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GRANT NO: DAMD17-89-Z-9021

TITLE: DISCOVERY AND DEVELOPMENT OF THERAPEUTIC DRUGS AGAINST
          LETHAL HUMAN RNA VIRUSES: A MULTIDISCIPLINARY ASSAULT

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REPORT DATE: July 16, 1991

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
                Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
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91-08214

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION	1b RESTRICTIVE MARKINGS
Unclassified	

2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION / AVAILABILITY OF REPORT
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	Approved for public release; distribution unlimited

4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)

6a. NAME OF PERFORMING ORGANIZATION	6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
Arizona State University		

6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
Cancer Research Institute Tempe, Arizona 85287-2404	

8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command	8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER
		Grant No. DAMD17-89-Z-9021

8c. ADDRESS (City, State, and ZIP Code)	10. SOURCE OF FUNDING NUMBERS			
Fort Detrick Frederick, Maryland 21702-5012	PROGRAM ELEMENT NO. 62787A	PROJECT NO. 3M1- 62787A871	TASK NO. AB	WORK UNIT ACCESSION NO. WUDA317987

11. TITLE (Include Security Classification)
DISCOVERY AND DEVELOPMENT OF THERAPEUTIC DRUGS AGAINST LETHAL HUMAN RNA VIRUSES: A MULTIDISCIPLINARY ASSAULT

12. PERSONAL AUTHOR(S)
George R. Pettit

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final Report	FROM 2/6/89 TO 7/5/91	1991 July 16	

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RAI; BD; Antiviral; Natural Products; RNA Viruses; Discovery of RNA-type antiviral drugs; Naturally occurring antiviral drugs
06	03		
06	13		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

See next page

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☐ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Virgina M. Miller	301-663-7325	SGRD-RMI-S

DD Form 1473, JUN 86   Previous editions are obsolete.   SECURITY CLASSIFICATION OF THIS PAGE

19. ABSTRACT

A total of 5,799 samples were submitted for prescreen RNA-type antiviral evaluation over the grant period to USAMRIID. After confirmation of activity, a good number of high priority extracts of plant and animal origin (and synthetic compounds) were identified for further research (fractionation, isolation and characterization of new antiviral compounds). The continued fractionation of these leads is in progress.

In addition to the natural products research, further development of the scale-up isolation of pancratistatin, an active lead against Japanese Encephalitis from both plant sources by greenhouse cultivation and semi-synthetic transformation of another plant product, narciclasine, occurred during the grant period. For the semi-synthetic research, seven tons of *Narcissus incomparabilis* has been obtained and is at the initial stage of scale-up isolation.

In short, progress continues to be excellent and we have a promising number of new antiviral leads to pursue.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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___N/A___ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

___N/A___ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

___N/A___ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

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_____   7/16/91
PI - Signature   Date

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I. Introduction

A long-term USAMRIID research program directed at the isolation and structural elucidation of new and potentially useful antiviral drugs from marine animals and plants has been substantially advanced by the two years of USAMRIID financial assistance. The financial support provided by the USAMRIID program was used to isolate and characterize new antiviral chemotherapeutic drugs from confirmed active extracts of marine invertebrates and vertebrates as well as marine and terrestrial plants including fungi, algae and other microorganisms. The research was sharply directed at marine animal and plant species yielding extracts with an outstanding level of antiviral activity in the USAMRIID's programs (RNA viruses).

II. Current Advances

A. Fractionation of Active Leads from Natural Products

Over the grant period, a total of 5,799 samples were submitted for antiviral evaluation (see Appendix A). Of this total 1,612 were from crude plant extracts and 3,826 were from crude marine extracts with another 288 from microorganism mycellium extracts as listed in Appendix A, Table I. Prescreen activity was determined in 1,420 of the total number and are listed in Tables II and III. Full screen submissions of fractions from 26 plant and 21 marine species are listed in Table IV. Table V lists samples submitted either in response to a specific request or as possible actives from other sources.

The ten highest priority antiviral actives determined by November, 1990 and beyond are as follows:

Marine Animal Sources

AVS-709 *Styela plicata* B 705028 (DO48)

AVS-7438   Unknown sponge (Papua New Guinea)   B 723123

AVS-8374 & 9217   Unknown sponge (Antarctic)   B 722902

Plant Sources

AVS-6976-6979   *Cryptocarya multipaniculata*   B 611679 (FO09-FO12)

AVS-6986 & 6988   *Virola oleifera*   B 619315 (FO12 & FO14)

AVS-7032   *Eucalyptus spathulata*   B 827298 (FO08)

AVS-7067-7068   *Ruprechtia tangarana*   B 836749 (FO05-FO06)

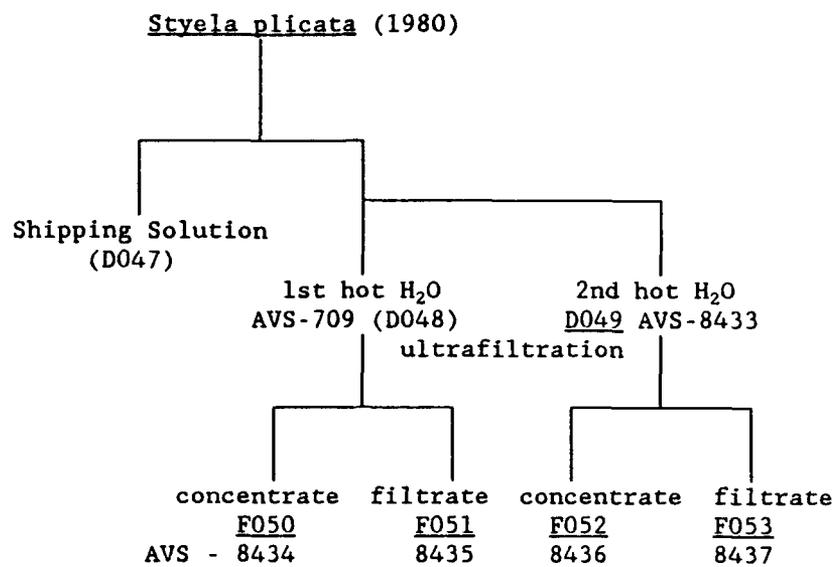AVS-7083-7087   *Phyllanthus anisolobus*   B 848528 (FO11-FO15)

AVS-7092   *Notelaea ligustrina*   B 853791 (FO17)

AVS-8259   Unknown Phaeophyta (Papua New Guinea)   B 848990

The accompanying data sheets and flow sheets for fractionations of active extracts follow, and provide a status report at end of this report period.

B705028.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT.CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-000709	B705028 D048	HIV	CEM	2/20/91	0.32	12.6	22.6	0	> 100	> 100	100	> 4.43	> 4.43	> 27.98
AVS-000709	B705028 D048	HIV	MT2	2/20/91	0.32	2.77	13.7	0	> 100	> 100	100	> 7.295	> 7.29	> 33.98
AVS-000709	B705028 D048	HIV	CEM	7/5/90	0.32	6.14	20.4	0	> 100	> 100	100	> 4.902	> 4.9	> 22.97
AVS-008433	B 705028-D049	HIV	CEM	1/4/91	3.2	107	0	0	969	> 1000	1000	0		> 13.64
AVS-008433	B 705028-D049	HIV	CEM	12/11/90	0.32	2.26	14.7	0	> 100	> 100	100	> 6.786	6.79	> 30.99
AVS-008433	B 705028-D049	HIV	MT2	12/11/90	0.32	4.19	59.6	0	> 100	> 100	100	> 1.677	1.68	> 24.19
AVS-008434	B 705028-F050	HIV	CEM	1/4/91	3.2	26.4	545	0	> 1000	> 1000	1000	> 1.833	1.83	> 19.9
AVS-008434	B 705028-F050	HIV	CEM	12/11/90	0.32	22.3	66.4	0	> 100	> 100	100	> 1.507	1.51	> 7.38
AVS-008434	B 705028-F050	HIV	MT2	12/11/90	0.32	1	2.11	0	> 100	> 100	100	> 47.445	47.44	> 54.02
AVS-008435	B 705028-F051	HIV	CEM	12/11/90	0.32	0	0	0	0.145	0.291	100	0		0
AVS-008435	B 705028-F051	HIV	MT2	12/11/90	0.32	0	0	0	> 100	> 100	100	0		> 0.83
AVS-008436	B 705028-F052	HIV	CEM	12/11/90	0.32	8.5	0	0	2.74	> 100	100	0		> 5.48
AVS-008436	B 705028-F052	HIV	MT2	12/11/90	0.32	1.38	14.1	0	> 100	> 100	100	> 7.087	7.09	> 38.71
AVS-008437	B 705028-F053	HIV	CEM	12/11/90	0.32	16.6	0	0	0.178	> 100	100	0		> 11.66
AVS-008437	B 705028-F053	HIV	MT2	12/11/90	0.32	1.66	37.7	0	> 100	> 100	100	> 2.656	2.66	> 34.06

B705028

<u>Styela plicata</u> (1980)

Shipping Solution
(D047)

1st hot H$_2$O
AVS-709 (D048)
ultrafiltration

2nd hot H$_2$O
<u>D049</u> AVS-8433

concentrate   filtrate   concentrate   filtrate
<u>F050</u>   <u>F051</u>   <u>F052</u>   <u>F053</u>
AVS - 8434   8435   8436   8437

B723123.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007438	B723123	JE	VERO	9/20/90	1	4.06	0	0	105	222	320	0		11.9
AVS-007438	B723123	JE	VERO	10/25/90	1	1.61	0	0	107	>190	320	0		12.23
AVS-007438	B723123	PT	VERO	9/20/90	1	0	0	0	17.3	>140	320	0		0.13
AVS-007438	B723123	PT	VERO	10/25/90	1	56.6	0	0	146	222	320	0		8
AVS-007438	B723123	SF	VERO	10/25/90	1	0	0	0	96.8	171	305	0		1.03
AVS-007438	B723123	SF	VERO	9/20/90	1	0	0	0	32	>190	320	0		0
AVS-007438	B723123	VEE	VERO	9/21/90	1	0	0	0	78.1	214	320	0		0.63
AVS-007438	B723123	VEE	VERO	10/26/90	1	0	1.94	0	70.6	>183	320	0		33.12
AVS-007438	B723123	YF	VERO	9/20/90	1	1	0	0	108	206	320	106.013	55.49	24.71
AVS-007438	B723123	YF	VERO	10/25/90	1	1	2.39	0	72.8	158	313	66.221	30.43	25
AVS-009453	B723123 F003	YF	VERO	4/24/91	1	0	0	0	49.8	77.7	283	0		0
AVS-009453	B723123 F003	JE	VERO	4/24/91	1	0	0	0	46.6	72.8	274	0		0
AVS-009453	B723123 F003	SF	VERO	4/23/91	1	0	0	0	36.3	57.5	95.8	0		0
AVS-009453	B723123 F003	PT	VERO	4/23/91	1	0	0	0	128	192	307	0		0
AVS-009453	B723123 F003	VEE	VERO	4/26/91	1	0	0	0	56.9	98.3	298	0		11.11
AVS-009454	B723123 F004	JE	VERO	4/24/91	1	32	0	0	>320	>320	320	0		0
AVS-009454	B723123 F004	YF	VERO	4/24/91	1	1	2.45	0	>320	>320	320	130.79	>130.79	46.75
AVS-009454	B723123 F004	SF	VERO	4/23/91	1	0	0	0	195	>320	320	0		1.33
AVS-009454	B723123 F004	PT	VERO	4/23/91	1	0	0	0	>320	>320	320	0		0
AVS-009454	B723123 F004	VEE	VERO	4/26/91	1	0	0	0	>320	>320	320	0		4.99
AVS-009455	B723123 F005	JE	VERO	4/24/91	1	0	0	0	203	>320	320	0		0
AVS-009455	B723123 F005	YF	VERO	4/24/91	1	1.2	4.68	0	227	>320	320	68.399	48.5	34.36
AVS-009455	B723123 F005	SF	VERO	4/23/91	1	0	0	0	17.3	108	320	0		0
AVS-009455	B723123 F005	PT	VERO	4/23/91	1	150	282	0	304	>320	320	1.135	1.08	6.42
AVS-009455	B723123 F005	VEE	VERO	4/26/91	1	0	0	0	296	>320	320	0		0
AVS-009456	B723123 F006	YF	VERO	4/24/91	1	0	0	0	7.67	>19.4	90	0		0.5
AVS-009456	B723123 F006	JE	VERO	4/24/91	1	0	0	0	15.8	>23.4	84.5	0		0
AVS-009456	B723123 F006	SF	VERO	4/23/91	1	0	0	0	8.91	>16.5	30.5	0		0
AVS-009456	B723123 F006	VEE	VERO	4/26/91	1	0	0	0	17.8	>30	92.6	0		0.1
AVS-009456	B723123 F006	PT	VERO	4/23/91	1	32	0	0	15.9	>28.1	91.9	0		0.01
AVS-009457	B723123 F007	YF	VERO	4/24/91	1	0	0	0	241	>320	320	0		2.4
AVS-009457	B723123 F007	JE	VERO	4/24/91	1	0	0	0	204	>320	320	0		0
AVS-009457	B723123 F007	SF	VERO	4/23/91	1	0	0	0	10.6	26.8	287	0		0
AVS-009457	B723123 F007	VEE	VERO	4/26/91	1	0	0	0	267	>320	320	0		0.13
AVS-009457	B723123 F007	PT	VERO	4/23/91	1	0	0	0	>320	>320	320	0		0

B723123

Unknown sp. (Porifera)

AVS-7438

$CH_2Cl_2-H_2O$

```
                    |
        +-----------+-----------+
        |           |           |
     CH₂Cl₂        H₂O       insolubles
     F003          F004        F005
  AVS- 9453        9454        9455
        |
   hexane-
   9:1 MeOH-H₂O
        |
   +----+----+
   |         |
 hexane   MeOH-H₂O
  F007      F006
AVS- 9457   9456
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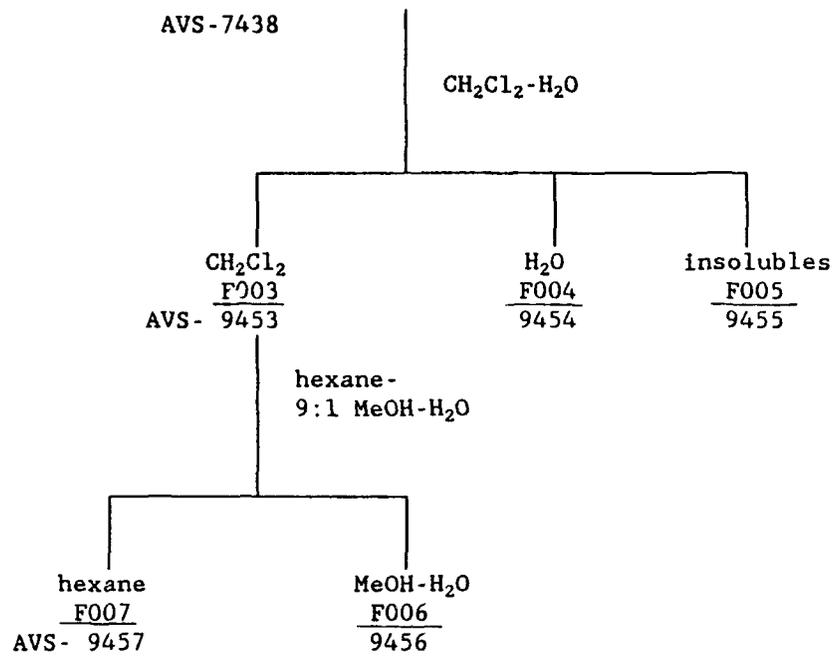
B722902.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-008374	B 722902	JE	VERO	12/13/90	10	481	824	0	1160	1840	3060	2.235	1.41	5
AVS-008374	B 722902	PT	VERO	12/13/90	10	346	566	0	1240	1890	3070	3.342	2.18	10.26
AVS-008374	B 722902	SF	VERO	12/13/90	10	264	443	922	1550	2100	3090	4.739	3.5	20.91
AVS-008374	B 722902	VEE	VERO	12/14/90	10	553	957	0	974	1780 >	3200	1.856	1.02	3.14
AVS-008374	B 722902	VV	VERO	1/10/91	1	0	0	0	320 >	320 >	320	0		0
AVS-008374	B 722902	YF	VERO	1/17/91	10	108	210	929	1700	2470 >	3200	11.77	8.12 >	35.14
AVS-008374	B 722902	SF	VERO	1/16/91	10	327	554	0	1480	2050 >	3090	3.709	2.67 >	16.87
AVS-008374	B 722902	HIV	MT2	2/5/91	0.32	0	0	0	100 >	100 >	100	0		1.08
AVS-008374	B 722902	HIV	CEM	2/5/91	0.32	0	0	0	9.03 >	100 >	100	0		0
AVS-008374	B 722902	SF	VERO	3/21/91	10	224	477	0	887	1670	3050	3.507	1.86	11.5
AVS-008374	B 722902	PT	VERO	3/21/91	10	320	0	0	1190	1860	3070	0		11.79 >
AVS-008374	B 722902	YF	VERO	3/21/91	10	131	827	0	1000	1730	3050	2.096	1.21 >	10.36 >
AVS-008374	B 722902	JE	VERO	3/21/91	10	512	0	0	1550	2100	3090	0		10.49 >
AVS-008374	B 722902	VEE	VERO	3/22/91	10	1020	1880	0	3200 >	3200 >	3200 ^	1.704 ^	1.7 >	8.87 >
AVS-009217	B722902	JE	VERO	2/27/91	10	100	0	0	1500	2170 >	3200	0		21.58 >
AVS-009217	B722902	YF	VERO	2/13/91	10	164	305	0	1330	1950	3080	6.403	4.35	17.01
AVS-009217	B722902	SF	VERO	2/12/91	10	160	255	861	1570	2130	3150	8.353	6.13	27.17
AVS-009217	B722902	VEE	VERO	2/15/91	10	616	0	0	898	2310 >	3200	0		1.5
AVS-009217	B722902	PT	VERO	2/12/91	10	779	0	0	1550	2100	3090	0		3.94
AVS-009217	B722902	VV	VERO	3/28/91	1	0	0	0	320 >	320 >	320	0		0

B611679.TXT

AVS #	CTR #	VIRUS	CELL	DATE	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-006975	B611679-F008	HIV	MT2	8/8/90	0	0	0	100 ^	100 ^	100	0		7.89 ^
AVS-006975	B611679-F008	JE	VERO	6/21/90	0	0	0	149	229 ^	320 ^	0		0
AVS-006975	B611679-F008	PT	VERO	6/21/90	0	0	0	164	231 ^	320 ^	0		0.92 ^
AVS-006975	B611679-F008	SF	VERO	6/21/90	0	0	0	130	197	317	0		0
AVS-006975	B611679-F008	VEE	VERO	6/22/90	0	0	0	212 ^	320 ^	320	0		0.1
AVS-006975	B611679-F008	YF	VERO	6/21/90	0	0	0	172	245 ^	320	0		2.5
AVS-006976	B611679-F008	HIV	MT2	8/8/90	0	0	0	100 ^	100 ^	100	0		4.6 ^
AVS-006976	B611679-F009	JE	VERO	6/21/90	0	0	0	128	192	307	0		0
AVS-006976	B611679-F009	PT	VERO	6/21/90	0	0	0	155	210	309	0		0
AVS-006976	B611679-F009	SF	VERO	8/2/90	28.5	53.5	0	153	209	309	3.905	2.87	13.98
AVS-006976	B611679-F009	SF	VERO	6/21/90	34.1	75.2	0	108	179	306	2.38	1.44	8.88
AVS-006976	B611679-F009	VEE	VERO	6/22/90	0	0	0	121	187	307	0		0.12
AVS-006976	B611679-F009	YF	VERO	6/21/90	0	0	0	143	203	310	0		4.03
AVS-006977	B611679-F010	HIV	MT2	8/8/90	0	0	0	100 ^	100 ^	100	0		2.59 ^
AVS-006977	B611679-F010	JE	VERO	6/21/90	0	0	0	156	211	311	0		0
AVS-006977	B611679-F010	PT	VERO	8/2/90	12.3	28.9	0	155	210	309	7.272	5.37	19.33
AVS-006977	B611679-F010	PT	VERO	6/21/90	53.4	0	0	155	210	309	0		8.51
AVS-006977	B611679-F010	SF	VERO	8/2/90	9.22	30.5	89	155	210	309	6.875	5.07	32.48
AVS-006977	B611679-F010	SF	VERO	6/21/90	22.8	43.9	96.6	155	210	309	4.787	3.53	21.98
AVS-006977	B611679-F010	VEE	VERO	6/22/90	0	0	0	156	211	311	0		2.4
AVS-006977	B611679-F010	YF	VERO	8/2/90	90.2	0	0	155	210	309	0		7.21
AVS-006977	B611679-F010	YF	VERO	6/21/90	42.1	0	0	156	211	311	0		13.28
AVS-006978	B611679-F011	HIV	MT2	8/8/90	0	0	0	100 ^	100 ^	100	0		2.77 ^
AVS-006978	B611679-F011	JE	VERO	8/9/90	0	0	0	1000 ^	1000 ^	1000	0		1.88 ^
AVS-006978	B611679-F011	JE	VERO	6/21/90	0	0	0	320 ^	320 ^	320	0		0
AVS-006978	B611679-F011	PT	VERO	6/21/90	60.3	172	0	320 ^	320 ^	320	1.865	1.86 ^	16.74 ^
AVS-006978	B611679-F011	SF	VERO	8/7/90	447	0	0	1000 ^	1000 ^	1000	0		4.88 ^
AVS-006978	B611679-F011	SF	VERO	6/21/90	107	187	0	320 ^	320 ^	320	1.711	1.71 ^	11.45 ^
AVS-006978	B611679-F011	VEE	VERO	8/10/90	0	0	0	1000 ^	1000 ^	1000	0		0.5 ^
AVS-006978	B611679-F011	VEE	VERO	6/22/90	0	0	0	320 ^	320 ^	320	0		0
AVS-006978	B611679-F011	YF	VERO	8/8/90	0	0	0	1000 ^	1000 ^	1000	0		0
AVS-006978	B611679-F011	YF	VERO	6/21/90	0	0	0	320 ^	320 ^	320	0		0.03
AVS-006979	B611679-F012	HIV	MT2	8/8/90	0	0	0	100 ^	100 ^	100	0		5.16 ^
AVS-006979	B611679-F012	JE	VERO	8/9/90	0	0	0	490	660	966	0		4.1 ^
AVS-006979	B611679-F012	JE	VERO	6/21/90	0	0	0	320 ^	320 ^	320	0		0
AVS-006979	B611679-F012	PT	VERO	6/21/90	70.6	164	0	320 ^	320 ^	320	1.954	1.95 ^	15.06 ^
AVS-006979	B611679-F012	SF	VERO	8/7/90	105	155	315	490	660	966	4.25	3.16 ^	19.32 ^
AVS-006979	B611679-F012	SF	VERO	6/21/90	77.3	167	0	320 ^	320 ^	320	1.92	1.92 ^	13.46 ^
AVS-006979	B611679-F012	VEE	VERO	8/10/90	0	0	0	490	660	966	0		1.66

B611679.TXT

AVS #	CTR #	VIRUS	CELL	DATE	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-008979	B611679-F012	VEE	VERO	6/22/90	0	0	0	>320	>320	>320	0		0.35
AVS-008979	B611679-F012	YF	VERO	8/8/90	294	0	0	>480	>653	>965	0		2.41
AVS-008979	B611679-F012	YF	VERO	6/21/90	32	88.5	0	>320	>320	>320	3.616	3.62	>17.36
AVS-009439	B611679 F013	YF	VERO	4/24/91	201	0	0	>320	>320	>320	0		1.81
AVS-009439	B611679 F013	SF	VERO	4/23/91	264	0	0	>320	>320	>320	0		2.12
AVS-009439	B611679 F013	JE	VERO	4/24/91	66.9	158	0	>320	>320	>320	2.019	2.02	>16.1
AVS-009439	B611679 F013	PT	VERO	4/23/91	0	0	0	>320	>320	>320	0		0
AVS-009440	B611679 F014	VEE	VERO	4/26/91	0	0	0	>320	>320	>320	0		0.22
AVS-009440	B611679 F014	YF	VERO	4/24/91	0	0	0	143	202	308	0		0
AVS-009440	B611679 F014	SF	VERO	4/23/91	0	0	0	148	205	309	0		1.08
AVS-009440	B611679 F014	JE	VERO	4/24/91	0	0	0	155	210	309	0		4.05
AVS-009440	B611679 F014	PT	VERO	4/23/91	0	0	0	155	210	309	0		0.2
AVS-009441	B611679 F015	VEE	VERO	4/28/91	0	0	0	160	220	320	0		0.01
AVS-009441	B611679 F015	YF	VERO	4/24/91	24.3	0	0	51	70.7	235	0		0.1
AVS-009441	B611679 F015	SF	VERO	4/23/91	68.4	0	0	24.7	50	95	0		0
AVS-009441	B611679 F015	JE	VERO	4/24/91	56.6	0	0	58.2	84.3	289	0		0.49
AVS-009441	B611679 F015	PT	VERO	4/23/91	0	0	0	114	183	306	0		3.98
AVS-009442	B611679 F016	VEE	VERO	4/26/91	0	0	0	155	210	309	0		0.5
AVS-009442	B611679 F016	YF	VERO	4/24/91	0	0	0	41.2	70	274	0		0
AVS-009442	B611679 F016	SF	VERO	4/23/91	0	0	0	7.33	15	30.9	0		0.16
AVS-009442	B611679 F016	JE	VERO	4/24/91	90.9	0	0	55.4	84.8	290	0		0
AVS-009442	B611679 F016	PT	VERO	4/23/91	72.6	0	0	54.4	76.7	274	0		0.52
AVS-009443	B611679 F017	VEE	VERO	4/26/91	0	0	0	77.3	151	303	0		0.05
AVS-009443	B611679 F017	YF	VERO	4/24/91	0	0	0	70.9	145	303	0		0
AVS-009443	B611679 F017	SF	VERO	4/23/91	0	0	0	8.69	21.6	89.4	0		0
AVS-009443	B611679 F017	JE	VERO	4/24/91	49.8	84.5	0	62.2	100	298	1.928	1.05	0
AVS-009443	B611679 F017	PT	VERO	4/23/91	0	0	0	88.7	163	304	0		4.38
AVS-009444	B611679 F018	VEE	VERO	4/26/91	0	0	0	119	187	309	0		1
AVS-009444	B611679 F018	YF	VERO	4/24/91	0	0	0	45.2	63.4	96.3	0		0
AVS-009444	B611679 F018	SF	VERO	4/23/91	2.71	0	0	13.7	28.9	92.4	0		10.53
AVS-009444	B611679 F018	JE	VERO	4/24/91	0	0	0	44	62.6	96.3	0		0.03
AVS-009444	B611679 F018	PT	VERO	4/23/91	17.2	0	0	49	66	96.6	0		7.25
AVS-009445	B611679 F019	VEE	VERO	4/26/91	70.4	0	0	59	86	290	0		1.14
AVS-009445	B611679 F019	YF	VERO	4/24/91	11.7	30.8	0	30.4	54.7	99	1.776	0.99	7.88
AVS-009445	B611679 F019	SF	VERO	4/23/91	1	0	0	2.61	8.12	29.4	0		3.64
AVS-009445	B611679 F019	JE	VERO	4/24/91	0	0	0	18.6	28.7	265	0		0
AVS-009445	B611679 F019	PT	VERO	4/23/91	4.15	6.38	0	17.5	25.1	89.5	3.927	2.75	14.12
AVS-009445	B611679 F019	VEE	VERO	4/26/91	57.5	0	0	22.6	68.6	309	0		0.3
AVS-009446	B611679 F020	JE	VERO	4/24/91	46.1	66.4	0	137	199	310	2.994	2.06	8.88

B611679.TXT

AVS #	CTR #	VIRUS	CELL	DATE	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-009446	B611679 F020	YF	VERO	4/24/91	47.8	81	0	128	193	310	2.386	1.59	7.75
AVS-009446	B611679 F020	SF	VERO	4/23/91	0	0	0	7.65	19.3	94.6	0		0
AVS-009446	B611679 F020	PT	VERO	4/23/91	41.1	58.2	0	124	190	309	3.269	2.13	11.73
AVS-009446	B611679 F020	VEE	VERO	4/26/91	0	0	0	151	> 225	320	0	>	0.15

B611679

Cryptocarya multipaniculata
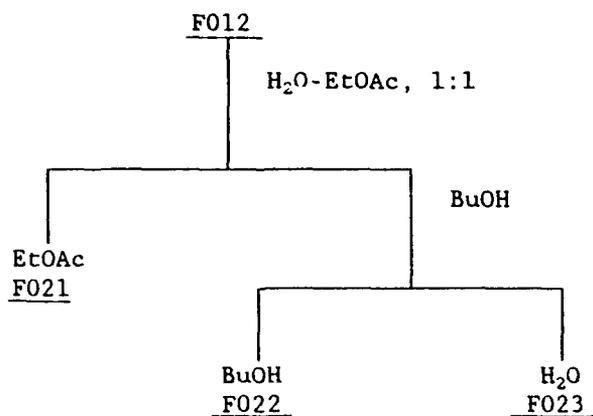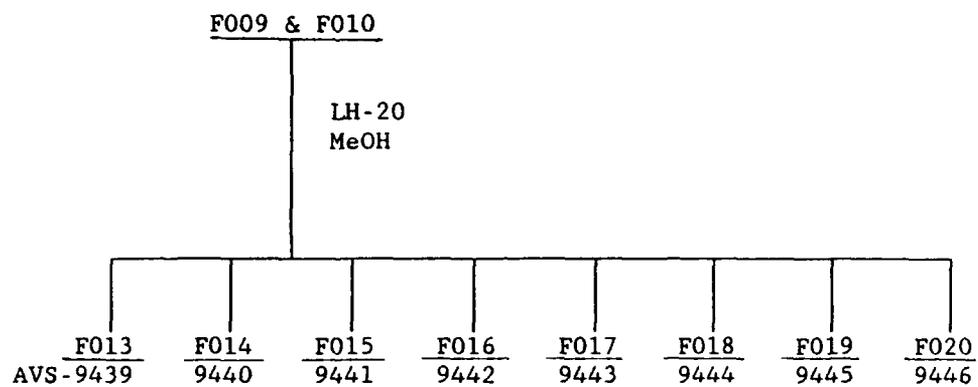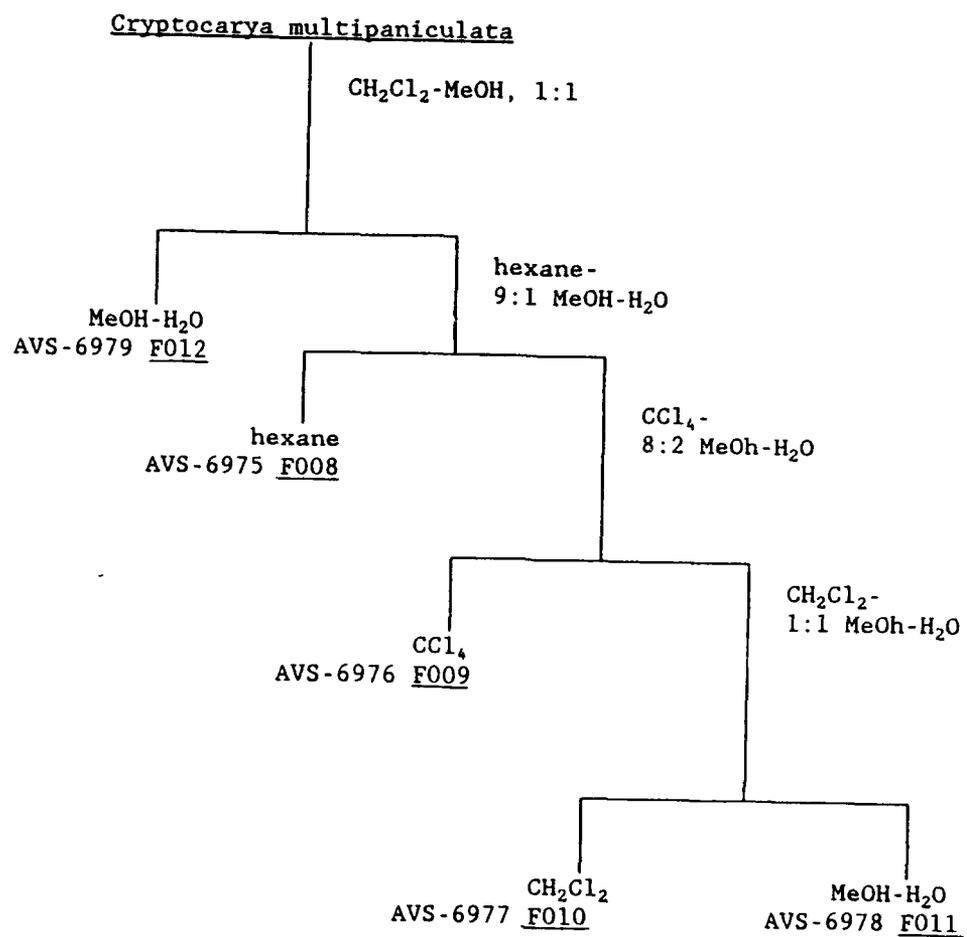
<u>F009 & F010</u>

LH-20
MeOH

| <u>F013</u> | <u>F014</u> | <u>F015</u> | <u>F016</u> | <u>F017</u> | <u>F018</u> | <u>F019</u> | <u>F020</u> |
| AVS-9439 | 9440 | 9441 | 9442 | 9443 | 9444 | 9445 | 9446 |

<u>F012</u>

H_2O-EtOAc, 1:1

BuOH

EtOAc
<u>F021</u>

BuOH
<u>F022</u>

H_2O
<u>F023</u>

B611679

Cryptocarya multipaniculata

CH_2Cl_2-MeOH, 1:1

MeOH-H_2O
AVS-6979 F012

hexane-
9:1 MeOH-H_2O

hexane
AVS-6975 F008

CCl_4-
8:2 MeOh-H_2O

CCl_4
AVS-6976 F009

CH_2Cl_2-
1:1 MeOh-H_2O

CH_2Cl_2
AVS-6977 F010

MeOH-H_2O
AVS-6978 F011

B619315.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT.CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-006984	B619315-F010	HIV	MT2	8/8/90	0.32	1.47	0	0	100	> 100	100	0		> 30.31
AVS-006984	B619315-F010	JE	VERO	6/21/90	1	0	0	0	144	203	308	0		0
AVS-006984	B619315-F010	PT	VERO	6/21/90	1	0	0	0	139	199	308	0		> 1.1
AVS-006984	B619315-F010	SF	VERO	6/21/90	1	0	0	0	130	194	307	0		0.55
AVS-006984	B619315-F010	VEE	VERO	6/22/90	1	0	0	0	140	> 265	320	0		0
AVS-006984	B619315-F010	YF	VERO	6/21/90	1	0	0	0	158	216	320	0		4.45
AVS-006985	B619315-F011	HIV	MT2	8/16/90	0.32	0	0	0	49.3	66.7	97.9	0		1.06
AVS-006985	B619315-F011	JE	VERO	6/21/90	1	0	0	0	40.1	60.3	96.8	0		0
AVS-006985	B619315-F011	PT	VERO	6/21/90	1	0	0	0	28.9	52.8	95.3	0		> 0.04
AVS-006985	B619315-F011	SF	VERO	6/21/90	1	0	0	0	38.6	59	95.9	0		0
AVS-006985	B619315-F011	VEE	VERO	6/22/90	1	0	0	0	8.06	70.9	286	0		0
AVS-006985	B619315-F011	VV	VERO	7/19/90	1	0	0	0	13.5	27.9	247	0		0
AVS-006985	B619315-F011	YF	VERO	6/21/90	1	0	0	0	49	66	96.6	0		> 0.1
AVS-006986	B619315-F012	HIV	MT2	8/16/90	0.32	0	0	0	70.6	100	100	0	2.75	2.01
AVS-006986	B619315-F012	JE	VERO	8/9/90	3.2	134	179	302	492	663	973	3.709		> 16.01
AVS-006986	B619315-F012	JE	VERO	6/21/90	1	235	0	0	220	> 320	320	0		0
AVS-006986	B619315-F012	PT	VERO	6/21/90	1	21.3	0	0	135	224	320	0		> 14.36
AVS-006986	B619315-F012	SF	VERO	8/7/90	3.2	15.2	28.3	88.1	452	634	963	22.437	15.97	41.73
AVS-006986	B619315-F012	SF	VERO	6/21/90	1	14.6	30.2	88.8	157	216	320	7.151	5.19	25.88
AVS-006986	B619315-F012	VEE	VERO	8/10/90	3.2	129	227	0	283	520	970	2.288	1.25	8.26
AVS-006986	B619315-F012	VEE	VERO	6/22/90	1	87.7	208	0	222	> 320	320	1.54	1.07	6.48
AVS-006986	B619315-F012	VV	VERO	8/9/90	1	34.2	59.8	0	86.8	162	310	2.715	> 1.45	9.84
AVS-006986	B619315-F012	VV	VERO	7/19/90	1	32	46.8	92.7	182	264	320	5.647	> 3.89	21.69
AVS-006986	B619315-F012	YF	VERO	8/8/90	3.2	228	0	0	193	286	917	0		0.33
AVS-006986	B619315-F012	YF	VERO	6/21/90	1	45.1	63.6	0	174	255	320	4.016	2.74	12.38
AVS-006987	B619315-F013	HIV	MT2	8/16/90	0.32	0	0	0	100	100	100	0		1.2
AVS-006987	B619315-F013	JE	VERO	6/21/90	1	0	0	0	> 320	> 320	320	0		0
AVS-006987	B619315-F013	PT	VERO	6/21/90	1	0	0	0	> 320	> 320	320	0		5.92
AVS-006987	B619315-F013	SF	VERO	6/21/90	1	0	0	0	> 320	> 320	320	0		3.73
AVS-006987	B619315-F013	VEE	VERO	6/22/90	1	0	0	0	87.6	> 320	320	0		0
AVS-006987	B619315-F013	VV	VERO	7/19/90	1	184	0	0	200	300	320	0		0.64
AVS-006987	B619315-F013	YF	VERO	6/21/90	1	0	0	0	> 320	> 320	320	0		0
AVS-006988	B619315-F014	HIV	MT2	8/16/90	0.32	0	0	0	100	100	100	0		2.39
AVS-006988	B619315-F014	JE	VERO	8/9/90	3.2	0	0	0	527	735	1000	0		4.75
AVS-006988	B619315-F014	JE	VERO	6/21/90	1	0	0	0	142	259	320	0		0
AVS-006988	B619315-F014	PT	VERO	6/21/90	1	44.1	60.7	0	290	> 320	320	5.272	4.77	> 23.2
AVS-006988	B619315-F014	SF	VERO	8/7/90	3.2	140	220	0	497	674	993	3.068	2.26	> 12.59
AVS-006988	B619315-F014	SF	VERO	6/21/90	1	47.8	71.4	0	277	> 320	320	4.482	3.89	> 17.8

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AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-006988	B619315-F014	VEE	VERO	8/10/90	3.2	0	0	0	544	767 >	1000	0		2.36
AVS-006988	B619315-F014	VEE	VERO	6/22/90	1	0	0	0	189	277 >	320	0		0
AVS-006988	B619315-F014	VV	VERO	7/19/90		0	0	0	195	290 >	320	0		3.35
AVS-006988	B619315-F014	YF	VERO	8/8/90	3.2	166	0	0	513	706 >	1000	0		8.78
AVS-006988	B619315-F014	YF	VERO	6/21/90	1	100	0	0	320 >	320 >	320	0		11.16
AVS-009447	B619315 F015	JE	VERO	4/24/91	1	0	0	0	90.3	271	320	0		0
AVS-009447	B619315 F015	YF	VERO	4/24/91	1	294	0	0	154	276 >	320	0		0.34
AVS-009447	B619315 F015	SF	VERO	4/23/91	1	53.1	100	0	66	152	310	1.524	0.66	1.09
AVS-009447	B619315 F015	PT	VERO	4/23/91	1	143	0	0	186	272 >	320	0		2.98
AVS-009447	B619315 F015	VEE	VERO	4/26/91	1	208	0	0	61.3	90.6	320	0		0.25
AVS-009448	B619315 F016	YF	VERO	4/24/91	1	143	224	0	256 >	320 >	320	1.43	1.14 >	3.55
AVS-009448	B619315 F016	JE	VERO	4/24/91	1	148	219	0	88.7 >	320 >	320	1.458	0.4 >	2.39
AVS-009448	B619315 F016	SF	VERO	4/23/91	1	20.4	0	0	40	60	96	0		4.09
AVS-009448	B619315 F016	PT	VERO	4/23/91	1	63	136	0	320 >	320 >	320	2.356	2.36 >	14.43
AVS-009448	B619315 F016	VEE	VERO	4/26/91	1	156	265	0	320 >	320 >	320	1.21	1.21 >	6.5
AVS-009449	B619315 F017	YF	VERO	4/24/91	1	0	0	0	51.2	73	281	0		0
AVS-009449	B619315 F017	JE	VERO	4/24/91	1	44.9	63.1	0	89.8	169	311	2.683	1.42	6.89
AVS-009449	B619315 F017	SF	VERO	4/23/91	1	0	0	0	8.54	16.7	30.5	0		0
AVS-009449	B619315 F017	PT	VERO	4/23/91	1	0	0	0	49.9	67.8	100	0		1.33
AVS-009449	B619315 F017	VEE	VERO	4/26/91	1	39.8	57.4	0	160	220 >	320	3.825	2.78 >	16.13
AVS-009450	B619315 F018	YF	VERO	4/24/91	1	0	0	0	48	73	272	0		0
AVS-009450	B619315 F018	JE	VERO	4/24/91	1	0	0	0	51.3	76.7	279	0		0
AVS-009450	B619315 F018	SF	VERO	4/23/91	1	0	0	0	3.86	5.9	9.59	0		0
AVS-009450	B619315 F018	PT	VERO	4/23/91	1	0	0	0	55.9	79.9	282	0		4.6
AVS-009450	B619315 F018	VEE	VERO	4/26/91	1	39.1	79.6	0	70.6	124	300	1.552	0.89 >	6.15
AVS-009451	B619315 F019	YF	VERO	4/24/91	1	0	0	0	47.5	66.8	173	0		0.79
AVS-009451	B619315 F019	JE	VERO	4/24/91	1	0	0	0	44.8	66	222	0		0
AVS-009451	B619315 F019	SF	VERO	4/23/91	1	0	0	0	4.9	6.6	9.66	0		1.36
AVS-009451	B619315 F019	PT	VERO	4/23/91	1	13.4	17.9	30.2	50.7	69.4	198	3.877	2.83 >	15.36
AVS-009451	B619315 F019	VEE	VERO	4/26/91	1	22.3	0	0	53	74	271	0		6.22
AVS-009452	B619315 F020	YF	VERO	4/24/91	1	0	0	0	48.5	74.2	283	0		0.07
AVS-009452	B619315 F020	JE	VERO	4/24/91	1	0	0	0	42.9	73.3	287	0		0.04
AVS-009452	B619315 F020	SF	VERO	4/23/91	1	0	0	0	3.63	5.75	9.58	0		
AVS-009452	B619315 F020	PT	VERO	4/23/91	1	14.9	23	0	57.4	82.7	293	3.596	2.49 >	11.71
AVS-009452	B619315 F020	VEE	VERO	4/26/91	1	27.7	90.9	0	66.7	105	315	1.151	0.73 >	6.99

B619315

Virola oleifera

CH_2Cl_2-MeOH, 1:1

MeOH-H_2O
AVS-6988 F014

hexane-
9:1 MeOH-H_2O

hexane
AVS-6984 F010

CCl_4-
8:2 MeOH-H_2O

CCl_4
AVS-6985 F011

CH_2Cl_2-
1:1 MeOH-H_2O

CH_2Cl_2
AVS-6986 F012

MeOH-H_2O
AVS-6987 F013

LH-20
MeOH

F015	F016	F017	F018	F019	F020
AVS-9447	9448	9449	9450	9451	9452
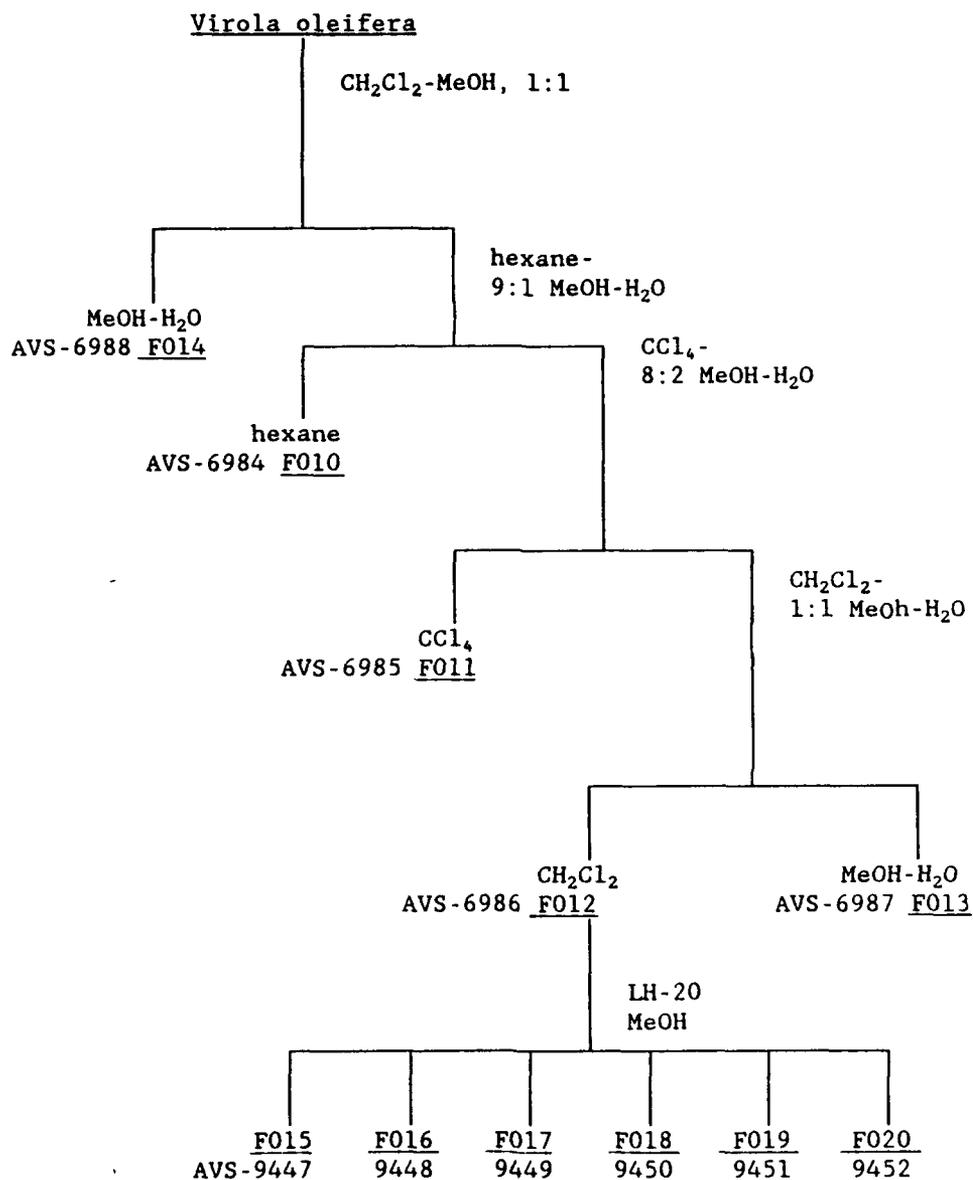
B827298.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007029	B827298-F005	HIV	MT2	8/22/90	0.32	100	0	0	100	100	100	0		6.58
AVS-007029	B827298-F005	JE	VERO	8/1/90	1	0	0	0	117	>200	320	0		0
AVS-007029	B827298-F005	PT	VERO	7/31/90	1	0	0	0	135	>245	320	0		0
AVS-007029	B827298-F005	SF	VERO	7/31/90	1	0	0	0	115	>208	320	0		0
AVS-007029	B827298-F005	VEE	VERO	8/3/90	1	0	0	0	186	>279	320	0		0.68
AVS-007029	B827298-F005	VV	VERO	7/26/90	1	0	0	0	124	>212	320	0		0
AVS-007029	B827298-F005	YF	VERO	8/1/90	1	0	0	0	30.2	>188	320	0		0
AVS-037030	B827298-F005	HIV	MT2	8/22/90	0.32	0	0	0	100	100	100	0		2.3
AVS-007030	B827298-F006	JE	VERO	8/1/90	1	0	0	0	62	>169	320	0		0
AVS-007030	B827298-F006	PT	VERO	7/31/90	1	0	0	0	40.7	71.6	279	0		0
AVS-007030	B827298-F006	SF	VERO	7/31/90	1	0	0	0	44.8	80.2	289	0		0
AVS-007030	B827298-F006	VEE	VERO	8/3/90	1	281	0	0	47.7	283	320	0		0
AVS-007030	B827298-F006	VV	VERO	7/26/90	1	0	0	0	320	>320	320	0		0
AVS-007030	B827298-F006	YF	VERO	8/1/90	1	0	0	0	14.9	>61.9	320	0		0
AVS-007031	B827298-F007	HIV	MT2	8/22/90	0.32	0	0	0	52.5	73	100	0		0.8
AVS-007031	B827298-F007	JE	VERO	8/1/90	1	0	0	0	57.4	82.7	299	0		0
AVS-007031	B827298-F007	JE	VERO	10/24/90	1	0	0	0	21.4	51.4	265	0		0
AVS-007031	B827298-F007	PT	VERO	7/31/90	1	0	0	0	44.6	63	96.3	0		3.99
AVS-007031	B827298-F007	SF	VERO	7/31/90	1	0	0	0	38.6	59	95.9	0		
AVS-007031	B827298-F007	VEE	VERO	8/3/90	1	49.6	76.9	0	220	>320	320	4.162	>2.86	12.26
AVS-007031	B827298-F007	VV	VERO	8/1/90	1	21.1	0	293	10.7	19	69.1	0		2.49
AVS-007031	B827298-F007	YF	VERO	8/1/90	1	64.1	0	290	35.6	57.9	98.2	0		0.05
AVS-007032	B827298-F008	HIV	MT2	8/22/90	0.32	151	0	0	100	100	100	0		2.5
AVS-007032	B827298-F008	JE	VERO	8/1/90	1	0	0	0	320	>320	320	0		0
AVS-007032	B827298-F008	JE	VERO	10/24/90	1	0	0	0	320	>320	320	0		0.32
AVS-007032	B827298-F008	PT	VERO	7/31/90	1	0	133	0	320	>320	320	2.414	>2.41	24.46
AVS-007032	B827298-F008	SF	VERO	7/31/90	1	0	119	0	320	>320	320	2.68	>2.68	18.27
AVS-007032	B827298-F008	VEE	VERO	8/3/90	1	0	273	0	320	>320	320	1.17	>1.17	9.14
AVS-007032	B827298-F008	VV	VERO	9/27/90	1	0	0	0	25.4	63.8	283	0		0
AVS-007032	B827298-F008	YF	VERO	8/1/90	1	0	230	0	320	>320	320	1.394	>1.39	6.98
AVS-007033	B827298-F009	HIV	MT2	8/22/90	0.32	0	0	0	95	100	100	0		1.77
AVS-007033	B827298-F009	JE	VERO	8/1/90	1	0	0	0	174	249	320	0		0.1
AVS-007033	B827298-F009	JE	VERO	9/27/90	3.2	0	0	0	427	618	962	0		0
AVS-007033	B827298-F009	PT	VERO	7/31/90	1	38	61	0	171	241	320	3.949	>2.79	13.42
AVS-007033	B827298-F009	SF	VERO	7/31/90	1	0	0	0	194	296	320	0		0.28
AVS-007033	B827298-F009	VEE	VERO	8/3/90	1	0	0	0	320	>320	320	0		3.79
AVS-007033	B827298-F009	VV	VERO	7/26/90	1	128	0	0	193	286	320	0		3.26
AVS-007033	B827298-F009	YF	VERO	8/1/90	1	0	0	0	168	236	320	0		0.15
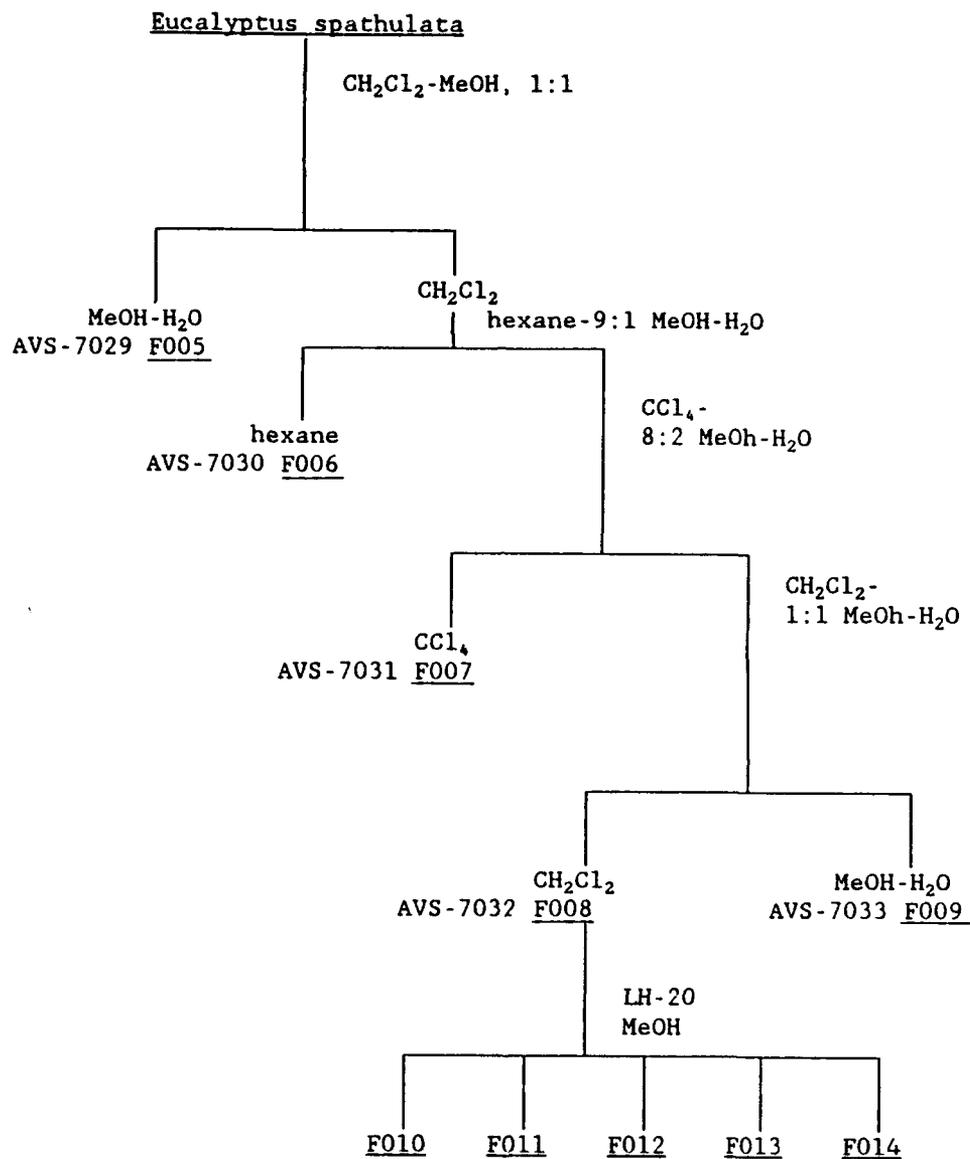
B827298

<u>Eucalyptus spathulata</u>

CH_2Cl_2-MeOH, 1:1

MeOH-H_2O
AVS-7029 <u>F005</u>

CH_2Cl_2
hexane-9:1 MeOH-H_2O

hexane
AVS-7030 <u>F006</u>

CCl_4-
8:2 MeOh-H_2O

CCl_4
AVS-7031 <u>F007</u>

CH_2Cl_2-
1:1 MeOh-H_2O

CH_2Cl_2
AVS-7032 <u>F008</u>

MeOH-H_2O
AVS-7033 <u>F009</u>

LH-20
MeOH

<u>F010</u>   <u>F011</u>   <u>F012</u>   <u>F013</u>   <u>F014</u>

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AVS #	CTR #	VIRUS	CELL	DATE	INIT.CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI		TAI
AVS-007065	B836749-F003	JE	VERO	8/31/90	1	0	0	0	26.5	100	320	0			0
AVS-007065	B836749-F003	JE	VERO	10/25/90	3.2	0	0	0	72.2	276	926	0			0
AVS-007065	B836749-F003	JE	VERO	10/3/90	3.2	0	0	0	43	97.8	913	0			0
AVS-007065	B836749-F003	PT	VERO	8/30/90	1	0	0	0	56.7	149	320	0			0
AVS-007065	B836749-F003	PT	VERO	10/25/90	3.2	0	0	0	60.9	115	876	0			0
AVS-007065	B836749-F003	PT	VERO	10/2/90	3.2	241	0	0	64.1	96.2	906	0		^	1.4
AVS-007065	B836749-F003	SF	VERO	8/30/90	1	0	0	0	52	173	320	0			0
AVS-007065	B836749-F003	SF	VERO	10/25/90	3.2	0	0	0	34.1	87.3	691	0			0
AVS-007065	B836749-F003	SF	VERO	10/2/90	3.2	0	0	0	57.9	98.4	800	0			0
AVS-007065	B836749-F003	VEE	VERO	8/28/90	1	0	0	0	69.8	249	320	0			0
AVS-007065	B836749-F003	VEE	VERO	10/26/90	3.2	0	0	0	168	382	938	0			0
AVS-007065	B836749-F003	VEE	VERO	10/5/90	3.2	0	0	0	100	283	924	0			0
AVS-007065	B836749-F003	VV	VERO	10/4/90	1	0	0	0	123	220	320	0			0
AVS-007065	B836749-F003	YF	VERO	8/31/90	1	41.1	100	0	10	130	320	1.303	0.1	^	0.11
AVS-007065	B836749-F003	YF	VERO	10/25/90	3.2	9.45	18.3	0	40.3	81.8	869	4.456	2.2	^	13.92
AVS-007065	B836749-F003	YF	VERO	10/3/90	3.2	13.7	23.9	0	44	94	918	3.929	1.84		7.19
AVS-007066	B836749-F004	JE	VERO	8/31/90	1	0	0	0	152	320	320	0			0
AVS-007066	B836749-F004	PT	VERO	8/30/90	1	215	0	0	320	320	320	0			2.07
AVS-007066	B836749-F004	SF	VERO	8/30/90	1	0	0	0	320	320	320	0			3.01
AVS-007066	B836749-F004	VEE	VERO	8/28/90	1	0	0	0	320	320	320	0			1.74
AVS-007066	B836749-F004	VV	VERO	10/4/90	1	0	0	0	175	251	320	0		^	0.4
AVS-007066	B836749-F004	YF	VERO	8/31/90	1	0	0	0	61.1	320	320	0			0
AVS-007067	B836749-F005	JE	VERO	8/31/90	1	0	0	0	27.6	154	320	0			0
AVS-007067	B836749-F005	JE	VERO	10/25/90	1	306	0	0	157	320	320	0		^	0
AVS-007067	B836749-F005	JE	VERO	10/3/90	3.2	0	0	0	131	259	911	0			0
AVS-007067	B836749-F005	PT	VERO	8/30/90	1	0	0	0	135	196	308	0			0
AVS-007067	B836749-F005	PT	VERO	10/25/90	1	189	0	0	151	279	320	0		^	0.45
AVS-007067	B836749-F005	PT	VERO	10/2/90	3.2	73.3	0	0	140	218	800	0			5.45
AVS-007067	B836749-F005	SF	VERO	8/30/90	1	66.4	0	0	109	180	309	0			0.88
AVS-007067	B836749-F005	SF	VERO	10/25/90	1	0	0	0	15.5	138	309	0			0
AVS-007067	B836749-F005	SF	VERO	10/2/90	3.2	0	0	0	29	58	251	0		^	0.2
AVS-007067	B836749-F005	VEE	VERO	8/28/90	1	192	0	0	73.8	320	320	0		^	0
AVS-007067	B836749-F005	VEE	VERO	10/26/90	1	0	0	0	245	320	320	0		^	0.44
AVS-007067	B836749-F005	VEE	VERO	10/5/90	3.2	0	0	0	115	206	821	0			0
AVS-007067	B836749-F005	VV	VERO	10/4/90	1	0	0	0	63.6	137	302	0			1.43
AVS-007067	B836749-F005	YF	VERO	8/31/90	1	11.8	21.7	0	5.75	155	320	7.138	0.26		9.34
AVS-007067	B836749-F005	YF	VERO	10/25/90	1	3.5	6.12	0	53.6	192	320	31.334	8.77	^	32.54
AVS-007067	B836749-F005	YF	VERO	10/3/90	3.2	4.89	9.48	28	93.5	218	906	22.952	9.86	^	33.97

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AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007068	B836749-F006	JE	VERO	8/31/90	1	0	0	0	69.1	165	310	0		0
AVS-007068	B836749-F006	JE	VERO	10/25/90	1	0	0	0	170	261	320	0		0
AVS-007068	B836749-F006	JE	VERO	10/3/90	1	0	0	0	63.4	140	302	0		0.99
AVS-007068	B836749-F006	PT	VERO	8/30/90	1	57.7	0	0	155	210	309	0		6.08
AVS-007068	B836749-F006	PT	VERO	10/25/90	1	0	0	0	123	194	320	0		0.3
AVS-007068	B836749-F006	PT	VERO	10/2/90	1	66.1	0	0	75.6	140	302	0		6.86
AVS-007068	B836749-F006	SF	VERO	8/30/90	1	32	51.9	0	152	208	309	4.006	2.92	19.21
AVS-007068	B836749-F006	SF	VERO	10/25/90	1	0	0	0	38.5	124	300	0		0
AVS-007068	B836749-F006	SF	VERO	10/2/90	1	0	0	0	25.6	48.7	95.8	0		0
AVS-007068	B836749-F006	VEE	VERO	8/28/90	1	0	0	0	120	205	320	0		0.64
AVS-007068	B836749-F006	VEE	VERO	10/26/90	1	0	0	0	222	320	320	0		0.22
AVS-007068	B836749-F006	VEE	VERO	10/5/90	1	0	0	0	76.7	143	302	0		0.88
AVS-007068	B836749-F006	VV	VERO	10/4/90	1	0	0	0	86.8	161	304	0		0
AVS-007068	B836749-F006	YF	VERO	8/31/90	1	29.3	54.1	0	111	181	306	3.339	2.05	8.13
AVS-007068	B836749-F006	YF	VERO	10/25/90	1	11.4	18.4	0	103	189	320	10.288	5.62	26.73
AVS-007068	B836749-F006	YF	VERO	10/3/90	1	6.45	13.2	31	50.8	87	293	6.564	3.83	19.91
AVS-007069	B836749-F007	JE	VERO	8/31/90	1	0	0	0	43.1	77.8	288	0		0
AVS-007069	B836749-F007	PT	VERO	8/30/90	1	0	0	0	41.7	66	247	0		0
AVS-007069	B836749-F007	SF	VERO	8/30/90	1	0	0	0	61.7	92.6	295	0		0
AVS-007069	B836749-F007	VEE	VERO	8/28/90	1	283	0	0	38.5	79	320	0		0.65
AVS-007069	B836749-F007	VV	VERO	10/4/90	1	0	0	0	52.6	78.4	282	0		0
AVS-007069	B836749-F007	YF	VERO	8/31/90	1	0	0	0	8.49	62.8	279	0		0
AVS-007070	B836749-F009	JE	VERO	8/31/90	1	0	0	0	22.4	100	320	0		0
AVS-007070	B836749-F009	PT	VERO	8/30/90	1	0	0	0	10	121	320	0		0
AVS-007070	B836749-F009	SF	VERO	8/30/90	1	0	0	0	61.3	94.7	296	0		0
AVS-007070	B836749-F009	VEE	VERO	8/28/90	1	0	0	0	32	320	320	0		0
AVS-007070	B836749-F009	VV	VERO	10/4/90	1	0	0	0	84.3	320	320	0		0
AVS-007070	B836749-F009	YF	VERO	8/31/90	1	0	0	0	3.2	100	320	0		0
AVS-007071	B836749-F010	JE	VERO	8/31/90	0.3	0	0	0	100	100	100	0		0
AVS-007071	B836749-F010	JE	VERO	10/25/90	3.2	136	246	0	296	534	953	2.009	0.94	3.39
AVS-007071	B836749-F010	JE	VERO	10/3/90	3.2	55.2	0	0	142	259	908	0		4.33
AVS-007071	B836749-F010	PT	VERO	8/30/90	0.3	0	0	0	100	100	100	0		0
AVS-007071	B836749-F010	PT	VERO	10/25/90	3.2	320	100	0	232	495	959	1	1	4.23
AVS-007071	B836749-F010	PT	VERO	10/2/90	3.2	0	0	0	198	296	923	0		0
AVS-007071	B836749-F010	SF	VERO	8/30/90	0.3	0	0	0	100	100	100	0		0
AVS-007071	B836749-F010	SF	VERO	10/25/90	3.2	0	0	0	143	320	932	0		0
AVS-007071	B836749-F010	SF	VERO	10/2/90	3.2	0	0	0	135	222	845	0		0
AVS-007071	B836749-F010	VEE	VERO	8/28/90	0.3	0	0	0	29.6	100	100	0		0

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AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007071	B836749-F010	VEE	VERO	10/26/90	3.2	0	0	0	394	599	967	0		0.24
AVS-007071	B836749-F010	VEE	VERO	10/5/90	3.2	0	0	0	120	202	757	0		0
AVS-007071	B836749-F010	VV	VERO	10/4/90	1	0	0	0	13.7	88.7	296	0		0
AVS-007071	B836749-F010	YF	VERO	8/31/90	0.3	0	0	0	24.7	100	100	0		0
AVS-007071	B836749-F010	YF	VERO	10/25/90	3.2	51.7	226	0	227	495	959	2.193	1.01 >	10.07
AVS-007071	B836749-F010	YF	VERO	10/3/90	3.2	56.6	0	0	109	216	869	0		1.34
AVS-009458	B836749 F016	YF	VERO	4/24/91	1	39.2	57.9	0	145	211 >	320 >	3.649	2.5 >	13.51
AVS-009458	B836749 F016	JE	VERO	4/24/91	1	0	0	0	185	274 >	320 >	0		0.05
AVS-009458	B836749 F016	SF	VERO	4/23/91	1	0	0	0	23	46.9	94.7	0	>	1.36
AVS-009458	B836749 F016	VEE	VERO	4/26/91	1	0	0	0	238 >	320 >	320 >	0		0
AVS-009458	B836749 F016	PT	VERO	4/23/91	1	0	0	0	165	229 >	320 >	0		1.27
AVS-009459	B836749 F017	YF	VERO	4/24/91	1	0	0	0	52.4	94.9 >	320 >	0		0
AVS-009459	B836749 F017	JE	VERO	4/24/91	1	0	0	0	59.2	135 >	320 >	0		0
AVS-009459	B836749 F017	SF	VERO	4/23/91	1	0	0	0	28.9	64.6	274	0		0
AVS-009459	B836749 F017	VEE	VERO	4/26/91	1	208	0	0	180	320 >	320 >	0		0
AVS-009459	B836749 F017	PT	VERO	4/23/91	1	0	0	0	73.5	137	320 >	0		0
AVS-009460	B836749 F018	YF	VERO	4/24/91	1	10.9	16	32	87	196 >	320 >	12.287	5.45 >	26.42
AVS-009460	B836749 F018	JE	VERO	4/24/91	1	0	0	0	128	299 >	320 >	0		0
AVS-009460	B836749 F018	SF	VERO	4/23/91	1	0	0	0	23.2 >	51	137	0	>	0.01
AVS-009460	B836749 F018	VEE	VERO	4/26/91	1	0	0	0	320	320 >	320 >	0	>	0.4
AVS-009460	B836749 F018	PT	VERO	4/23/91	1	0	0	0	131	200 >	320 >	0	>	0

B836749

Ruprechtia tangarana

CH_2Cl_2-MeOH, 1:1

crude extract
AVS-7065 F003

MeOH-H_2O
AVS-7066 F004

CH_2Cl_2
AVS-7067 F005
AVS-9460 F018

hexane-
9:1 MeOH-H_2O

CCl_4-
8:2 MeOH-H_2O

hexane
AVS-7068 F006
AVS-9458 F016

CCl_4
AVS-7069 F007

CH_2Cl_2-
1:1 MeOh-H_2O

CH_2Cl_2
F008
AVS-9459 F017

aq. MeOH
AVS-7070 F009
F013

LH-20
MeOH

F010   F011   F012   F013   F014
AVS-7071

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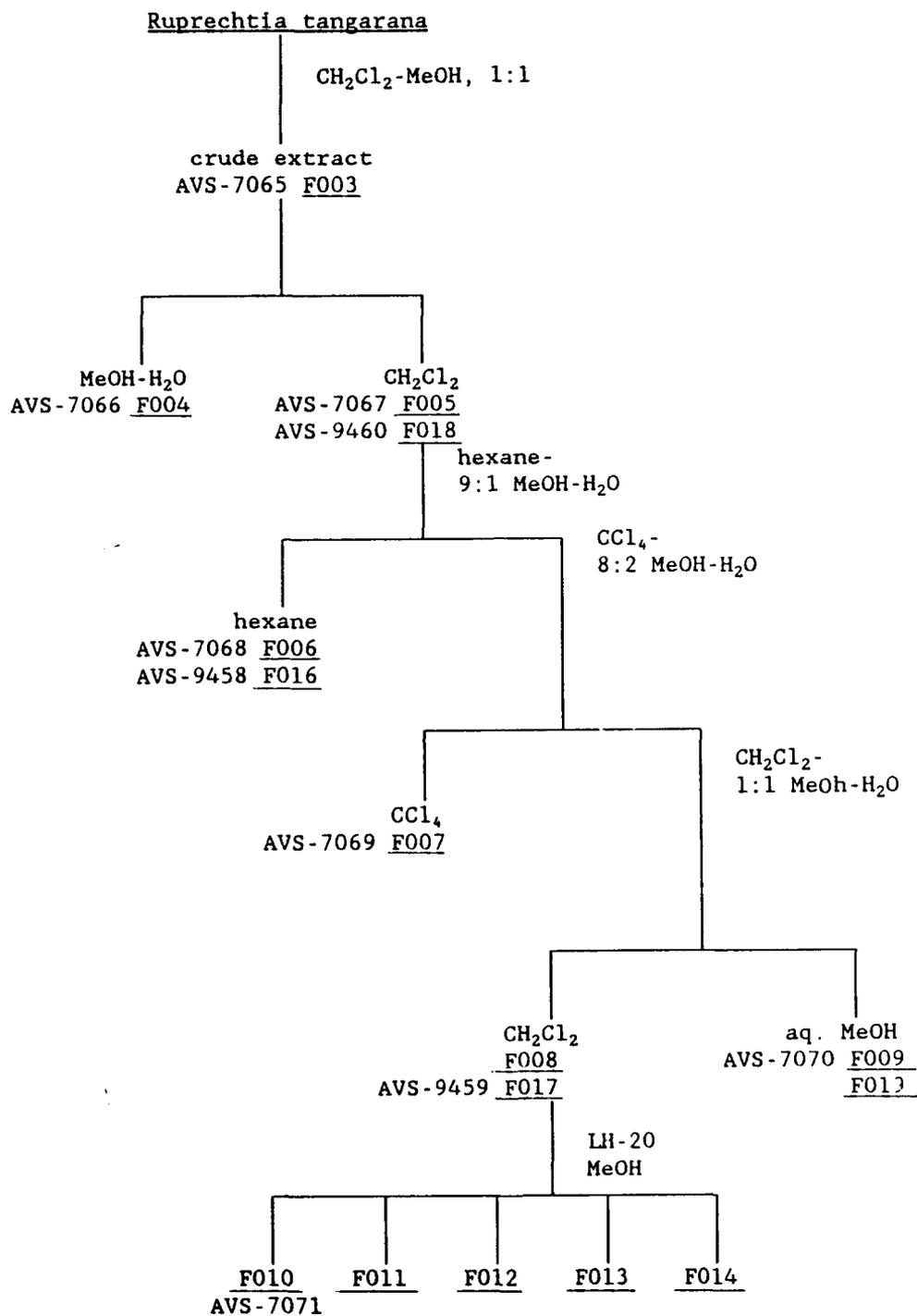
AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007083	B848528-F011	JE	VERO	8/31/90	1	51.2	95.2	0	50.9	144	309	1.513	0.53	0.87
AVS-007083	B848528-F011	JE	VERO	10/3/90	1	47.1	83.3	0	126	191	307	2.287	1.51	7.55 ^
AVS-007083	B848528-F011	JE	VERO	10/25/90	1	61.1	0	0	88.7	192 ^	320	0		1.19
AVS-007083	B848528-F011	PT	VERO	8/30/90	1	52.1	0	85.7	52.1	124	300	0		2.34
AVS-007083	B848528-F011	PT	VERO	10/25/90	1	13.5	23.8	0	148	208	315	8.727	6.21	29.69 ^
AVS-007083	B848528-F011	PT	VERO	10/2/90	1	35.1	75.8	0	97.4	171	305	2.261	1.28	12.67 ^
AVS-007083	B848528-F011	SF	VERO	8/30/90	1	4.31	8.5	31.1	86.8	161	304	18.897	10.22	35.95 ^
AVS-007083	B848528-F011	SF	VERO	10/25/90	1	8.27	16.8	0	50.1	112	299	6.706	2.99	12.33
AVS-007083	B848528-F011	SF	VERO	10/2/90	1	12.9	19.8	0	62.5	97.2	297	4.905	3.15	16.41 ^
AVS-007083	B848528-F011	VEE	VERO	8/28/90	1	0	0	0	320	320	320			2.04
AVS-007083	B848528-F011	VEE	VERO	10/26/90	1	53.5	93.5	0	192	320 ^	320	3.422	2.06	9.89 ^
AVS-007083	B848528-F011	VEE	VERO	10/5/90	1	39.2	73.8	0	80.6	168	314	2.276	1.09	4.52
AVS-007083	B848528-F011	VV	VERO	10/11/90	1	0	0	0	37	78.4	290	0		0
AVS-007083	B848528-F011	YF	VERO	8/31/90	1	10	21.9	0	52	147	309	6.694	2.37	17.13 ^
AVS-007083	B848528-F011	YF	VERO	10/25/90	1	20.1	77.6	0	41.1	145	313	1.865	0.53	7.35
AVS-007083	B848528-F011	YF	VERO	10/3/90	1	13.5	41.5	0	96.9	171	305	4.133	2.34	15.83 ^
AVS-007084	B848528-F012	JE	VERO	8/31/90	0.32	20.1	0	0	14.2	29	100	0		0
AVS-007084	B848528-F012	JE	VERO	10/25/90	0.32	16.4	0	0	27.2	51.1	98.9	0		5.58
AVS-007084	B848528-F012	JE	VERO	10/3/90	0.32	24.8	0	0	21	50.3	98.9	0		0.04
AVS-007084	B848528-F012	PT	VERO	8/30/90	0.32	4.02	9.07	9.31	8.3	27	100	2.981	0.92	6.82
AVS-007084	B848528-F012	PT	VERO	10/25/90	0.32	0.856	2	0	25.7	47.9	95.8 ^	24.006	12.88	42.4 ^
AVS-007084	B848528-F012	SF	VERO	10/2/90	0.32	1.5	20.1	0	29.5	53.4	97.1	2.656	1.47	23.51 ^
AVS-007084	B848528-F012	SF	VERO	8/30/90	0.32	0.769	1.87	8.32	22.8	40.7	96.3	21.78	12.21	38.97
AVS-007084	B848528-F012	SF	VERO	10/25/90	0.32	0.58b	1.49	0	2.47	33.3	93.3	22.348	1.65	17.48
AVS-007084	B848528-F012	SF	VERO	10/2/90	0.32	1.84	6.73	0	15.5	28	91.9	4.162	2.3	16.6 ^
AVS-007084	B848528-F012	VEE	VERO	8/28/90	0.32	0	0	0	100	100	100	0		0
AVS-007084	B848528-F012	VEE	VERO	10/26/90	0.32	18.6	0	0	17.3	34 ^	100 ^	0		0
AVS-007084	B848528-F012	VEE	VERO	10/5/90	0.32	29	0	0	12.9	27.4	100	0		0
AVS-007084	B848528-F012	VV	VERO	10/11/90	1	0	0	0	10.4	21	85.8	0		0
AVS-007084	B848528-F012	YF	VERO	8/31/90	0.32	1.89	7.44	0	13.9	27.6	100	3.709	1.86	13.21
AVS-007084	B848528-F012	YF	VERO	10/25/90	0.32	4.02	0	0	20.2	44.6 ^	100	0		11.84
AVS-007084	B848528-F012	YF	VERO	10/3/90	0.32	9.27	0	0	21.3	41.7	96.4	0		5.56 ^
AVS-007085	B848528-F013	JE	VERO	8/31/90	0.32	15.3	0	0	21.8	44	100	0		5.24
AVS-007085	B848528-F013	JE	VERO	10/25/90	0.32	11.9	24.6	0	32	65.3	100	2.652	1.3	7.29
AVS-007085	B848528-F013	JE	VERO	10/3/90	1	17.6	0	0	40.7	84.3	308	0		5.72
AVS-007085	B848528-F013	PT	VERO	8/30/90	0.32	0	0	0	16.6	25.8	93.4	0		6.23 ^
AVS-007085	B848528-F013	PT	VERO	10/25/90	0.32	5.56	12.4	0	41.3	64.6 ^	100	5.229	3.34	21.13 ^
AVS-007085	B848528-F013	PT	VERO	10/2/90	1	17.9	0	0	7.11	57	262	0		0.77 ^
AVS-007085	B848528-F013	SF	VERO	8/30/90	0.32	1.79	3.74	9.06	24.1	45.4	100	12.113	6.44	30.82

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AVS #	CTR #	VIRUS	CELL	DATE	INIT.CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007085	B848528-F013	SF	VERO	10/25/90	0.32	1.89	3.69	0	0.83	42.1	100	11.417	0.22	14.87
AVS-007085	B848528-F013	SF	VERO	10/2/90	1	4.28	7.85	0	22.2	40.7	273	5.189	2.83	14.51
AVS-007085	B848528-F013	VEE	VERO	8/28/90	0.32	0	0	96.8	100	100 >	100	0		1.11
AVS-007085	B848528-F013	VEE	VERO	10/26/90	0.32	15.1	25.5	0	100 >	100 >	100	3.927 >	3.93	18.41 ^
AVS-007085	B848528-F013	VV	VERO	10/5/90	1	21.2	59.3	0	23.8	177	320	2.984	0.4	4.51 ^
AVS-007085	B848528-F013	VV	VERO	11/1/90	0.32	20.1	0	0	10.8	30.4	98.5	0		0
AVS-007085	B848528-F013	YF	VERO	10/25/90	0.32	3.02	21.1	0	33	59.2	100	2.803	1.56	16.05 ^
AVS-007085	B848528-F013	YF	VERO	10/3/90	1	10.9	0	0	23.1	59.2	298	0		4.83
AVS-007086	B848528-F014	JE	VERO	8/31/90	1	5.12	0	0	9.07	21.5	320	0		2.78
AVS-007086	B848528-F014	JE	VERO	10/25/90	0.32	4.81	0	0	19.7	30.3	100	0		12.32 ^
AVS-007086	B848528-F014	JE	VERO	10/3/90	0.32	0	0	0	18.7	31.5	100	0		2.06
AVS-007086	B848528-F014	PT	VERO	8/30/90	1	7.59	0	0	7.66	16.4	78.8	0		1.88
AVS-007086	B848528-F014	PT	VERO	10/25/90	0.32	3.94	6.06	0	19.2	28.3	93	4.674	3.16	17.33 ^
AVS-007086	B848528-F014	PT	VERO	10/2/90	0.32	0	0	0	18.7	28.2	100	0		2.17 ^
AVS-007086	B848528-F014	SF	VERO	8/30/90	1	1	1.25	2.91	6.18	9.16	29.9	7.317	4.94	21.69 ^
AVS-007086	B848528-F014	SF	VERO	10/25/90	0.32	1.06	1.74	0	13.7	23.9	88.7	13.734	7.87	26.98 ^
AVS-007086	B848528-F014	SF	VERO	10/2/90	0.32	4.68	7.71	0	15.2	23.3	85.2	3.017	1.97	8.81
AVS-007086	B848528-F014	VEE	VERO	8/28/90	1	0	0	0	68.3	130	320	0		0.46
AVS-007086	B848528-F014	VEE	VERO	10/26/90	0.32	6.04	21.7	0	13.9	100 >	100	4.605	0.64	10.69 ^
AVS-007086	B848528-F014	VV	VERO	10/5/90	0.32	24.9	0	0	6.78	15.7	100	0		0
AVS-007086	B848528-F014	VV	VERO	10/11/90	1	0	0	0	14.1	22.5	92.8	0		0.79
AVS-007086	B848528-F014	YF	VERO	10/25/90	0.32	5.31	0	0	15.8	26.2	100	0		8.12 ^
AVS-007086	B848528-F014	YF	VERO	10/3/90	0.32	6.84	0	0	16.4	24.9	100	0		4.42
AVS-007087	B848528-F015	JE	VERO	8/31/90	0.32	4.54	8.11	0	8.79	30	100	3.698	1.08	8.05 ^
AVS-007087	B848528-F015	JE	VERO	10/25/90	0.32	5.31	0	0	25.7	100 >	100	0		10.98
AVS-007087	B848528-F015	JE	VERO	10/3/90	0.32	14.9	0	0	29	94.1	100	0		8.08 ^
AVS-007087	B848528-F015	PT	VERO	8/30/90	0.32	4.21	0	0	7.09	29.9	100	0		2.91
AVS-007087	B848528-F015	PT	VERO	10/25/90	0.32	1.76	3.62	9.03	24.1	94.3	100	26.091	6.67	33.38 ^
AVS-007087	B848528-F015	PT	VERO	10/2/90	0.32	5.01	0	0	22.5	51.4	100	0		10.96
AVS-007087	B848528-F015	SF	VERO	8/30/90	0.32	0.477	0.97	0	9.5	31.1	100	31.982	9.77	35.17
AVS-007087	B848528-F015	SF	VERO	10/25/90	0.32	0.702	1.41	0	5.75	23.3	100	16.531	4.07	22.8
AVS-007087	B848528-F015	SF	VERO	10/2/90	0.32	3.39	4.86	0	19.6	29.3	100	6.026	4.04	23.48
AVS-007087	B848528-F015	VEE	VERO	8/28/90	0.32	28.6	0	9.3	6.78	33.3	93.3	0		0
AVS-007087	B848528-F015	VEE	VERO	10/26/90	0.32	11.3	20.7	0	40	100 >	100	4.834	1.93	13.41 ^
AVS-007087	B848528-F015	VEE	VERO	10/5/90	0.32	18.1	0	0	11.8	43.3	100	0		0
AVS-007087	B848528-F015	VV	VERO	10/11/90	1	17.9	0	0	9.06	24	238	0		0
AVS-007087	B848528-F015	YF	VERO	8/31/90	0.32	1.35	3.88	0	18	31.5	100	8.099	4.65	24.2 ^
AVS-007087	B848528-F015	YF	VERO	10/25/90	0.32	3.66	0	0	19.3	40.2	100	0		12.79
AVS-007087	B848528-F015	YF	VERO	10/3/90	0.32	6.96	0	0	21.8	56	100	0		7.65

B848528

Phyllanthus anisolobus
|
CH_2Cl_2-MeOH, 1:1
extraction

MeOH-H_2O
AVS-7087 <u>F015</u>

(CH_2Cl_2)
|
hexane-
9:1 MeOH-H_2O

hexane
AVS-7083 <u>F011</u>

CCl_4-
8:2 MeOH-H_2O

CCl_4
AVS-7084 <u>F012</u>

CH_2Cl_2-
1:1 MeOH-H_2O

CH_2Cl_2
AVS-7085 <u>F013</u>

MeOH-H_2O
AVS-7086 <u>F014</u>

LH-20-MeOH

<u>F018</u> <u>F019</u>   <u>F020</u> <u>F021</u>

B853791.TXT
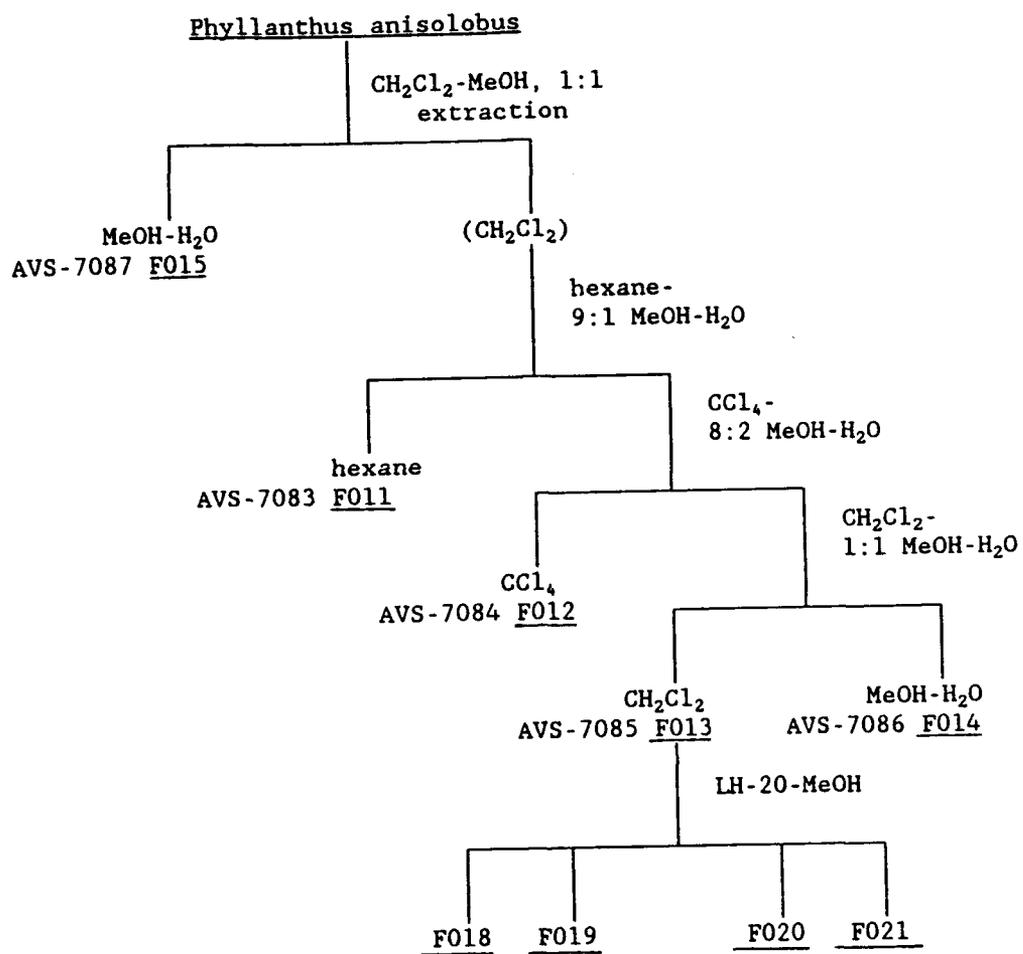
AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007088	B853791-F011	JE	VERO	8/31/90	1	0	0	0	19.8	48.2	320	0		0
AVS-007088	B853791-F011	PT	VERO	8/30/90	1	0	0	0	51.3	70.6	240	0		2.18
AVS-007088	B853791-F011	SF	VERO	8/30/90	1	0	0	0	24.9	55.3	320	0		0
AVS-007088	B853791-F011	VEE	VERO	8/28/90	1	0	0	0	58.2	158	310	0		0
AVS-007088	B853791-F011	VV	VERO	10/11/90	1	0	0	0	20.1	31.5	272	0		0
AVS-007088	B853791-F011	YF	VERO	8/31/90	1	0	0	0	23.4	45.9	320	0		0
AVS-007089	B853791-F012	JE	VERO	8/31/90	1	0	0	0	218	320	320	0		0.06
AVS-007089	B853791-F012	JE	VERO	10/3/90	3.2	0	0	0	459	> 845	1000	0		0
AVS-007089	B853791-F012	PT	VERO	8/30/90	1	264	0	0	320	320	320	0		1.16
AVS-007089	B853791-F012	PT	VERO	10/2/90	3.2	402	0	0	505	> 1000	1000	0		1.4
AVS-007089	B853791-F012	SF	VERO	8/30/90	1	163	0	0	320	320	320	0		2.94
AVS-007089	B853791-F012	SF	VERO	10/2/90	3.2	0	0	0	87	228	903	0		0
AVS-007089	B853791-F012	VEE	VERO	8/28/90	1	0	0	0	320	320	320	0		0.01
AVS-007089	B853791-F012	VEE	VERO	10/5/90	3.2	0	0	0	830	> 1000	1000	0		0
AVS-007089	B853791-F012	VV	VERO	10/11/90	1	247	0	0	320	> 320	320	0		3.79
AVS-007089	B853791-F012	YF	VERO	8/31/90	1	0	0	0	320	320	320	0		0
AVS-007089	B853791-F012	YF	VERO	10/3/90	3.2	0	0	0	391	745	1000	0		0
AVS-007090	B853791-F013	HIV	MT2	9/12/90	0.32	0	0	0	100	100	100	0		1.7
AVS-007090	B853791-F013	VV	VERO	10/11/90	1	0	0	0	320	> 320	320	0		0.31
AVS-007091	B853791-F014	VV	VERO	10/11/90	1	0	0	1030	320	> 320	320	0		0
AVS-007092	B853791-F017	JE	VERO	10/25/90	3.2	0	0	0	1000	> 1000	1000	0		0.8
AVS-007092	B853791-F017	JE	VERO	10/3/90	3.2	0	0	0	1000	> 1000	1000	0		1.6
AVS-007092	B853791-F017	PT	VERO	10/25/90	3.2	24.8	93.8	0	1000	> 1000	1000	10.666	> 10.67	36.77
AVS-007092	B853791-F017	PT	VERO	10/2/90	3.2	63.4	146	1070	1000	> 1000	1000	6.864	> 6.86	31.69
AVS-007092	B853791-F017	SF	VERO	10/25/90	3.2	26.3	98.6	0	2.67	> 1000	1000	10.144	> 0.03	32.11
AVS-007092	B853791-F017	SF	VERO	10/2/90	3.2	147	420	947	1000	> 1000	1000	2.382	> 2.38	22.55
AVS-007092	B853791-F017	VEE	VERO	10/26/90	1	927	0	0	1000	> 1000	1000	0		1.3
AVS-007092	B853791-F017	VEE	VERO	10/5/90	3.2	0	0	0	90.9	> 1000	1000	0		0
AVS-007092	B853791-F017	VV	VERO	11/1/90	3.2	0	0	0	320	> 320	320	0		3.62
AVS-007092	B853791-F017	YF	VERO	10/25/90	1	290	473	984	1000	> 1000	1000	2.114	> 2.11	20.15
AVS-007092	B853791-F017	YF	VERO	10/3/90	3.2	120	176	0	1000	> 1000	1000	5.676	> 5.68	22.36
AVS-007093	B853791-F018	VV	VERO	10/11/90	1	0	0	0	36.3	57.8	97	0		0
AVS-007094	B853791-F019	JE	VERO	10/25/90	3.2	132	0	0	214	445	955	0		1.84
AVS-007094	B853791-F019	JE	VERO	10/3/90	3.2	0	0	0	171	241	845	0		1.55
AVS-007094	B853791-F019	PT	VERO	10/25/90	3.2	41.5	63.8	0	171	269	924	4.213	> 2.67	13.64
AVS-007094	B853791-F019	PT	VERO	10/2/90	3.2	88.1	0	0	166	233	800	0		7.45
AVS-007094	B853791-F019	SF	VERO	10/25/90	3.2	0	0	0	39.2	146	830	0		0
AVS-007094	B853791-F019	SF	VERO	10/2/90	3.2	0	0	0	67.9	144	811	0		0
AVS-007094	B853791-F019	VEE	VERO	10/26/90	3.2	179	0	0	361	> 614	1000	0		3.77

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AVS #	CTR #	VIRUS	CELL	DATE	INIT. CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-007094	B853791-F019	VEE	VERO	10/5/90	3.2	261	0	0	110	229	909	0		0
AVS-007094	B853791-F019	VV	VERO	10/11/90	1	51.8	89.6	0	187	275	320	3.064	2.09	8.43
AVS-007094	B853791-F019	VV	VERO	11/1/90	1	49.3	75.9	0	177	>255	>320	3.36	2.34	10.37
AVS-007094	B853791-F019	YF	VERO	10/25/90	3.2	302	0	0	173	288	921	0	>	1.93
AVS-007094	B853791-F019	YF	VERO	10/3/90	3.2	0	0	0	165	238	845	0		2.73
AVS-007095	B853791-F020	JE	VERO	10/3/90	3.2	0	0	0	613	>906	1000	0		0.32
AVS-007095	B853791-F020	PT	VERO	10/2/90	3.2	0	0	0	544	>767	1000	0	>	7.62
AVS-007095	B853791-F020	SF	VERO	10/2/90	3.2	0	0	0	506	>739	1000	0		5.5
AVS-007095	B853791-F020	VEE	VERO	10/5/90	3.2	581	0	0	>597	>912	1000	0		0
AVS-007095	B853791-F020	VV	VERO	11/1/90	1	0	0	0	320	>320	1000	0		0
AVS-007095	B853791-F020	YF	VERO	10/3/90	3.2	0	0	0	>715	>1000	1000	0	>	2.8
AVS-007096	B853791-F021	VV	VERO	11/1/90	1	0	0	0	>320	>320	320	0	>	1.21
AVS-007097	B853791-F022	PT	VERO	9/5/90	1	0	0	0	>320	>320	320	0	>	7.54
AVS-007097	B853791-F022	VV	VERO	10/11/90	1	0	0	0	>320	>320	320	0	>	2.66
AVS-007098	B853791-F023	PT	VERO	9/5/90	1	0	0	0	>320	>320	320	0	>	5.21
AVS-007098	B853791-F023	VV	VERO	10/11/90	1	0	0	0	>320	>320	320	0	>	0

B853791

Notelaea ligustrina

Roots

CH$_2$Cl$_2$-MeOH, 1:1
extraction

MeOH-H$_2$O
BuOH

CH$_2$Cl$_2$
hexane-
9:1 MeOH-H$_2$O

BuOH
AVS-7096 F021

H$_2$O
AVS-7097 F022

insolubles
AVS-7092 F017
F024

hexane
AVS-7093 F018

CH$_2$Cl$_2$-
3:2 MeOH-H$_2$O

CH$_2$Cl$_2$
AVS-7094 F019

BuOH
1:4 MeOH-H$_2$O

BuOH
AVS-7095 F020

aqueous
layer
AVS-7098 F023

B853791

Notelaea ligustrina

Stem bark

CH_2Cl_2-MeOH, 1:1
extraction

MeOH-H_2O CH_2Cl_2
          BuOH   hexane-
                                  9:1 MeOH-H_2O

                                           CH_2-Cl_2
                                           3:2 MeOH-H_2O

BuOH H_2O
AVS-7090 F013   AVS-7091 F014

                        hexane

                                                    BuOH-
                                                    1:3 MeOH-H_2O

                              CH_2Cl_2
                        AVS-7088 F011

                                          BuOH   aqueous
                                    AVS-7089 F012   layer

B848990.TXT

AVS #	CTR #	VIRUS	CELL	DATE	INIT.CONC.	IC25	IC50	IC95	TC25	TC50	TC95	TI50	SI	TAI
AVS-008259	B 848990	HIV	OEM	12/20/90	3.2	3.75	8.27	28 >	1000	1000 >	1000 >	120.914 >	120.914 >	73.07
AVS-008259	B 848990	JE	VERO	12/12/90	10	0	0	0	1500	3200 >	3200	0		0
AVS-008259	B 848990	PT	VERO	12/11/90	10	61.1	128	0	600	3200 >	3200 >	24.94	4.68	19.8
AVS-008259	B 848990	SF	VERO	12/11/90	10	0	0	0	560	3200	3200	0		0
AVS-008259	B 848990	VEE	VERO	12/11/90	10	0	0	0	733	2930 >	3200	0		7.57
AVS-008259	B 848990	VV	VERO	12/21/90	1	0	0	0	304	320 >	320	0	^	0
AVS-008259	B 848990	YF	VERO	12/12/90	10	2170	0	0	1330	3200 >	3200	0		2.26
AVS-008259	B 848990	HIV	MT2	1/17/91	0.32	11.3	16	29.9 >	100	100 >	100 >	6.245 >	6.25 ^	34.17
AVS-008259	B 848990	HIV	OEM	1/17/91	0.32	11.6	16.3	29.9 >	100	100 >	100 >	6.145 >	6.15 ^	32.76
AVS-008259	B 848990	SF	VERO	3/22/91	10	0	0	0	422	2350 >	3200	0		0
AVS-008259	B 848990	YF	VERO	3/20/91	10	2170	0	0	137	2320 >	3200	0		0
AVS-008259	B 848990	PT	VERO	3/19/91	10	56.6	179	0	200	2570 >	3200	14.375	1.12	9.71
AVS-008259	B 848990	JE	VERO	3/20/91	10	0	0	0	2760 >	3200 >	3200	0	^	1.43
AVS-008259	B 848990	VEE	VERO	3/22/91	10	0	0	0	1440 >	3200 >	3200	0	^	8.33

B848990

Unknown phaeophyta

AVS - 8259 H_2O extract

H_2O-EtOAc
(1:1)

EtOAc
extract

n-BuOH-H_2O
(1:1)

n-BuOH
extract
F003

H_2O
layer
F004
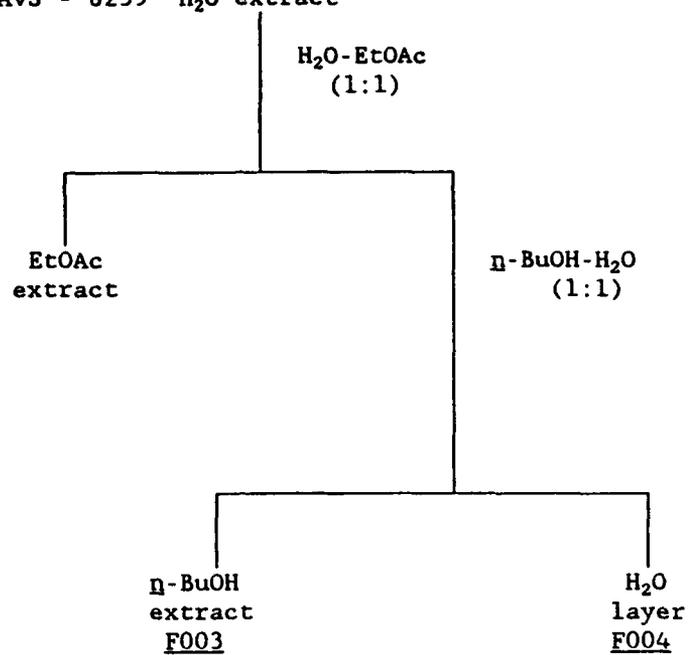
B.   Scale-up Isolation of Narciclasine and Pancratistatin and Synthetic Modifications of Narciclasine

We have pursued the pancratistatin family of antiviral leads as a top priority. The research results here have been very encouraging and pancratistatin, isonarciclasine, cis-dihydronarciclasine as well as trans-dihydronarciclasine have proved to be quite promising. The most exciting antiviral result has been the discovery in USAMRIID's laboratories that pancratistatin will effectively treat the in vivo experimental version of Japanese Encephalitis.

We have completed reisolation of pancratistatin (1) from 1/2 ton of Pancratium littorale collected in the Republic of Seychelles. Enough pancratistatin is now available for the next series of in vivo experiments.

In order to ensure that pancratistatin is available for future clinical trials, we have been expanding our botanical research aimed at this objective. Presently we have grown 900 Pancratium littorale plants and are preparing to triple that amount in the coming year. In turn, that will ensure a steady clinical supply. In the botanical research, we have also been exploring tissue culture methods of cloning Pancratium littorale and this avenue is also becoming increasingly productive. The evaluation of other Pancratium and Hymenocallis species for pancratistatin and related compounds has continued and led to discovery of a new source for pancratistatin, namely, Hymenocallis calatay from a Singapore collection.

Related plants of the Amaryllidaceae were evaluated for antiviral constituents. We completed the isolation and structural elucidation of trans-dihydronarciclasine (2) as the active (Japanese encephalitis in vitro) and anticancer (P388 lymphocytic leukemia) constituent of Zephyranthes candida.

Meanwhile we have undertaken a world-wide procurement of Amaryllidaceae plants in the *Pancratium* and *Hymenocallis* genera. Twenty such species obtained were evaluated for antiviral constituents related to pancratistatin. One of these collected in Singapore in the *Hymenocallis* genus (unpublished) yielded 7-deoxy-<u>trans</u>-dihydronarciclasine (3) as the principle antiviral constituent. Interestingly this new natural product was just prepared by us using a semi-synthetic route from 7-deoxy-narciclasine isolated from *Pancratium littorale* and represents one of those rare examples where the synthetic product preceded discovery of the natural product.

A major effort has been devoted to scaling-up the isolation of narciclasine from a *Narcissus* species.

We have isolated narciclasine (4) from a large scale collection (10,000 bulbs) of *Narcissus in ʌrᵢarabilis*. So far about 50 grams of narciclasine has been isolated by this procedure and another 100 grams is in progress. The narciclasine is being employed in a semi-synthetic approach to pancratistatin. For experimental details of the semi-synthetic approach to pancratistatin from narciclasine, please refer to Appendix B. Epoxide (5) has been prepared and converted to ketone 6. The ketone reduction step is being studied in detail to increase yields of the β-alcohol corresponding to pancratistatin. Additional quantities of narciclasine are being converted to isonarciclasine (7) another antiviral derivative of pancratistatin found by USAMRIID to have <u>in vitro</u> antiviral activity. While total synthesis of pancratistatin was recently achieved it involved some 30 steps and was not practical for scale-up. Thus, we plan to use the semi-synthesis from narciclasine for pancratistatin and related compounds to meet future clinical needs. Toward that end some 30,000 bulbs (about seven tons) of *Narcissus incomparabilis* was obtained in September, 1990,
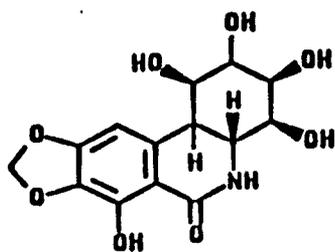
PANCRATISTATIN
1
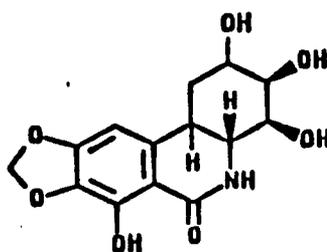
trans-Dihydronarciclasine
2
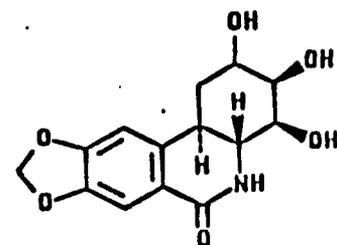
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3
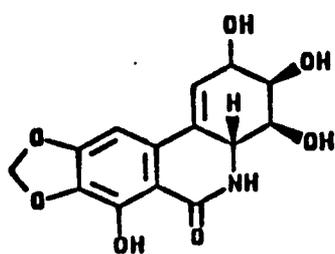
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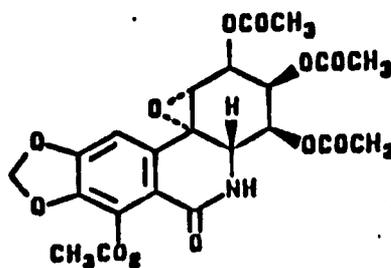
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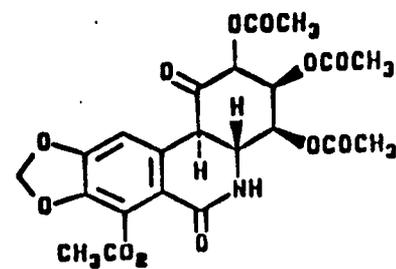
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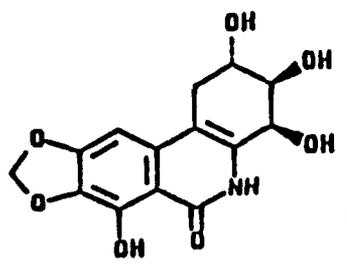
Isonarciclasine

7
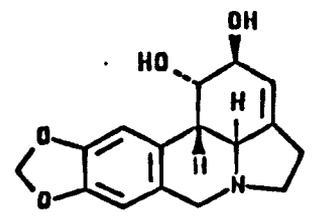
Lycorine

8

and the scale-up isolation of additional narciclasine has begun.

Other structure/modification studies will be directed at the Amaryllidaceae antiviral constituent lycorine (*8*). Here modification will involve various epoxidation and hydroxylation procedures along with conversion to glycoside derivatives. Fortunately we have already isolated 50 grams of lycorine for these investigations from *Narcissus incomparabilis*.

Detailed reports have been included in the manuscript section of this final summary of our research directed at evaluating structure/activity relationships in our bryostatin series of marine animal constituents. Bryostatins 1 and 2 have been found active against the HIV-1 <u>in vitro</u> syncytia screen. The contract renewal beginning June 28 will allow each of the most important RNA antiviral leads noted above to be vigorously pursued.

III. Near Term Plans

The discovery that pancratistatin will effectively retard USAMRIID's _in vivo_ Japanese Encephalitis has opened the way to a new generation of antiviral drugs. The combination of isolation and synthesis from narciclasine will ensure eventual large-scale production of pancratistatin and related compounds.

Very importantly for the future, we have now underway the fractionation research on a number of high priority USAMRIID RNA antiviral actives along with the HIV actives noted above.

In short the USAMRIID research program at the ASU-CRI has been making, as usual, excellent progress concerned with discovery and development of potentially useful antiviral drugs. Again, this was only made possible by the most necessary USAMRIID support and collaborative endeavors.

IV. Publications, Meeting Abstracts, Personnel

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AMER. SOC. OF HEMATOLOGY, Annual Meeting, Atlanta, GA, December 1989:

"Bryostatin 1 Induces Differentiation of B-CLL Cells," H. G. Drexler, S. M. Gignac, R. A. Jones, C. S. Scott, G. R. Pettit, and A. V. Hoffbrand

"Activation and Differentiation of Normal B-Cells Induced by Bryostatin 1," H. G. Drexler, S. M. Gignac, G. R. Pettit, and A. V. Hoffbrand

"Differential Effects of Bryostatin-1 on the Growth of Myeloid Leukemia and Normal Hematopoietic Cells in the Lewis x Brown Norway Hybrid (LBN) Rat," K. S. Durham, A. M. Yeager, D. Reardon, D. T. Kasper, W. S. May, and G. R. Pettit

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"Modulation of the Response of Highly Purified Human Hematopoietic Progenitor Cells (MY-10$^+$) to Hematopoietic Growth Factors by Bryostatin 1," C. McCrady, F. Lei, G. Pettit, and S. Grant

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PERSONNEL RECEIVING PAY

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Mr. Phillip J. Daschner	100	12/1/89 - 2/5/90
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	100	10/16/90 - 1/31/91
Ms. Christine H. Duplissa	100	11/1/89 - 1/31/90
	100	10/16/90 - 1/31/91
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	100	7/1/89 - 2/5/91
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Mr. Larry P. Tackett	75	11/1/89 - 2/5/90
Dr. Bruce E. Tucker	100	2/6/89 - 2/5/91

Graduate Degrees from Grant Support -- None

Patent Disclosures

Patent disclosures being filed by the University emanating from funds provided by USAMRIID continue to be reported by the University's Director of Technology Transfer.

ANTINEOPLASTIC AGENTS, 162.[1] *ZEPHYRANTHES CANDIDA*

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Abstract.—The Chinese medicinal plant *Zephyranthes candida* was found to contain a cytostatic constituent. Separation of a *n*-BuOH extract directed by results of a bioassay employing the P-388 lymphocytic leukemia led to *trans*-dihydronarciclasine [2] as the principal cytostatic agent with ED_{50} 3.2×10^{-3} μg/ml.

Amaryllidaceous plants such as *Narcissus poeticus* were recorded in the Bible as well-established treatments for cancer (1), and others were in use by the Greek physicians of the fourth century BC (2). The first isolation, in 1877 (3), of a biologically active Amaryllidaceae constituent, the now well-known lycorine (4), was an early achievement of organic chemistry, and such studies have been intensifying (4–7). In 1984, we reported discovery and structural elucidation of a strongly antineoplastic phenanthridone designated pancratistatin [1] produced by plants of *Pancratium littorale* (2,8) and *Zephyranthes grandiflora* (5).

In 1964, extracts of the medicinal (5) *Zephyranthes candida* (Lindl.) Herb. (obtained in Hong Kong) had already proved active (KB cell line from a human epidermoid carcinoma of the nasopharynx) in the U.S. National Cancer Institute's exploratory research program, but we were unable to obtain a re-collection (People's Republic of China) until 1982. Earlier (1955) Boit and Ehmke (9) isolated four alkaloids from the Dutch *Z. candida* representing the pyrrolo[*de*]phenanthridine (lycorine), pretazettine (tazettine), and 5,10b-ethanophenanthridine (haemanthidine and nerinine) ring systems. The study was extended in 1964–65 (10,11) to isolation of dihydrolycorine and zephyranthine from a Japanese variety and in 1978 to a flavone glycoside (12). We now have found the principal cytostatic (murine P-388 lymphocytic

leukemia, PS system) (12,13) constituent of *Z. candida* to be *trans*-dihydronarciclasine [2] previously (14) prepared by hydrogenation of narciclasine [6] and heretofore unknown as a biosynthetic product.
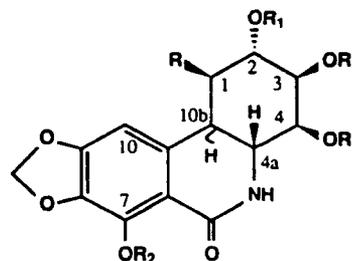
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2   R=R_1=R_2=H, 10b=α-H
3   R=H, R_1=R_2=Ac, 10b=α-H
4   R=R_2=H, R_1=Ac, 10b=α-H
5   R=R_1=R_2=H, 10b=β-H

6
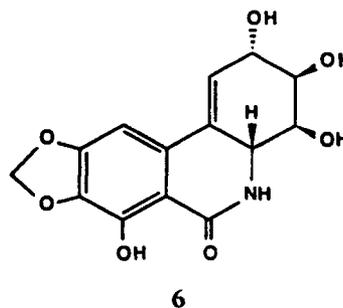
Ground bulbs of *Z. candida* were extracted with CH_2Cl_2-MeOH (1:1) at ambient temperature. After addition of H_2O, the aqueous phase was concentrated and extracted with *n*-BuOH. The PS-active (cell line) *n*-BuOH extract was concentrated and triturated with MeOH to provide a fraction that was separated (guided by PS bioassay) by successive

Sephadex LH-20 and Si gel cc steps. The resulting enriched active (ED$_{50}$ 0.0034 μg/ml) fraction was devoid of acetate groups (ir), and the major component coeluted [tlc, CH$_2$Cl$_2$-MeOH-H$_2$O (90:10:0.1)] with an authentic synthetic specimen of *trans*-dihydronarciclasine. The fraction was acetylated and separated on a column of Si gel to yield *trans*-dihydronarciclasine peracetate [3] (PS ED$_{50}$ 3.2 × 10^{-3} μg/ml) as the major component. The structure of the peracetate was established by detailed spectral analysis (2) and comparison with an authentic sample as well as with the product obtained by catalytic hydrogenation (Adam's catalyst in HOAc at 50 psi) of narciclasine, followed by acetylation. Hydrogenation afforded as the major product the expected *cis*-dihydronarciclasine accompanied by the trans isomer. Facile deacetylation of the phenolic acetoxy group was observed during chromatography and in MeOH solutions to give the 7-hydroxy-2,3,4-triacetoxy derivative 4. *Trans*-dihydronarciclasine (prepared from the acetate) was found to strongly inhibit the PS leukemia with ED$_{50}$ 0.0032 μg/ml, while the synthetic *cis*-dihydro analogue 5 led to PS ED$_{50}$ 0.024 μg/ml.

Isolation of *trans*-dihydronarciclasine [2] as the major antineoplastic constituent of *Z. candida* has revealed another interesting and potentially useful Amaryllidaceae biosynthetic product. Further study of this very productive plant family for anticancer and other medically useful components will doubtless prove rewarding and is in progress.

EXPERIMENTAL

GENERAL METHODS.—Details of general procedures and chromatographic techniques were provided in our earlier summaries (2,5).

PLANT MATERIAL.—*Z. candida* PR #55337, NSCB657832 was re-collected in China in 1981 (received February 1982) as part of the NCI-USDA collaborative program directed by Drs. J.L. Hartwell and M. Suffness. A voucher specimen is maintained at the USDA, Beltsville, MD, and in the ASU-CRI.

Extraction.—Freshly ground bulbs (18 kg) were stored in MeOH-CH$_2$Cl$_2$ (1:1) (32 liters) for 10 days. Addition of H$_2$O (15% by volume) caused separation of the CH$_2$Cl$_2$ phase. MeOH and CH$_2$Cl$_2$ were added to the aqueous phase to increase the original total volume by 50 and 25%, respectively. The plant was extracted with this mixture (2:1:0.5 ratio of MeOH-H$_2$O to added MeOH and CH$_2$Cl$_2$) for a further 80 days. Addition of H$_2$O (25% by volume) allowed the CH$_2$Cl$_2$ phase to separate, which was combined with the first CH$_2$Cl$_2$ extract and concentrated to a 109-g residue (PS ED$_{50}$ 3.5 μg/ml). The aqueous phase was concentrated and partitioned between H$_2$O (6 liters) and *n*-BuOH (4 × 6 liters). Concentration of the *n*-BuOH extract to a small volume and addition of MeOH (2 liters) gave an active MeOH-soluble fraction (149 g, PS ED$_{50}$ 0.27 μg/ml). Upon further dilution with MeOH (600 ml) and CH$_2$Cl$_2$ (400 ml) the solution was filtered to yield 28 g of a solid (PS ED$_{50}$ 1.6 μg/ml). The filtrate was chromatographed on a column of Sephadex LH-20 (2.5 kg) using MeOH-CH$_2$Cl$_2$ (3:2) as eluent.

ISOLATION OF *TRANS*-DIHYDRONARCICLASINE [2].—Elution (the preceding LH-20 column) between volumes 7215–16950 ml gave a 6.2-g fraction (PS ED$_{50}$<0.02 μg/ml). Trituration with Me$_2$CO (50 ml) provided a light orange solid (2.72 g, PS ED$_{50}$ 0.016 μg/ml) and a soluble fraction (3.5 g, PS ED$_{50}$ 0.0043 μg/ml). When the orange solid was triturated with MeOH-CH$_2$Cl$_2$ (1:1) (3 × 10 ml, 1 day), followed by MeOH (5 ml, 2 days), a soluble fraction (2.58 g) was obtained similar (by tlc) to the Me$_2$CO-soluble fraction. An aliquot of the Me$_2$CO-soluble fraction (1.76 g) and the latter soluble fraction (2.58 g) were combined and the mixture subjected to rapid chromatography on a column of Si gel (200 g). Gradient elution with CH$_2$Cl$_2$ (1 liter) and CH$_2$Cl$_2$-MeOH (99:1 to 95:5 to 9:1) (2 liters) gave a fraction (1.05 g) which was triturated with MeOH (5 ml, 1 day) to give a buff-colored solid [0.20 g, PS ED$_{50}$ 0.0034 μg/ml, ir (KBr) 3350, 1660, 1460, 1340, 1280, 1225, 1060, 1025 cm^{-1}]. Half of the solid was acetylated [Ac$_2$O-pyridine (1:1) (6 ml), 24 h, room temperature], and the product (0.12 g) was chromatographed on a column of Si gel (Lobar B column). Development with CH$_2$Cl$_2$ (200 ml) and CH$_2$Cl$_2$-MeOH (99:1) (400 ml) followed by CH$_2$Cl$_2$-MeOH (49:1) (all affording between 675 and 725 ml) total eluent volume, *trans*-dihydronarciclasine-2,3,4-triacetate [4] (16 mg) which recrystallized from MeOH-CH$_2$Cl$_2$ as small colorless needles: mp 309–311° [lit. (13) mp 293°]; [α]^{32}D +81.94° (c = 0.72, CHCl$_3$); uv λ max MeOH (log ε) 231 (4.04), 239 (4.01), 280 (3.75), 310 (3.33) nm; ^{1}H nmr (400 MHz, CDCl$_3$) 1.914 (1H, ddd, *J* = 14.0, 12.5, 3.0

Hz, H-1β), 2.086 (6H, s, 2 × Ac), 2.137 (3H, s, Ac), 2.432 (1H, ddd, J = 14.0, 3.5, 3.2 Hz, H-1α), 3.134 (1H, ddd, J = 12.7, 12.5, 3.5 Hz, H-10b), 3.777 (1H, dd, J = 12.7, 11.8 Hz, H-4a), 5.175 (1H, dd, J = 11.8, 3.0 Hz, H-4), 5.189 (1H, m, H-3), 5.438 (1H, dd, J = 3.2, 3.0 Hz), 5.855 (1H, s, NH), 6.037, 6.049 (1H, each, d, J = 1.2 Hz, OCH$_2$O), 6.323 (1H, s, H-10), 9.704 (1H, s, ArOH). Acetylation [Ac$_2$O-pyridine (1:1)] led to *trans*-dihydronarciclasine peracetate [3] identified by tlc and ir spectra (in CHCl$_3$) with an authentic specimen.

Continued elution between volumes 725–760 ml gave a mixture (14 mg) of the above triacetate and *trans*-dihydronarciclasine peracetate and between volumes 760–810 ml *trans*-dihydronarciclasine peracetate (80 mg).

Recrystallization from MeOH/CH$_2$Cl$_2$ afforded a pure specimen of **3** as colorless needles: mp 181–182° [lit. (14) 188–189°]; [α]^{51}D + 123.9° (c = 1.13, CHCl$_3$) [lit. (14) [α]^{20}D + 128.5° (c = 0.82, CHCl$_3$)]; uv λ max MeOH (log ε) 231 (4.10), 239 (4.09), 280 (3.82), 310 (3.40) nm; ir (KBr) v max 3600, 3500, 3330 (sh), 3310, 1760, 1730 (sh), 1670, 1634, 1505, 1487, 1460, 1371, 1345, 1298, 1255, 1235, 1172, 1080, 1051, 1031, 930 cm^{-1}; ^{1}H nmr (400 MHz, CDCl$_3$) 1.906 (1H, ddd, J = 14.0, 12.7, 3.0 Hz, H-1), 2.054, 2.071, 2.139 (3H each, Ac), 2.364 (3H, ArOAc), 2.428 (1H, ddd, J = 14.0, 3.5, 3.2 Hz, H-1α), 3.140 (1H, ddd, J = 12.7, 12.0, 3.5 Hz, H-10b), 3.762 (1H, dd, J = 12.0, 10.8 Hz, H-4a), 5.156 (1H, dd, J = 10.8, 3.0 Hz, H-4), 5.192 (1H, m, H-3), 5.416 (1H, dd, J = 3.2, 3.0 Hz, H-2), 5.810 (1H, s, NH), 6.065, 6.073 (1H each, d, J = 1.2 Hz, -O-CH$_2$-O), 6.642 (1H, s, H-10); ^{13}C nmr (22.63 MHz, CDCl$_3$) 170.34, 169.35, 169.14 (4C, 4 × OCOMe), 163.35 (C-6), 152.40 (C-9), 139.63 (C-7), 137.00 (C-10a), 134.33 (C-8), 116.04 (C-6a), 102.91 (OCH$_2$O), 102.00 (C-10), 71.62, 68.60, 67.43 (3 × CHOAc), 52.41 (C-4a), 35.61 (C-10b), 27.00 (C-1), 21.02, 20.86, 20.70 (4c, 4 × OCOCH$_3$) ppm; hreims *m/z* [M]$^+$ 477.1258 (3.09%) (calcd 477.1271 for C$_{22}$H$_{23}$NO$_{11}$), 435.1165 (100%) (calcd 435.1166 for C$_{20}$H$_{21}$NO$_{10}$).

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Antineoplastic Agents. 206. Structure of the Cytostatic Macrocyclic Lactone Combretastatin D-2[1]

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Received November 14, 1989

The South African tree *Combretum caffrum* (Combretaceae) has been found to contain two new and cytostatic (P388 lymphocytic leukemia) macrocyclic lactones designated combretastatin D-1 (1, ED_{50} 3.3 μg/mL) and D-2 (2, ED_{50} 5.2 μg/mL). With the X-ray crystal structure of combretastatin D-1 (1) serving as an unequivocal reference point ^{13}C NMR and high field (400 MHz) ^1H NMR spectral techniques were employed to assign structure 2 to combretastatin D-2.

The South African tree *Combretum caffrum* (Combretaceae) has been found to produce two *cis*-stilbenes, combretastatins A-1 and A-4, that strongly inhibit growth of the P-388 lymphocytic leukemia cell line (PS system) and tubulin polymerization.[2] Recently, we reported[3] the iso-

lation and structure determination of an unexpected 17-membered macrocyclic lactone designated combretastatin D-1 (1) from the same plant. We now summarize the

1   2

isolation and structural elucidation of another PS cell line inhibitory member of this unusual series of macrocyclic lactones named combretastatin D-2 (2) along with chemical
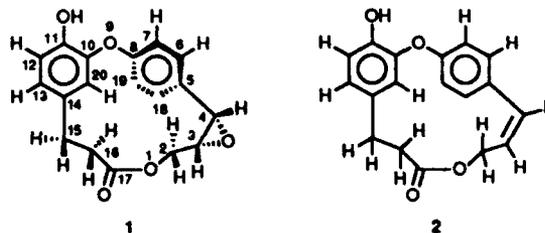
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Table I. ¹H NMR Assignments for Combretastatin D-2 (2) and Derivatives 5a–c in Deuteriochloroform Solution in δ Value (ppm) with Chloroform as Internal Standard

position	2	5a[a]	5b	5c
2α	4.64, d, 6.8	4.64, d, 6.9	3.91, d, 11.4	4.05, d, 11.9
2β	4.64, d, 6.8	4.26, m	4.31, dd, 11.4, 7.0	4.56, dd, 12, 7.6
3	6.06, dt, 10.6, 6.8	2.09, 2.38, m	4.24, m	4.27, m
4	7.11, d, 10.6	3.72, m	2.81, dd, 12.9, 8.4	2.93, dd, 13.2, 11.4
		4.06, m	3.26, dd, 12.9, 5.1	3.60, dd, 13.2, 5.3
6	7.33, d, 8.4	7.33, br d, 9.7	7.35, dd, 8.3, 2.3	7.35, dd, 8.3, 2.1
7	7.09, d, 8.4	7.09, dd, 8.0, 1.7	7.05, dd, 8.4, 2.5	7.10, dd, 8.3, 2.5
12	6.85, d, 8.0	6.83, d, 8.2	6.84, d, 8.2	6.84, d, 8.2
13	6.63, ddd, 8, 1.8, 1.7	6.61, dd, 8.4, 1.7	6.61, dd, 8.3, 1.6	6.61, dd, 8.3, 1.8
15α	2.87, t, 5.0	2.82, m	2.72, br dd, 17.1, 8	2.61, br dd, 17.1, 7.2
15β	2.87, t, 5.0	2.85, m	2.96, br dd, 16.4, 9.9	3.05, br dd, 16.6, 10.6
16α	2.29, dt, 5.0, 1.7	2.25, m	2.23, ddd, 17, 10.5, 1.7	2.15, ddd, 15.2, 12, 1.4
16β	2.29, dt, 5.0, 1.7	2.30, m	2.35, ddd, 17, 8.2, 1.8	2.40, ddd, 16.8, 7.4, 1.4
18	7.33, d, 8.4	7.31, dd, 8.0, 1.9	7.31, dd, 8.0, 2.5	7.31, dd, 8.1, 2.2
19	7.09, d, 8.4	7.02, dd, 8.3, 2.0	7.02, dd, 8.2, 2.5	7.00, dd, 8.1, 2.5
20	5.07, d, 1.8	5.30, d, 2.0	5.23, d, 1.8	5.21, d, 1.8
11-OH	5.47, s	5.51, br s	5.50, br s	5.48, s
3-OH			2.07, d, 6.1	

[a] Two major conformers; the chemical shift of the major conformer is reported.

transformations of combretastatin D-1 undertaken as part of the original structural elucidation.[3]

A methylene chloride–methanol (1:1) extract of *Combretum caffrum* stem wood was initially fractionated and separated as described.[2a,b] The fraction that previously yielded[2b] combretastatin A-2 was subjected to a similar PS bioassay guided chromatographic separation sequence (a series of Sephadex LH-20 partition chromatograms using hexane–toluene–methanol, 3:1:1, and silica gel column chromatographic procedures employing various combinations of hexane–ethyl acetate as eluant) afforded combretastatin D-2 (2, 5.8 mg from 77 kg of wood), which exhibited PS ED_{50} 5.2 μg/mL.

As with combretastatin D-1 (1) mass spectral analysis of combretastatin D-2 indicated a molecular formula ($C_{18}H_{16}O_4$) with 11 double-bond equivalents. The infrared spectrum of lactone 2 showed absorption due to a lactone or an ester carbonyl (at 1728 cm⁻¹), hydroxyl group (3436, 3429 cm⁻¹), and aromatic rings. The ¹H NMR spectrum contained signals corresponding to methylene adjacent to carbonyl, a benzylic methylene, an oxymethylene, eight olefinic and/or aromatic protons, and a shielded aromatic proton (Table I). The proton NMR spectrum was assigned on the basis of 2D ¹H NMR and ¹H-COSY techniques.[4] The spin systems were (a) ArCH₂CH₂CO-; (b) -OCH₂CH=CH-; (c) a *para*-substituted aromatic ring; and (d) an ortho,ortho,meta-substituted aromatic ring. On the assumption that combretastatin D-2 had a lactone ring, all the double-bond equivalents were thereby accounted for. The ¹³C NMR spectrum of olefin 2 was consistent with this deduction. The ¹H NMR spin systems were assembled[3] on the basis of NOEDS experiments.

Comparison of the ¹³C NMR spectrum (Table II) of combretastatin D-2 with the spectrum of combretastatin D-1 (1, confirmed by X-ray crystal structure determination) provided unequivocal support for the proposed structure. The carbon-13 spectrum of lactone 2 was found to be essentially identical with that of combretastatin D-1, except for the olefinic carbon signals. Since the original ¹³C assignments for combretastatin D-1 were based on direct one-bond ¹H, ¹³C correlation using the ¹H, ¹³C-COSY[5] experiment (ambiguous for the quaternary carbons), it became necessary to assign the carbon resonances of the more abundant combretastatin D-1. Therefore, all

Table II. ¹³C NMR Assignments for Combretastatin D-1 (1 with HMBC Correlations) and Combretastatin D-2 (2) in Deuteriochloroform

position	1	2	1 (HMBC)
2	62.56	59.06	C-2 → H-3
3	52.99	137.74	C-3 → H-2α, H-2β, H-4
4	55.84	135.45	C-4 → H-2α, H-2β, H-6
5	132.44	132.01	C-5 → H-4, H-7, H-19
6	128.83	129.09	C-6 → H-4, H-18
7	123.95	123.89	C-7 → H-19
8	156.01	155.6	C-8 → H-7, H-19
10	149.09[a]	149.32[a]	C-10 → H-12, 11-OH, H-20
11	142.62[a]	142.48[a]	C-11 → H-12, 11-OH, H-20
12	115.38	115.39	C-12 → H-13, 11-OH
13	122.03	121.89	C-13 → H-15α, H-12, H-20
14	131.90	131.14	C-14 → H-12, H-15α,β, H-16α,β
15	26.97	26.89	C-15 → H-16α,β, H-20
16	31.24	32.42	C-16 → H-15α,β
17	172.53	173.30	C-17 → H-2α,β, H-15β, H-16α,β
18	126.34	125.68	C-18 → H-4, H-6
19	123.14	123.89	C-19 → H-7
20	112.24	112.58	C-20 → H-12, H-13, H-15α

[a] Assignments with identical superscripts in vertical columns may be interchanged.

the carbon resonances of epoxide 1 were assigned by heteronuclear multiple bond connectivity (HMBC) experiments.[6] The observed connectivities are recorded in Table II. The previous assignments[3] remain unchanged except for reversal of the quaternary carbon signals at C-5 and C-14. Both protons at C-2 gave a strong cross correlation with the carbonyl group, clearly confirming presence of the lactone. Similarly, assignment of the chemical shift of C-8 was confirmed by correlation with H-7 and H-19. Definite assignment of C-10 and C-11 remains uncertain because of common correlation cross peaks but this is of little consequence. Combretastatin D-2 must have structure 2 and this was further corroborated by the mass spectral fragmentation pattern (structure 4).

Attempts to convert combretastatin D-1 (1) into D-2 (2) and thereby provide further support for the D-2 structure were unsuccessful. For example, reaction of combretastatin D-1 with Zn/Cu couple[7] gave hydrocarbon derivative 5a and alcohol 5b. Several other reagents such as P_2I_4[8]

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3 NOE

4 MS
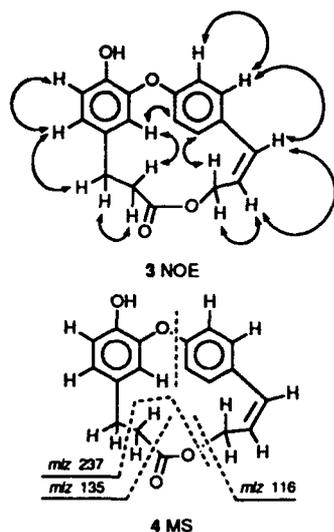
or sodium iodide with acetonitrile and trifluoroacetic anhydride[9] were either unreactive under the conditions studied or caused decomposition. When the epoxide group of combretastatin D-1 was hydrogenated to give alcohol **5b**, subsequent treatment with thionyl chloride in pyridine yielded 3,4-deoxy-3-chlorocombretastatin D-1 (**5c**).

5a: $R_1 = R_2 = H$
5b: $R_1 = OH$, $R_2 = H$
5c: $R_1 = H$, $R_2 = Cl$
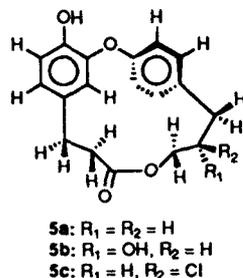
Combretastatins D-1 and D-2 both contain a new oxygen heterocyclic ring (17-membered exterior and 15-atom interior). We propose the designation caffrane for this new macrocyclic ring system. Combretastatin D-2 is probably a penultimate biosynthetic precursor of combretastatin D-1 and may originate biosynthethically as noted earlier[3] from two units of tyrosine or equivalent via *o*-phenol coupling, deamination, partial reduction, and lactonization. When additional quantities of combretastatins D-1 and D-2 become available, the biological properties of these biosynthetic products will be further ascertained.

Experimental Section

Synthetic intermediates were used as received from Sigma-Aldrich Co. All chromatographic solvents were redistilled. Sephadex LH-20 (particle size 25–100 μm) was obtained from Pharmacia Fine Chemicals AB (Uppsala, Sweden) and silica gel 60 (70–230 mesh) was supplied by E. Merck, (Darmstadt, Germany). Analtech, Inc. (Newark, DE) silica gel GHLF U (0.25-mm layer thickness) was employed for thin layer chromatograms. Development was performed with ceric sulfate–sulfuric acid spray reagent (heated at approximately 150 °C for 5–10 min) and/or by use of ultraviolet light. Solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate.

All melting points are uncorrected and were observed with a Kofler-type hot-stage apparatus. Ultraviolet spectra were obtained on a Hewlett-Packard Model 8540A UV/VIS spectrophotometer. Infrared spectra were measured with a Nicolet FT-IR Model MX-1

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unit. Nuclear magnetic resonance spectra were obtained with a Bruker AM-400 instrument using deuteriochloroform as solvent and the residual chloroform signal as an internal standard (δ 7.256). The [13]C NMR multiplicities were determined by using the APT sequence. Mass spectral measurements were performed with a MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln, NE.

Isolation of Combretastatins D-1 (1) and D-2 (2). Fraction A (28.6 g),[2a–c] obtained after extraction of *Combretum caffrum* (77 kg) stem wood, was further separated on a column of Sephadex LH-20 (2.5 kg) by partition chromatography using hexane–toluene–methanol (3:1:1) to afford two active fractions (1.97 g, PS ED$_{50}$ 1.8 × 10^{-2} μg/mL, and 0.54 g, PS ED$_{50}$ 1.9 μg/mL). The latter fraction (0.54 g) was chromatographed on a silica gel (0.04–0.063 μm) flash column (3.0 × 20.0 cm). The column was packed and eluted with hexane–chloroform–acetone (3:2:0.25) to give combretastatin D-1 (1, 180 mg, (2.3 × 10^{-4})% yield). For the physical data, consult ref 3.

The fraction weighing 1.97 g was dissolved in hexane–toluene–methanol (3:1:1, 20 mL) and the solution was filtered. The filtrate was chromatographed on a Sephadex LH-20 (200 g) column using the same solvent system. The resulting active fraction (1.35 g, PS ED$_{50}$ 2.4 × 10^{-2} μg/mL) was dissolved in hexane–ethyl acetate (1:1, 5 mL) and chromatographed on a column (60 × 2.5 cm) of silica gel (60 g). Gradient elution from 4:1 → 1:1 hexane–ethyl acetate afforded in a 3:1 fraction the PS-active (0.7 g, ED$_{50}$ 1.0 × 10^{-2} μg/mL) material. Rechromatography in acetone (2 mL) over a long silica gel column (100 × 1.2 cm, 45 g) and gradient elution with hexane–ethyl acetate (9:1 → 4:1) furnished in the 4:1 fraction pure combretastatin D-2 (2, 5.8 mg, (7.5 × 10^{-6})% yield based on dried plant material), needles from acetone–hexane: mp 148–51 °C; PS ED$_{50}$ 5.2 μg/mL; UV λ_{max} (nm) 235 (ϵ 7300), 274 (2260), 339 (1050); IR (NaCl) ν_{max} 3436, 3429, 1728, 1519, 1503, 1440, 1215, 1186, 1159, 1110 cm^{-1}; HREIMS *m/z* 296.1052 (M$^+$, 100, calcd for $C_{18}H_{16}O_4$ 296.1049), 237.0916 (20, calcd for $C_{16}H_{13}O_2$ 237.0916), 180.0426 (5, calcd for $C_9H_8O_4$ 180.0423), 138.0321 (46, calcd for $C_7H_6O_3$ 138.0317), 135.0450 (50 calcd for $C_8H_7O_2$ 135.0446), 116.0620 (30, calcd for C_9H_8 116.0626), 91.0545 (35, calcd for C_7H_7 91.0548); for [1]H and [13]C NMR data see Tables I and II, respectively.

Benzyl Bond Hydrogenolysis of Combretastatin D-1 (1 → 5b). Method A. To a solution of combretastatin D-1 (1, 10 mg) in a mixture of ethyl acetate–methanol (5:3, 10 mL) was added 5% Pd/C (10 mg). The mixture was hydrogenated under ambient temperature and pressure for 72 h. Catalyst was removed (filtration) and the filtrate was concentrated to give pure alcohol **5b** (10 mg, quantitative yield) as needles from ethyl acetate–hexane: mp 191–93 °C; [α]$^{30}_D$ – 12.6° (*c* 0.95, CHCl$_3$/CH$_3$OH, 1:1); IR (KBr) ν_{max} 3200, 1740, 1719, 1521, 1504, 1285, 1220, 1163, 1155, 1142, 1103 cm^{-1}; HREIMS *m/z* 314.1153 (M$^+$, 100, calcd for $C_{18}H_{18}O_5$ 314.1154), 271.0966 (69, calcd for $C_{16}H_{15}O_4$ 271.0970), 226.0995 (55, calcd for $C_{15}H_{14}O_2$ 226.0994); for [1]H NMR data see Table I.

Method B. Combretastatin D-1 (5.0 mg) in ethanol (2 mL) was treated with freshly prepared[7] zinc/copper couple (100 mg) for 10 days. The solution was filtered and the filtrate concentrated to give a mixture of unreacted starting material and two products. Separation on a preparative silica gel plate using hexane–acetone (7:3) as solvent afforded the less polar hydrocarbon product (**5a**, 0.8 mg) as a viscous oil (for [1]H NMR data see Table I): HREIMS *m/z* 298 (M$^+$, 6), 135 (10), 115 (60), 107 (65), 91 (100). Unreacted combretastatin D-1 (1.0 mg) was recovered, and the most polar product (2.0 mg) was identified as alcohol **5b** by direct comparison (TLC, [1]H NMR) with the product of method A.

Chlorination of Alcohol 5b. To a cooled (0 °C) solution of alcohol **5b** (1.0 mg) in pyridine (0.2 mL) was added thionyl chloride (0.1 mL), and the solution was stirred for 1 h at 0 °C and overnight at room temperature. The solvent was evaporated under a stream of nitrogen and the residue was chromatographed by using a pipet filled with silica gel. Elution of the pipet column with hexane–acetone (3:1) gave 3-chloro-3,4-deoxycombretastatin D-1 (**5c**, 1.0 mg) as an amorphous powder from acetone–hexane: mp 170–172 °C; IR (NaCl) ν_{max} 3450, 1740, 1597, 1520, 1506, 1205 cm^{-1}; HREIMS *m/z* 332.0812 (M$^+$, 100, calcd for $C_{18}H_{17}O_4{}^{35}Cl$ 332.0816), 334.0798 (31, calcd for $C_{18}H_{17}O_4{}^{37}Cl$ 334.0786), 297.1132 (29, calcd for $C_{18}H_{17}O_4$ 297.1127); for [1]H NMR data see Table I.

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ANTINEOPLASTIC AGENTS, 178. ISOLATION AND STRUCTURE OF LYCHNOSTATINS 1 AND 2 FROM THE SOUTH AMERICAN *LYCHNOPHORA ANTILLANA*[1]

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ABSTRACT.—Bioassay-guided (P388 lymphocytic leukemia cell line) separation of a CH_2Cl_2/MeOH extract of *Lychnophora antillana* led to the isolation of two cytostatic (P-388, ED_{50} 2.0 and 0.19 µg/ml, respectively) germacranolides designated lychnostatins 1 [1] and 2 [2]. Structural elucidation was based initially upon high field (400 MHz) nmr and electron impact mass spectral interpretations and unequivocally completed by X-ray crystal structure determinations.

Although many species of the large plant family Compositae are well-known for a variety of reasons, including primitive medical applications, some occur in a few small and relatively unexplored tropical genera. One such genus, the *Lychnophora* of the subtribe Lychnophorinae (2,3), contain some twenty-three species indigenous primarily to Brazil. More than twenty years ago, as part of the U.S. National Cancer Institute's (NCI) world-wide exploratory programs directed (by Jonathan L. Hartwell) toward the discovery of new anticancer drugs, specimens of *Lychnophora antillana* Urb. (also known as *Piptocoma antillana*) were collected and evaluated. By 1974, an EtOH extract was found to provide 32–34% life extension against the NCI murine P-388 lymphocytic leukemia (PS system) at 4.9↦16 mg/injection. A 1979 Puerto Rican collection gave analogous biological results (including PS cell line ED_{50} 0.72 µg/ml) and led to the present study.

The plant was extracted with CH_2Cl_2-MeOH (1:1), and the extract was partitioned (4) between MeOH-H_2O (9:1↦4:1↦3:2) with hexane↦CCl_4↦CH_2Cl_2 to yield an active CH_2Cl_2-soluble fraction (PS ED_{50} 0.15 µg/ml). Separation (PS bioassay-guided) of this fraction on a Si gel column resulted in isolation of lychnostatins 1 [1] and 2 [2] as the major PS-active (ED_{50} 2.0 and 0.19 µg/ml) constituents.

Initial structural investigations revealed both cytostatic compounds to be new sesquiterpene lactones of the germacranolide type. While varied biological activity has been reported for a number of such compounds from other genera (5–18), only one example of antineoplastic activity (10) has been reported for germacranolides isolated from the *Lychnophora* (19–24). Several germacranolides distantly related to lychnostatins 1 and 2 have been isolated from Brazilian *Lychnophora* (19), *Eremanthus* (25), and

1 R=OH
2 R=H
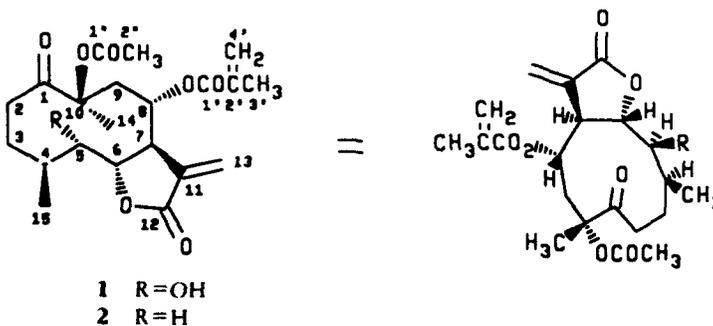
Piptolepis (21,26) species. One of these, isolated from *Lychnophora blanchetii* (19), was assigned structure 3 a structural isomer of lychnostatin 1.

Ir, ¹H-, ¹³C-nmr, and mass spectral analyses suggested the presence of an α-methylene lactone, as well as methacrylate, acetate, and ketone groups. From mass spectral data, it was determined that lychnostatin 1 [1] differed from lychnostatin 2 [2] only by having an additional oxygen atom. In addition, eims exhibited significant peaks corresponding to $[M - HOAc]^+$ and $[M - HOAc - CH_2 = C(CH_3)CO_2H]^+$ fragment ions, thereby confirming the presence of the ester groups. The spectral data and molecular formula were also consistent with a ten-membered carbon ring bearing the substituents just noted. Extensive ¹H-nmr and ¹³C-nmr decoupling experiments provided sufficient additional information to allow assignment of the α-methacrylate unit adjacent to the lactone. The nmr data also seemed to suggest that a hydroxyl group in lychnostatin 1 was adjacent to the lactone ring. From empirical formula data, the presence of macrocyclic ring unsaturation seemed to be excluded for both compounds. Because neither the complete regio nor stereo relationships of the macrocyclic ring substituents could be definitively ascertained from the above information alone, a number of structural possibilities remained.

In order to establish unambiguously the complete structures of lychnostatins 1 and 2, single crystal X-ray diffraction analyses were undertaken (Table 1). Cell parameters for both lychnostatins were nearly identical, suggesting that each of the compounds had similar cell packing characteristics and conformations. Indeed, this assumption proved to be correct. An X-ray-analysis-derived structure for lychnostatin 1 is shown in Figure 1. The absence of unsaturation in the 10-membered macrocyclic rings for both lychnostatins was thereby established. Although unusual, this result was not without precedent (19,25,27,28). Also established were the orientation of the macrocyclic ring and the relative stereochemistry of the ring substituents for both compounds. The β disposition of the C-4 and C-7 substituents, as well as the C-10 methyl, was readily apparent for the lychnostatins.

For lychnostatin 1, the additional oxygen atom was found to be present as a β-oriented C-5 hydroxy group. The two ester substituents attached to the C-8 and C-10 ring atoms of both compounds, as well as the C-6 oxygen atom (which forms part of the *trans*-fused α-methylene lactone ring) were all α-oriented with respect to the 10-membered ring. The more stable *trans*-fusion of the lactone ring to the 10-membered ring is a feature commonly observed for a majority of germacranolide sesquiterpene lactones. The α-methylene–γ-lactone rings of both lychnostatins 1 and 2 exhibited some nonplanarity (endocyclic torsion angle moduli sum of 49° and 55°, respectively). Examples

3 R = $\overset{\overset{\text{CH}_2}{\parallel}}{\text{CO}}$CCH₃

5 2,3-*trans*

4 R = CO—CH₃ / CH₃
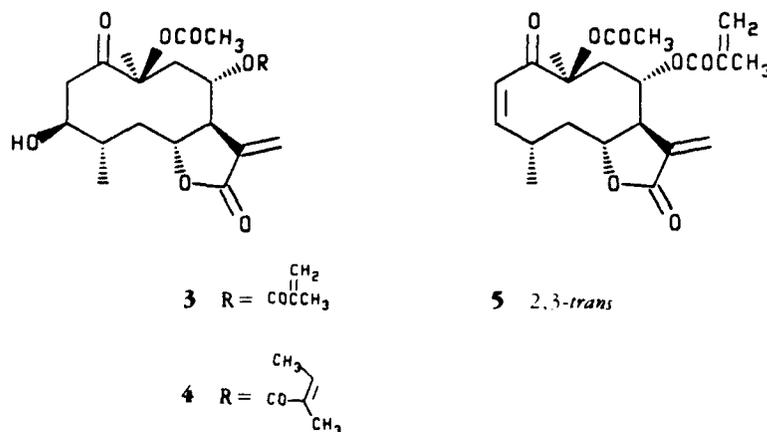
TABLE 1.   Crystal Data Experimental and Refinement Parameters for Lychnostatin 1 [1] and Lychnostatin 2 [2].

Parameters	Compound	
	1	**2**
Crystal data		
Molecular formula	$C_{21}H_{28}O_8$	$C_{21}H_{28}O_7$
F.W.	408.45	392.45
F(000)	872	840
Space group	$P2_12_12_1$	$P2_12_12_1$
Crystal dimensions (mm)	$0.07 \times 0.10 \times 0.32$	$0.08 \times 0.10 \times 0.45$
Radiation, Å	$CuK\alpha, \lambda = 1.54184$	$CuK\alpha, \lambda = 1.54184$
Temperature, °C	26 ± 1	26 ± 1
Cell constants		
a, Å	5.876(2)	5.785(4)
b, Å	8.865(1)	8.902(3)
c, Å	40.057(8)	40.457(6)
V, Å^3	2089.3	2083.4
Z	4	4
ρo, g/cm^3	1.289	1.245
ρc, g/cm^3	1.298	1.251
μ, cm^{-1}	7.9	7.4
Collection Parameters		
Instrument	Enraf-Nonius CAD4 diffractometer	Enraf-Nonius CAD4
Monochromator	Graphite crystal, incident beam	Graphite crystal, incident beam
Attenuator	Ni foil, factor 11.9	Ni foil, factor 11.9
Take-off angle, deg	2.8	2.8
Detector aperature, mm	4.0 to 5.9 horizontal, 4.0 vertical	4.0 to 5.9 horizontal, 4.0 vertical
Crystal-detector dist.	21 cm	21 cm
Scan type	ω–2θ	ω–2θ
Scan rate, °/min (in ω)	1 to 5	1 to 5
Scan width, deg	$0.9 + 0.140 \tan \theta$	$0.8 + 0.140 \tan \theta$
Maximum 2θ, deg	150.0	150.0
No. of refl. measured	2676 total, 2537 unique	2602, 2532 unique
Corrections made	Lorentz-polarization Linear decay, (0.815 to 1.185 on I)	Lorentz-polarization Linear decay, (0.985 to 1.129 on I) Empirical absorption, (0.88 to 0.99 on I)
Solution and Refinement Parameters		
Solution method	Direct Methods	Direct Methods
Hydrogen atoms	Refined, Uiso = 0.06 Å^2, restrained to ride	Refined, Uiso = 0.06 Å^2, restrained to ride
Refinement	Full matrix least-squares	Full matrix least-squares
Minimization function	$\Sigma w(|Fo|-|Fc|)^2$	$\Sigma w(|Fo|-|Fc|)^2$
Least-squares weights	$1/\sigma^2(Fo)$	$1/\sigma^2(Fo)$
Anomalous dispersion	All non-hydrogen atoms	All non-hydrogen atoms
Reflections included	2178 with $Fo^2 > 3.0\sigma(Fo^2)$	1297 with $Fo^2 > 3.0\sigma(Fo^2)$
Parameters refined	262	254
Unweighted R factor	0.045	0.049
Weighted R factor	0.044	0.038
EDS of obs. of unit wt.	2.30	2.74
Convergence, Largest shift, Å	0.07	0.06
High peak in final diff. map, e/Å^3	0.20(4)	0.20(5)
Computer hardware	PDP-11/23, MicroVax II	—
Computer software	SDP-PLUS (Enraf-Nonius & B.A. Frenz and Assoc., Inc.) CRYSTALS (CCL, Univ. of Oxford)	—
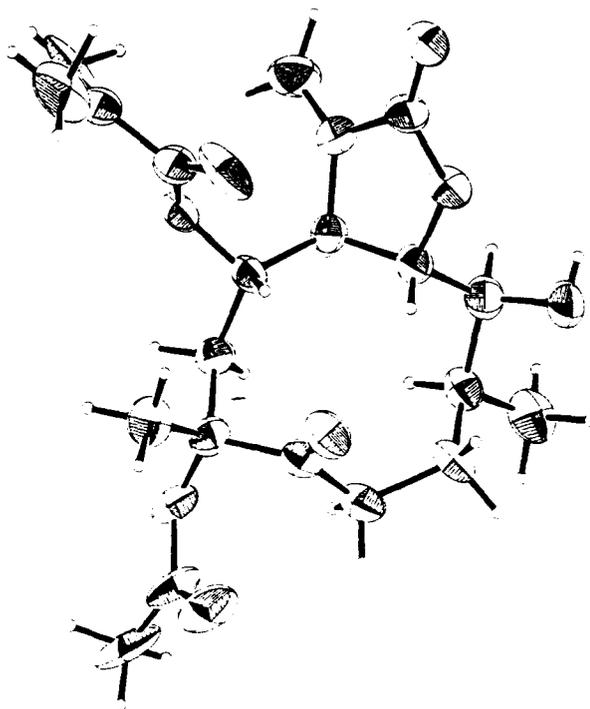
FIGURE 1. Crystal structure (ORTEP representation) of lychnostatin 1.

covering the range of nearly complete planarity to pronounced non-planarity of the *trans*-fused lactone ring have been reported (10, 27, 29–32).

No abnormalities were observed for the bond distances and angles of either compound, the values being in good agreement with those reported for similar substances (27, 29, 30, 33–35). Conformational analysis of the data for lychnostatins 1 and 2. with respect to the 10-membered macrocyclic ring, revealed a conformational deviation from that previously proposed and/or observed for cyclodecane/cyclodecanone rings (36–39). Among these are two boat-boat conformations, referred to as the "O-inside" and the "O-outside" conformations, as depicted in Figure 2 In the "O-inside" conformation, the oxygen is approximately perpendicular to the imagined plane of the cyclodecane ring, whereas in the "O-outside" conformation, the oxygen lies approximately in the plane of the ring. The "O-inside" is conformationally favored over the "O-outside," primarily due to the decreased number of destabilizing intra-annular hydrogen atom interactions present in this conformation (36). The conformation assumed by lychnostatins 1 and 2 is depicted in Figure 3 as a twist chair-boat conformation. Although subtly different from the boat-boat "O-inside" conformer, it still maintains one essential distinguishing feature of that conformer, i.e., positioning of the carbonyl oxygen in a perpendicular orientation to the plane of the 10-membered ring.

With lychnostatins 1 and 2, significant intra-annular hydrogen interactions (interatomic bond distance $<2 \times H$ van der Waals radii or ca. 2.30 Å) occur on both the α and β faces, as signified by arrows in Figure 3. Table 2 summarizes the intra-annular interatomic distances occurring in the lychnostatins. In each case the carbonyl oxygen, O-1, does not seem to participate in any significant intra-annular interactions. All intra-annular atomic distances involving O-1 were found to be 2.40 Å or greater. On the other hand, a greater number of hydrogen-hydrogen intra-annular interactions occur in the conformer adopted by lychnostatins 1 and 2, as compared to the "normal" boat-boat

O-inside

O-outside
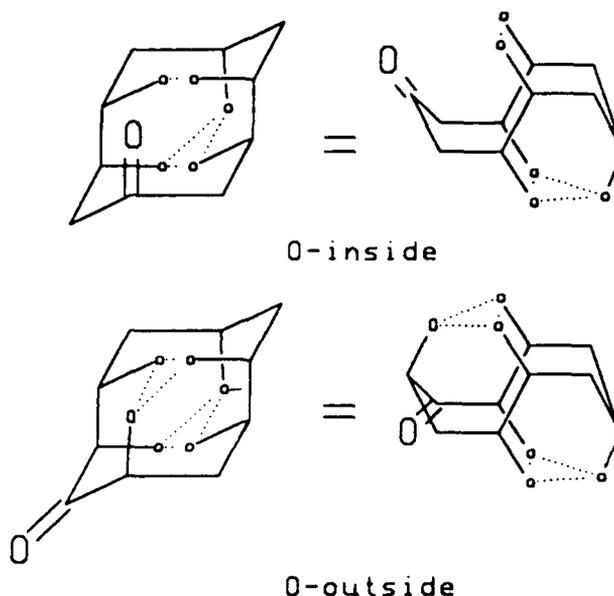
FIGURE 2.   Two possible cyclodecanone conformers.

O-inside conformer. Presumably, the *trans*-fusion of the α-methylene–γ-lactone ring to the 6,7 position of the cyclodecanone ring, as well as the α orientation of the ester side chains, provides steric factors contributing to this conformational modification or deviation.

The absolute configuration of lychnostatins 1 and 2 could not be ascertained from the X-ray data, only the relative configuration; nearly identical R values were obtained for both enantiomers. Thus, either the structures depicted by 1 and 2 or their mirror images are equally plausible. A less reliable method (27, 29–31, 40, 41) for affixing absolute stereochemistry about the ring juncture of the α-methylene–γ-lactone and the cyclodecanone ring, based upon cd data, also failed due to interference by the methacrylate moiety with the diagnostic n→π* transition curve of the lactone. Finally, utilization of information based solely on the possible biosynthetic pathway previously proposed for the generation of germacranolides must also be excluded, as there are no carbon-carbon double bonds in the 10-membered ring that might indicate its mode of origin. The aforementioned problems concerning absolute configurational assignments and correct classification (12→6 or 12→8 lactonization) have been encountered earlier (27,29).

As previously mentioned, Bohlmann *et al.* (19) have investigated *L. blanchetii* collected in northeast Brazil and assigned structures 3, 4, and 5 to three of the con-
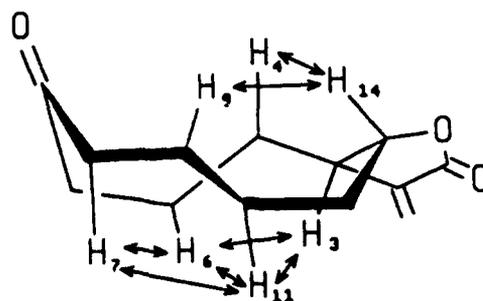
FIGURE 3.   Conformation assumed by the lychnostatins

TABLE 2.   Intra-annular Atomic Distances in Lychnostatin 1 [1] and Lychnostatin 2 [2] (<3.00 A).

Atoms	Lychnostatin 1		Lychnostatin 2	
	α-face	β-face	α-face	β-face
H-5–H-6	2.09 A		2.10 A	
H-3–H-11	2.36 A		2.43 A	
H-3–H-7	>3.00 A		>3.00 A	
H-4–H-9		>3.00 A		>3.00 A
H-4–H-14		2.12 A		2.13 A
H-4–O-1		2.54 A		2.58 A
H-6–H-7	2.31 Å		2.26 A	
H-6–H-11	2.30 A		2.29 A	
H-7–H-11	2.19 Å		2.20 A	
H-9–H-14		2.07 A		2.07 A
H-9–O-1		2.50 A		2.49 A
H-14–O-1		2.40 A		2.40 A

stituents. In addition to these germacranolides, they also identified the two pentacyclic triterpenes, lupeol and lupenone. In the present study, we found betulinic and ursolic acids as representatives of the latter group. More importantly, germacranolide **3** appears to be a structural isomer of lychnostatin 1. From biosynthetic considerations it seems likely that structure **3** may need further refinement.

Lychnostatins 1 [1] and 2 [2] now augment the small number of germacranolides known to exhibit cell growth inhibitory and/or antineoplastic activity (6, 10, 12, 13, 15–18). The lychnostatins are also unusual in that they don't completely satisfy the postulate proposed by Manchand and Blount (17) that antitumor activity requires, in addition to the α-methylene–γ-lactone, an oxygen function or double bond at C-4. Further experiments directed at biological evaluation and unambiguously defining absolute configuration of the lychnostatins are under way.

EXPERIMENTAL

Solvents used for chromatography were redistilled. Ambient cc procedures employed Si gel (70–230 mesh), supplied by E. Merck, Darmstadt. Cc under pressure was carried out using prepacked Lobar Lichro Prep Si gel 60 (40–63 um). Fraction collection was partially automated, using a Gilson microfractionator. Tlc was performed with Si gel GHLF from Analtech, Inc. The tlc plates were developed by uv light and/or a ceric sulfate spray reagent.

All melting points are uncorrected and were observed using a Koeffler-type melting point apparatus. Each substance was colorless. Ir spectra were recorded with a Perkin-Elmer Model 299 spectrophotometer. Optical rotations were determined with a Perkin-Elmer model 241 Automatic Polarimeter. The 100 MHz ^{1}H-nmr spectra were recorded with a Varian XL-100 instrument and the 400 MHz with a Bruker WH-400 nmr spectrophotometer. The ^{13}C spectra were obtained employing a Bruker WH-90 at 22.63 MHz. TMS was used as an internal reference, and δ values are reported in ppm. Mass spectra were obtained using an MAT 312 spectrophotometer.

PLANT MATERIAL.—*L. antillana* (stems and leaves, herbarium specimens NSC B 507 14 maintained by the USDA) was recollected in Puerto Rico in 1979, under auspices of the Economic Botany Laboratory, Agricultural Research Center East, USDA, Beltsville, Maryland, as part of a joint NCI-USDA program directed by Drs. M.J. Suffness and J.A. Duke.

EXTRACTION AND SOLVENT PARTITIONING.—The stems and leaves of *L. antillana* (54 kg) were extracted with CH_2Cl_2-MeOH (1:1) (320 liters) at ambient temperature for 4 days. Decantation of solvent and subsequent dilution with H_2O (25% by volume) allowed the chlorocarbon phase to separate. The CH_2Cl_2 was removed to yield a viscous, brown gum (1145 g) which was further purified by partitioning (4) employing the sequence MeOH-H_2O (9:1→4:1→3:2) against, respectively, hexane→CCl_4→CH_2Cl_2. An aliquot (40 g) of the CH_2Cl_2 fraction (total weight, 315 g) was chromatographed on a column of Si gel (800 g). Elution with hexane-Me_2CO (9:1) (6.0 liters to 10.0 liters) yielded fraction A (0.18 g) as a color-

less solid. Further elution (13.0 to 18.0 liters) resulted in isolation of lychnostatin 1 [1] as a colorless solid (0.31 g, 5.7 × 10^{-5}% yield).

Rechromatography of fraction A (see above) on a column of Si gel (18 g, dry packed column) and elution with CH_2Cl_2 (200 ml) afforded a single product, lychnostatin 2 [2] (20 mg, 3.7 × 10^{-6}% yield). Further elution with CH_2Cl_2-MeOH (44:1) gave a mixture (0.10 g) which was rechromatographed on Si gel (Lobar B column). Gradient elution with CH_2Cl_2-MeOH (99:1→44:1) (400 ml total) led to a pure compound (34 mg) that recrystallized from MeOH/CH_2Cl_2 to give betulinic acid (20 mg), mp 304–307°. Further elution with the same solvents yielded another minor product (22 mg), which proved to be ursolic acid, mp 260–265°. Both triterpene carboxylic acids were identified by comparison (tlc, ir, ^{1}H nmr) with authentic specimens.

LYCHNOSTATIN 1 [1].—Recrystallization from Me_2CO/hexane afforded crystals melting at 228–230°; tlc R_f 0.85 in CHCl$_3$-MeOH (9:1); [α]^{23}D +89° (c = 1.0, CHCl$_3$); ir (KBr) ν max 3580, 3430, 1780, 1732, 1710, 1702 (sh), 1660, 1633, 1460, 1380, 1308, 1276, 1267, 1253, 1154, 1120, 1095, 1075, 1060, 1020, 993, 960, 816, 805, 763, 700, 600 cm^{-1}; ^{1}H nmr (400 MHz, CDCl$_3$) δ 1.08 (d, 3H, J = 8 Hz, Me-15), 1.62 (1H, br s, -OH), 1.74 (3H, s, Me-14), 1.94 (3H, s, Me-3′), 1.70–2.10 (3H, m, -CH$_2$-), 2.03 (3H, s, Me-2″), 2.32 (1H, dd, J = 16, 2 Hz, -CH$_2$), 2.66–2.74 (3H, m, -CH-), 3.07 (1H, d, J = 10 Hz, H-7), 3.41 (1H, dd, J = 8, 7 Hz, H-5), 4.35 (1H, d, J = 8 Hz, H-6), 4.84 (1H, m, H-8), 5.65 (2H, br s, H-13a or H-13b, H-4′a or H-4′b), 6.16 (1H, br s, H-4′a or H-4′b), 6.24 (1H, br s, H-13a or H-13b); ^{13}C nmr (22.63 MHz, CDCl$_3$), 207.61 (s, C-1), 169.56 (s, C-12), 168.45 (s, C-1″), 165.82 (s, C-1′), 135.93 (s, C-11 or C-2′), 134.92 (s, C-11 or C-2′), 126.31 [t (2C), C-13 and C-4′], 84.91 (s, C-10), 82.44 (d, C-6), 77.50 (d, C-5), 70.77 (d, C-8), 44.29 (d, C-7), 41.07 (t, C-2 or C-9), 35.32 (t, C-2 or -9), 32.07 (d, C-4), 24.05 (t, C-3), 22.12 (q, C-14), 21.38 (q, C-2″), 20.24 (q, C-3′), 18.23 (q, C-15); eims m/z [M]$^+$ 408, [M − HOAc]$^+$ 348, [M − H$_2$O − C$_3$H$_5$O$_2$]$^+$ 305, [M − HOAc − C$_3$H$_5$O$_2$]$^+$ 262, [M − H$_2$O − HOAc − C$_3$H$_5$O$_2$]$^+$ 245. Anal. calcd for $C_{21}H_{28}O_8$, C 61.75, H 6.91; found C 61.66, H 6.67%.

LYCHNOSTATIN 2 [2].—Recrystallization of lychnostatin 2 [2] from Me_2CO/hexane provided fine needles: mp 190–193°; [α]^{30}D +20.9° (c = 0.67, CHCl$_3$); ir (KBr) ν max 2950, 1780, 1740, 1712, 1645, 1460, 1385, 1312, 1300, 1275, 1178, 1156, 1126, 1105, 1065, 1022, 955, 878, 810, 741, 614 cm^{-1}; ^{1}H nmr (100 MHz, CDCl$_3$) δ 1.04 (3H, d, J = 6 Hz, Me-15), 1.80 (3H, s, Me-14), 1.96 (3H, s, Me-3′), 1.4–2.2 (4H, m, -CH$_2$-), 2.06 (3H, s, Me-2″), 2.23 (1H, dd, J = 15, 2 Hz, -CH$_2$-), 2.70 (3H, m, -CH-), 3.05 (2H, m), 4.37 (1H, m, H-6), 4.96 (1H, dd, J = 8, 2 Hz, H-5), 5.73 (2H, d, J = 2 Hz, H-13a or H-13b, H-4′a or H-4′b), 6.18 (1H, br s, H-4′a or H-4′b), 6.35 (1H, br s, H-13a or H-13b); ^{13}C-nmr (22.63 MHz, CDCl$_3$) δ 208.29 (s, C-1), 169.62 (s, C-12), 168.97 (s, C-1″), 165.92 (s, C-1′), 135.86 (s, C-11 or C-2′), 134.69 (s, C-11 or C-2′), 126.53 (t, C-13 or C-4′), 124.88 (t, C-13 or C-4′), 84.26 (s, C-10), 77.86 (d, C-6), 68.24 (d, C-8), 46.99 (d, C-7), 43.44 (t, C-5), 30.09 (t, C-2 or C-9), 35.91 (t, C-2 or C-9), 29.70 (d, C-4), 27.13 (t, C-3), 23.98 (q, C-14), 21.51 (q, C-2″), 21.25 (q, C-3′), 18.20 (q, C-15); spsims m/z [M + Na]$^+$ 415; eims m/z [M]$^+$ 392, [M − CH$_3$CO]$^+$ 350, [M − HOAc]$^+$ 332, [M − C$_3$H$_5$O$_2$]$^+$ 263, [M − HOAc − C$_3$H$_6$O$_2$]$^+$ 246; hrfabms m/z [M + Li]$^+$ 399.1996 (calcd $C_{21}H_{28}O_7$ + Li, 399.1997).

X-RAY CRYSTAL STRUCTURE DETERMINATIONS OF LYCHNOSTATIN 1 [1] AND LYCHNOSTATIN 2 [2].—Preliminary examinations and data collections for lychnostatins 1 and 2 were performed at room temperature by the moving-crystal, moving-counter technique with background measurements made on both sides of the peak using an Enraf-Nonius CAD-4 automatic diffractometer. Crystal data, collection, and refinement parameters for the two compounds are summarized in Table 1. In each case, data was corrected for Lorentz and polarization effects. For lychnostatin 2, an additional semi-empirical absorption correction was also applied [the absorption correction being based on a series of psi scans (42)]. Space group assignments for each compound were derived on the basis of Laue symmetry and observed systematic extinctions. Cell dimensions were determined from least-squares refinement, using the setting angles of 25 carefully measured reflections. The structures were solved by direct methods (43). Scattering factors were taken from Cromer and Waber (44). Initial stages of refinement were performed using the SDP-PLUS (45) software package; final refinements were done with CRYSTALS (46). Anomalous dispersion corrections were made in Fc (47) for both compounds; the values of ΔF′ and ΔF″ were those of Cromer (48); extinction coefficients were refined on both compounds.^{1} A perspective view (49) displaying all essential conformational and configurational features for lychnostatins 1 and 2 appears in Figure 1.

Colorless crystals of lychnostatin 1, arising from MeOH-H$_2$O solution, were used in mass spectral,

density and X-ray data collections. The observed density and mass spectral data indicated a single molecule of lychnostatin 1 per asymmetric unit in space group $P2_12_12_1$. All unique reflections (one octant) were collected. Structural solution proceeded without incident, the nonhydrogen atoms being located readily. The remaining hydrogen atom coordinates were calculated at ideal positions and assigned fixed coordinates and isothermal parameters during subsequent structure-factor full-matrix least-squares refinements. Here the function minimized for least-squares was $\Sigma w(|F| - |Fc|)^2$ with the weight w defined as $1/\sigma^2(FO)$. Refinement was continued until convergence to a residual of R = 0.045 and R_w = 0.044.

The crystal structure of lychnostatin 2 was performed on a fine needle-shaped crystal obtained from Me_2CO/heptane solution. Observed density measurements again indicated one molecule per asymmetric unit corresponding to the $P2_12_12_1$ space group. Solution by direct methods proceeded with some difficulty. After a number of unsuccessful preliminary attempts, a starting set of seven reflections was used (from 400 reflections) with the largest E's (minimum E of 1.29) in order to generate 12,659 relationships. In addition, the lower limit of probability of acceptance of phases determined by the sigma 1 formula being included in the starting set was extremely low (i.e., 0.650). In this manner, a total of 200 possible phase sets were generated; the phase set with the highest overall figure of merit (2.99) provided an E map which revealed all 28 nonhydrogen atoms. Hydrogen atom coordinates again were calculated, fixed, and assigned isotropic thermal parameters in subsequent least-squares refinements. Anisotropic refinement was done on all nonhydrogen atoms by full matrix least-squares methods. Refinement converged to a residual of R = 0.049 and R_w = 0.038.

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ANTINEOPLASTIC AGENTS, 107. ISOLATION OF ACTEOSIDE AND ISOACTEOSIDE FROM *CASTILLEJA LINARIAEFOLIA*[1]

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ABSTRACT.—The southwestern Indian paintbrush, *Castilleja linariaefolia*, yielded extracts that displayed in vivo activity against murine P-388 (PS) lymphocytic leukemia. Separation guided by PS cell line inhibition led to isolation of cytotoxic compounds that were identified as the known glycosides acteoside [1] (ED_{50} 2.6 µg/ml) and isoacteoside [2] (ED_{50} 10 µg/ml). The identifications were established by spectral measurements and degradation studies. Mannitol was also found in this plant.

In mountainous areas of northern Arizona (2) and southern Utah, *Castilleja linariaefolia* Benth. is known as Indian paintbrush. While some 50 species of the large (3000 species and 220 genera) Scrophulariaceae family have been used in primitive cancer treatment, only one has been represented by the *Castilleja* genus (3), namely, the Mexican (Yucatan) *Castilleja communis* Benth. (4).

Extracts from *C. linariaefolia* gave confirmed activity against the Walker carcinosarcoma 256 (intramuscular WM system) in the rat. Each of the major plant parts appeared to contain the anticancer constituent(s), with the flowers showing the highest activity (90% inhibition of tumor growth at 266 mg/kg). When the murine P-388 lymphocytic leukemia (PS system) became available and a key fraction showed T/C 125–165% (10–40 mg/kg), we discontinued use of the WM system. [The PS in vitro studies were conducted in our laboratory according to procedures developed by the National Cancer Institute, and PS in vivo bioassays were performed under the auspices of the NCI (5).] Inconsistent results were obtained with both in vivo systems making fractionation difficult. Monitoring fractionation of *C. linariaefolia* with in vitro PS

eventually yielded two of the PS cytostatic constituents. These were found to be the known glycosides acteoside (6–9) and isoacteoside (8). These caffeoyl glycosides have been isolated from a Labiatae species (8), and acteoside also occurs in a Gesneriaceae (7) and two Oleaceae (6,9) species; neither glycoside had hitherto been found in a plant of the Scrophulariaceae. The research was completed using a series of recently developed (10) experimental procedures augmented by dccc. The dccc technique has previously been used in the separation of iridoid glycosides from *Castilleja miniata* (11). Interestingly, dccc was the only effective method found for separation of myricoside, a bioactive substance closely resembling acteoside in structure (12). As part of the current study, D-mannitol also was isolated.

Acteoside [1] was identified on the basis of detailed spectral analyses (uv, ir, etc.) and by identification of alkaline and acid hydrolysis products and confirmed by comparison with an authentic sample provided by Professor I. Nishioka, Faculty of Pharmaceutical Sciences, Kyushu University. The general spectral features of isoacteoside [2] closely resembled those of acteoside, except that the L-rhamnose unit methyl group signal in the ^{1}H-nmr spectrum was shifted from δ 1.12 to 1.27 ppm. Also, the C-6 and C-3 D-glucose unit carbon signals in the ^{13}C-nmr spectrum were shifted from δ 62.43 to 64.70 and from 81.64 to 84.15

1   R=R$_2$=H, R$_1$=COCH=CH—(ring: 2″,3″ OH, 4″ OH, 6″, 5″)

2   R=R$_1$=H, R$_2$=COCH=CH—(ring: 2″,3″ OH, 4″ OH, 6″, 5″)
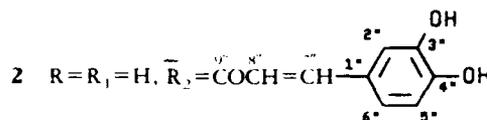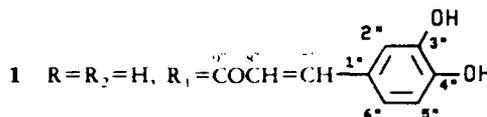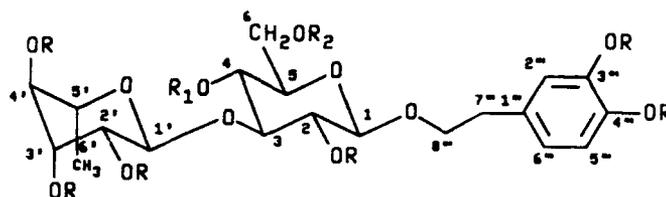
ppm, respectively. These data suggested an isomeric relationship, which was confirmed by a series of methylation, acetylation, and hydrolysis experiments (11,13,14).

Acteoside and isoacteoside exhibited moderate to weak cytotoxic activity in the PS in vitro system (ED$_{50}$ 2.6 and 10 μg/ml, respectively). Because of the antibacterial (12) and cAMP phosphodiesterase inhibitory (15) activity shown by several closely related natural products, these glycosides are worthy of further biological study.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Paper partition chromatography (ppc) was performed by the ascending method using Toyo Roshi No. 50 paper and *n*-BuOH–HOAc–H$_2$O (4:1:2). Mp's were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ir were recorded with an Hitachi EPI-G2 spectrometer. The ^{1}H-nmr spectra were measured with an Hitachi R 40 spectrometer at 90 MHz and the ^{13}C-nmr spectra with a JEOL JNM FX-200 spectrometer at 50.3 MHz. Chemical shifts are given in ppm (δ) downfield from TMS as an internal standard. Solution phase sims (16) mass spectra were obtained using a Varian MAT 312 spectrometer equipped with a modified capillaritron source and 0.14 M NaI in sulfolane as liquid phase. The eims were recorded using an Hitachi M-70 spectrometer.

PLANT MATERIAL.—The recollection (original in August 1966, Kaibab, N.F., Arizona) of *C. linariaefolia* (ca. 500 kg dry wt), used here was made in August 1978, in the Dixie National Forest, Garfield Co., Utah, at an elevation of

7000–8000 ft. Taxonomic identification was made by one of us (CPP) in the USDA Laboratory and by E. Lehto in the Department of Botany at Arizona State University. Herbarium specimens are maintained in the Department of Botany and in the Cancer Research Institute at Arizona State University.

PLANT EXTRACTION.—Dried plant material (leaves, stems, and roots, 36 kg) from the 1978 collection was extracted with a mixture (160 liters) of CH$_2$Cl$_2$ and MeOH (1:1) (10) at ambient temperature for 3 weeks. The extract was separated into CH$_2$Cl$_2$ and H$_2$O phases on addition of 25% by volume of H$_2$O. The aqueous phase was adjusted by the addition of MeOH and CH$_2$Cl$_2$ to achieve a 4:1:2 ratio for H$_2$O-MeOH-CH$_2$Cl$_2$, the plant was extracted with this mixture for 7 weeks. Addition of 15% by volume of H$_2$O resulted in separation of the CH$_2$Cl$_2$ phase which was combined with that obtained from the first partition. Concentration gave a CH$_2$Cl$_2$ extract (712 g; PS in vitro ED$_{50}$ 19 μg/ml; PS in vivo inactive at 12.5–100 mg/kg). The H$_2$O phase was concentrated to give an extract (3.5 kg) that was marginally active (in vivo T/C 120% at 25 mg/kg; PS in vitro ED$_{50}$>100 μg/ml).

SOLVENT PARTITION SEQUENCE.—A portion of the H$_2$O extract (180 g) was successively partitioned between MeOH-H$_2$O (9:1) (1800 ml)→(4:1)→(1:1) with hexane (3 × 1800 ml), CCl$_4$ (3 × 1800 ml), and CH$_2$Cl$_2$ (3 × 1800 ml), respectively. Concentration of the partitioned fractions gave hexane (1.5 g; PS in vitro ED$_{50}$ 6 μg/ml), CCl$_4$ (1.2 g; PS in vitro ED$_{50}$ 24 μg/ml), CH$_2$Cl$_2$ (4.6 g; PS in vitro ED$_{50}$ 51 μg/ml), and H$_2$O (142.8 g; PS in vitro ED$_{50}$ 36 μg/ml) fractions. None of these fractions exhibited activity in the PS in vivo system when tested at dose levels of 3.12–25 mg/kg. However, in a number of earlier experiments, fractions had been obtained at this stage showing T/C 165 at 40 mg/kg.

ISOLATION OF ACTEOSIDE [1] AND ISOAC-TEOSIDE [2].—An aliquot (10.22 g) of the H$_2$O fraction was chromatographed on Sephadex LH-20 (805 g; 77 × 8 cm) using MeOH-H$_2$O (4:1) as eluent. Fractions were monitored by ppc. After elution of 5.1 g of material, a fraction (2 g) was obtained from which D-mannitol (0.252 g) was isolated as colorless needles, mp 171–172°, and found to be identical (ir, tlc) with an authentic sample. Further elution gave 1.8 g of material (inactive against PS in vitro), followed by a fraction (1.3 g) that was active in vitro (ED$_{50}$ 4.1 μg/ml, PS in vivo inactive at 3.12–25 mg/kg). A 1.94-g portion of the in-vitro-active fraction (obtained after repeating the Sephadex LH-20 chromatographic step) was treated with MeOH. The soluble fraction (1.38 g) was dissolved in a minimum volume of the upper layer prepared from CHCl$_3$-MeOH-H$_2$O (5:5.7.3) and placed in the transfer tube of a dccc apparatus filled with the upper layer of the same solvent system as stationary phase. The flow rate of the moving lower phase was 0.66 ml/min. Three fractions were collected on the basis of monitoring by ppc.

Acteoside [1] (0.388 g) was isolated as a pale yellow amorphous powder from the third fraction. The first fraction (0.174 g) was again subjected to dccc to give isoacteoside [2] (0.107 g) as a light brown amorphous powder. Acteoside [1] exhibited the following physical properties: mp 145–149° [lit. (6) mp 147–150°]; solution phase sims m/z [M + H]$^+$ 625 (21%), 480 (10%), 472 (24%), [M + H − caffeoyl-3,4-dihydroxyphenethyl]$^+$ 325 (100%). The identity was confirmed by direct comparison of the ^{1}H- and ^{13}C-nmr spectra with those of an authentic sample. Treatment of acteoside with Ac$_2$O/pyridine followed by Si gel cc gave the peracetate as a colorless amorphous powder: uv (MeOH) λ max 283 (log ε 4.19) nm; the cd spectrum was identical with that already published (12). Isoacteoside [2] exhibited mp 136–139° and solution phase sims m/z 625 [M + H]$^+$ (22%), 480 (29%), 472 (30%), 325 [M + H − caffeoyl-3,4-dihydroxyphenethyl]$^+$ (100%). Acetylation and chromatography of the product gave the peracetate as a colorless amorphous powder: uv (MeOH) λ max 280 (log ε 4.53) nm. Acteoside [1] and isoacteoside [2] both showed activity against the PS cell line (ED$_{50}$ 2.6 and 10 μg/ml, respectively).

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ANTINEOPLASTIC AGENTS, 195. ISOLATION AND STRUCTURE OF ACERATIOSIDE FROM *ACERATIUM MEGALOSPERMUM*[1]

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ABSTRACT.—A new tetralin glucoside [1], named aceratioside, has been found to be a weakly cytostatic (PS ED_{50} 9 μg/ml) constituent in leaves produced by the Australian rain forest tree *Aceratium megalospermum*. Structural determination of aceratioside was primarily accomplished with results from a series of acetylation, methylation, and high field nmr (including heteronuclear multiple bond correlation) experiments.

The largest (200 of 350 species) genus, *Elaeocarpus*, of the subtropical to tropical Elaeocarpaceae (2) contains the *Elaeocarpus* alkaloids (3), a series of indolizidines that have received principal phytochemical attention. Chemical constituents of the genus *Aceratium* (4) in this family have remained essentially unexplored. *Aceratium* species occur primarily in the rain forests of Papuasia (5), and their relative inaccessibility probably accounts for the lack of prior chemical investigation.

Early (1962) in the U.S. National Cancer Institute's (NCI) world-wide evaluation of plants with potential anticancer constituents, extracts of the Queensland tree (to 15 m high) *Aceratium megalospermum* (F. v. M.) von Balgooy (6) began to be evaluated, and in the period 1964–66 they were confirmed as active against the NCI KB cell line (ED_{50} 0.17 μg/ml) and Walker carcinosarcoma (72% tumor reduction at 22 mg/kg). This lead was pursued with a 1979 re-collection of *Ac. megalospermum* leaves and the NCI P-388 lymphocytic leukemia cell line (PS system).

A CH_2Cl_2-MeOH (1:1) extract of the leaves (55 kg) was successively partitioned between MeOH-H_2O (9:1↦3:2) and hexane↦CH_2Cl_2. The PS activity was found concentrated in the CH_2Cl_2 (ED_{50} 0.07 μg/ml) and residual MeOH/H_2O (ED_{50} 0.16 μg/ml) fractions. The more pronounced cell growth inhibitory activity of the former fraction was due to the presence of cucurbitacins; cf. Bittner *et al.* (7). The latter fraction was separated by Si gel chromatography to afford the cytostatic constituent, aceratioside [1], in 5.0×10^{-5}% yield, as a colorless powder (PS ED_{50} 9 μg/ml) from $Me_2CO/CHCl_3$.

The high resolution fab mass spectrum of aceratioside [1] showed a molecular ion at *m/z* 371 $[M + H]^+$ corresponding to the molecular formula $C_{17}H_{22}O_9$ and suggested seven double bond equivalents. Uv and ir spectra indicated the presence of an aromatic ring system bearing a phenolic hydroxyl group (bathochromic shift by addition of NaOMe). The ir spectrum also exhibited absorption bands for hydroxyl groups (3446–3380 cm^{-1}), a carboxyl type carbonyl (1682 cm^{-1}) group, and an aromatic ring. The 400 MHz ^{1}H-nmr spectrum (Table 1) showed a complex pattern, and the proton spin systems required resolution employing 2D ^{1}H,^{1}H COSY (8). The latter 2D data revealed structural segments consisting of meta-coupled aromatic protons, ArCH$_2$CH$_2$CH(X)CH$_2$-Ar (subunit A), and -OCH(O)-CH(O)-CH(O)-CH(O)-CH(O)(CH$_2$O)- (subunit B).

The ^{13}C-nmr spectrum (Table 2) of 1 exhibited signals for seventeen carbons, deduced to be three simple methylenes, an oxymethylene, a shielded methine, five oxymethines, two olefinic methines (one relatively shielded at 102.7 ppm), two olefinic quaternary carbons, two oxygenated quaternary carbons, and a shielded carbonyl car-

[1]For part 194, see Drexler *et al.* (1).
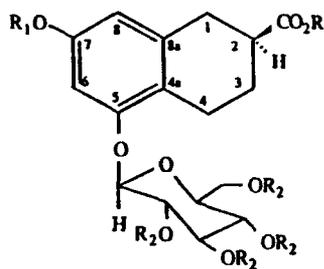
1   R=R$_1$=R$_2$=H
2   R=H, R$_1$=R$_2$=Ac
3   R=R$_1$=CH$_3$, R$_2$=H

4   **5**
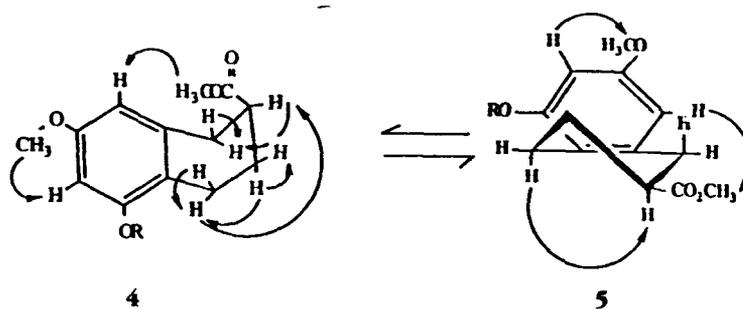
R = glucose

bon. The proton-bearing carbons were correlated to the protons attached to them by interpreting a ^{1}H-^{13}C COSY (9,10) spectrum. For example, the meta-coupled proton appeared at δ 6.95 and was correlated to the carbon at δ 102.7, reminiscent of an aromatic carbon ortho to two oxygen-bearing carbons.

Acetylation of aceratioside yielded a pentaacetate **2**, and methylation (MeI and K$_2$CO$_3$ in refluxing Me$_2$CO) gave the methyl ether **3**. The aromatic methyl ether and methyl ester signals appeared at δ 3.85 and 3.77 and in the ir at 1726 cm^{-1}. These results clearly suggested that subunit A contained a carboxyl group and subunit B a trihydroxypyranose with a hydroxymethyl group. Because the aromatic ring contained only one free hydroxy group, the other aromatic ring bonded oxygen atom must form an ether linkage. These facts suggested a 1,2,3,4-tetrahydronaphthalene-2-carboxylic acid glycoside. Placement of both oxygen atoms in a meta orientation in the aromatic ring was achieved using nOe methods. Irradiation of the isolated proton triplet at δ 3.42 (H-1) in the ^{1}H-nmr spectrum of aceratioside [1] significantly enhanced the signal at δ 6.65 (H-8) and placed oxygens at C-5 and C-7 (meta).

Exact position of the glycoside linkage was resolved on the basis of nOe studies (see **4**) and application of 2D nmr heteronuclear multiple bond connectivity (HMBC) (11–13) methods. With the methylation product **3**, nOe irradiation of methoxy singlets at δ 3.85 and 3.77 ppm enhanced signals for the aromatic protons at δ 6.35 and 6.32 ppm, respectively. The observed nOe between the methyl group of the methyl ester and H-8 was explained by a partial boat conformation **4** for the cyclohexene ring, thereby forcing the methyl ester closer to the aromatic ring. In this conformation H-2 and H-4a appear in a quasi 1,3 diaxial relationship or even closer if the molecule resides in a semi chair conformation [**5**]. One likely explanation of the nOe results depends on the two semi boat/chair conformations **4** and **5** existing in equilibrium.

TABLE 1. ^{1}H-nmr Assignments for Aceratioside [1], Aceratioside Pentaacetate [2] and the Methyl Ether 3.

Proton	Compound		
	1 (in $C_5D_5N + D_2O$)	2 (in $CDCl_3$)	3 (in $CDCl_3$)
H-1a	2.44, dt, 5.4, 12.6	2.30, m	2.39, dt, 4.4, 13
H-1b	3.42, dt, 5.4, 12.6	2.88, dt, 3.5, 10.2	3.02, dt, 4.6, 13
H-2	1.80, m	1.69, m	1.78, m
H-3a	1.75, m	1.78, m	1.87, m
H-3b	1.88, m	1.79, m	1.87, m
H-4a	2.29, dd, 2.3, 14.2	2.44, m	2.27, m
H-4b	2.54, dd, 5.3, 14.6	2.52, m	2.53, td, 4.2, 16
H-6	6.95, d, 2.8	6.78, d, 2.7	6.35, d, 2.8
H-8	6.65, d, 2.8	6.73, d, 2.7	6.32, d, 2.8
H-1'	4.81, d, 8	5.05, d, 6.8	4.30, d, 7.7
H-2'	4.20, dd, 8, 9	5.24, dd, 6.8, 8.6	3.63, t, 8.5
H-3'	4.14, dd, 9, 9	5.18, t, 8.6	3.53, t, 8.6
H-4'	3.84, dd, 9, 9	5.29, t, 10	3.51, t, 8.8
H-5'	4.10, ddd, 3, 9, 11	3.73, dt, 3.5, 10	3.65, m
H-6'a	4.77, dd, 11, 11	3.98, dd, 3.5, 12	4.49, dd, 12, 4
H-6'b	5.00, dd, 3, 11	4.40, dd, 3.5, 12	4.50, dd, 12, 12
Ac	—	2.02, 2.04, 2.07, 2.24, 2.29	—
OMe	—	—	3.77, 3.85
OH	—	—	2.40, 2.67, 4.84

The HMBC technique was used to assign (Table 2) all the carbon resonances. Strong and multiple correlation peaks were observed for all the protons two or three bonds away from carbon atoms. The H-1' (δ 4.81) proton was correlated to aromatic ring C-4a (δ 138.8), C-3' (74.6), and C-5' (δ 78.0). Relationship of the carboxyl group (δ 173.3) to H-3 (δ 1.75, 1.88 ppm), H-2 (δ 1.80), and H-4 (δ 2.29, 2.54 ppm) was also established. Proton H-6 (δ 6.95) was related to C-4a, -5, -7, and -8, and H-8 with C-1, -4a, -6, and -7. For other connectivities refer to Table 2. Interestingly, whenever a proton was arranged in a W-type spatial relationship with a carbon, a four-bond-apart HMBC correlation was observed. The HMBC experiments allowed placement of the glycoside at C-5 and confirmed the substitution pattern of the aromatic ring and carboxylic acid at position C-2. The glycoside linkage was assigned β on the basis of the anomeric proton coupling constants for the doublet H-1' in aceratioside [1] ($J = 8$ Hz), pentaacetate 2 ($J = 6.8$ Hz), and methyl ester 3 ($J = 7.7$ Hz). Acid hydrolysis of aceratioside led to the isolation of glucose and assignment of structure 1 to aceratioside.

The cd spectrum of methyl ester 3 in MeOH exhibited two relatively weak negative Cotton effect curves at λ max 230 and 270 nm due to the phenethyl chromophore (14). On the basis of direct comparison of the cd spectrum with that of the 1,2,3,4-tetrahydronaphthalene-(2S and 2R)-carboxylic acids (15), the absolute configuration of aceratioside [1] at C-2 was assigned S. [Cd spectra of 1,2,3,4-tetrahydronaphthalene-(2S and 2R)-carboxylic acids in MeOH are: 2S [α]D $-55°$; ϵ (nm) 0 (280), -1.2 (270), 0 (250), 0 (225), -33.3 (210); 2R: [α]D $+28.5°$; ϵ (nm) 0 (280), $+0.7$ (270), 0 (250), $+34.4$ (210).]

Certain tetralins have shown a variety of biological activities. For example, 6-substituted 1,2,3,4-tetrahydro-1-naphthoic acids have exhibited moderate anti-inflammatory activity (16) in mice. Other 1,2,3,4-tetrahydro-1-naphthoic acids have shown plant-growth-regulating activities (17), and 5,7-dihydroxy-2-aminotetralins have

TABLE 2. ^{13}C-nmr Assignments for Aceratioside [1] and Aceratioside Pentaacetate [2] and HMBC Correlations for Glucoside 1.

Carbon	Compound		
	1 (in C₅D₅N)	2 (in CDCl₃)	HMBC of 1 (in C₅D₅N)
C-1	31.2	29.1	H-1⟶C-2, -3, -4a, -8, -8a
C-2	31.5	28.7	H-2⟶C-3
C-3	25.8	25.6	H-3⟶C-1, -4, -9
C-4	34.8	34.1	H-4⟶C-2, -3, -9
C-4a	138.8	138.6	
C-5	152.4	146.8	
C-6	102.7	114.7	H-6⟶C-4a, -5, -7, -8
C-7	156.7	143.1	
C-8	107.8	120.5	H-8⟶C-1, -4a, -6, -7
C-8a	138.0	142.9	
C-9	173.3	173.5	
C-1'	108.4	100.5	H-1'⟶C-4a, -3', -5'
C-2'	75.4	72.2	H-2'⟶C-1', -5'
C-3'	74.6	71.0	H-3'⟶C-1', -2', -4'
C-4'	73.7	68.7	H-4⟶C-3', -5', -6'
C-5'	78.0	73.1	H-5'⟶C-6', -2', -4'
C-6'	64.7	60.6	H-6'⟶C-5'
Ac	—	20.6(×3), 20.8	
		21.1, 168.2	
		169.0, 169.2	
		169.4, 170.3	

exhibited dopaminergic and adrenergic action in dogs (18). Further biological evaluation of aceratioside is under way.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analtech Si gel GF (0.25 mm) plates were used for tlc and developed with either 3% ceric sulfate in 3 N H₂SO₄ spray and/or iodine vapor. Stationary phases used for gravity or flash cc were E. Merck (Darmstadt) Si gel (70–230 mesh for gravity and 40–63 mesh for flash), Whatman Si gel LPS-1 (13–24 mesh), or Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Melting points were observed with a Kofler-type hot stage apparatus. Optical rotation values were recorded using a Perkin-Elmer 241 polarimeter. The uv spectra were obtained in MeOH solution with a Hewlett-Packard 8450A UV/vis spectrophotometer. A Nicolett MX-1 FT spectrophotometer was employed for ir measurements. TMS, residual CHCl₃ (7.256 ppm), or CH₂Cl₂ (5.32 ppm) was used as an internal reference in all nmr experiments, which were determined with Bruker AM 400 (¹H, ¹³C), or WH 90 (¹H) instruments. Chemical shifts are recorded in ppm. CDCl₃ was used as nmr solvent unless otherwise mentioned. The hreims and sp-sims (fabms) were recorded with a Kratos MS 50 instrument in the NSF regional mass spectrometry facility at the University of Nebraska.

PLANT MATERIAL.—The leaves of *Ac. megalospermum*, earlier (1875) classified as *Aristotelia megalosperma*, were collected in the rain forests of Queensland, Australia in 1979 as part of the NCI-USDA programs directed by Drs. John L. Hartwell, Matthew Suffness, and J. Duke (USDA). The plant was assigned NCI B631963, and a herbarium specimen is maintained by the USDA, Beltsville, Maryland.

ISOLATION OF ACERATIOSIDE [1] (7-HYDROXY-1,2,3,4-TETRAHYDRONAPHTHALENE-2-CARBOXYLIC ACID-5-β-D-GLUCOSIDE].—Dried leaves (50 kg) of *Ac. megalospermum* were extracted at ambient temperature with 400 liters of CH₂Cl₂-MeOH (1:1), and the extract was converted to a CH₂Cl₂ fraction (2.07 kg, PS ED₅₀ 0.3 μg/ml) by addition of H₂O (100 liters). The CH₂Cl₂ fraction (1.55 kg) was dissolved in 8 liters of MeOH-H₂O (9:1) and extracted with hexane (4 × 4 liters). The MeOH-H₂O phase was adjusted to 3:2 by addition of H₂O and extracted with CH₂Cl₂ (3 × 4 liters) to give CH₂Cl₂ (358 g, PS

ED$_{50}$ 0.07 µg/ml) and residual aqueous fractions (93 g, PS ED$_{50}$ 0.16 µg/ml, PS T/C 121 at 25 mg/kg). An 11.8-g aliquot of the latter fraction was chromatographed on a column of Si gel employing gradient elution with CH$_2$Cl$_2$-MeOH (49:1↦17:3) to give an active fraction that crystallized from Me$_2$CO/CHCl$_3$ to furnish aceratioside [1] (0.32 g, 0.005% yield) as a buff colored powder: mp 273–275°, R_f 0.35 [Si gel, CH$_2$Cl-MeOH (9:1)]; [α]D + 10° (c=1.2, MeOH); hr fabms m/z [M + H]$^+$ 371.1366 (100%) for C$_{17}$H$_{23}$O$_9$ (calcd 371.1341); eims m/z [M]$^+$ 370 (8%), [M − H$_2$O]$^+$ 352 (5%), [M − 2H$_2$O]$^+$ 334 (10), [M − glucose + H]$^+$ 208 (73), [M − glucose − CO]$^+$ 180 (100), [M − aglycone]$^+$ 163 (24), [M − glucose − 2 × CO]$^+$ 152 (30); uv (MeOH) λ max 226 (ε 5124), 281 (2032); uv (MeOH + NaOCH$_3$) λ max 230 (ε 5442), 235 (5255), 290 (3097); ir (KBr) ν max 3446, 3380, 1682, 1607, 1492, 1343, 1165, 1147, 1084, 1071, 1054, 1037 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2. Anal. calcd for C$_{17}$H$_{22}$O$_9$·1.5 H$_2$O: C 51.38, H 6.09; found C 50.88, H 5.83.

ACERATIOSIDE PENTAACETATE [2].—A solution of glycoside 1 (50 mg) in pyridine/Ac$_2$O (1 ml each) was acetylated at room temperature (overnight). After addition of EtOH, the solvent was evaporated at reduced pressure. The pentaacetate (70 mg) crystallized from EtOH as an amorphous powder: mp 189–191°; R_f 0.2 or 0.72 [Si gel, hexane-EtOAc (9:5 or 1:1)]; [α]D − 10° (c = 1.2, MeOH); hreims m/z [M]$^+$ 580.1886 (2%) (calcd for C$_{27}$H$_{32}$O$_{14}$, 580.1792), [M − 2 × Ac − OHAc]$^+$ 436.1356 (6%) (calcd for C$_{21}$H$_{24}$O$_{10}$, 436.1370), [C$_{21}$H$_{22}$O$_9$]$^+$ 418.1281 (14), [M − 2 × OHAc − 2 × Ac]$^+$ 376.1144 (16), [C$_{17}$H$_{18}$O$_7$]$^+$ 334.1046 (7), [C$_{15}$H$_{16}$O$_6$]$^+$ 292.0948 (20), [C$_{13}$H$_{14}$O$_5$]$^+$ 250.0833 (80), [C$_{11}$H$_{12}$O$_4$]$^+$ 208.0739 (100), [C$_{10}$H$_{12}$O$_3$]$^+$ 180.0789 (61), [C$_8$H$_8$O$_3$]$^+$ 152.0474 (34); ir (NaCl) 1757, 1476, 1370, 1214, 1199, 1069, 1035 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

METHYLATION OF ACERATIOSIDE.—To a solution of aceratioside [1] (11.3 mg) in anhydrous Me$_2$CO (3 ml) was added anhydrous K$_2$CO$_3$ (50 mg) and MeI (1 ml). The mixture was heated at reflux for 3 h. The K$_2$CO$_3$ was removed by filtering the solution, and the filtrate was purified by chromatographic separation on a small Si gel column. Elution with EtOAc-hexane-MeOH (9:1:1) provided methyl ester 3 (8 mg) as an amorphous powder: mp 208–210°, R_f 0.32 [Si gel, hexane-CH$_2$Cl$_2$-MeOH-H$_2$O (10:80:10:1)], hreims m/z [M]$^+$ 398.1575 (3%) (calcd for C$_{19}$H$_{26}$O$_9$, 398.1577), [C$_{13}$H$_{16}$O$_4$]$^+$ 236.1047 (21); cd (MeOH) ε (nm) 0 (283), − 1.0 (270), 0 (256), 0 (240), − 7.6 (230), 0 (220); ir (NaCl) 3500, 1726, 1598, 1492, 1450, 1350, 1210, 1095, 1075, 1055, 1040 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

HYDROLYSIS OF ACERATIOSIDE.—The glucoside 1 (14.3 mg) was heated in a refluxing solution of 1 N HCl-MeOH (1:1) (5 ml) for 24 h. EtOAc (15 ml) was added, and the EtOAc phase was washed with H$_2$O (2 × 5 ml) and dried (anhydrous Na$_2$SO$_4$). Solvent was removed from both phases. The EtOAc extract was found to be a complex mixture, whereas the aqueous phase yielded glucose [identified by direct comparison with an authentic specimen using tlc on a cellulose plate with pyridine-EtOAc-HOAc-H$_2$O (5:5:1:3) as mobile phase and by ^{1}H-nmr comparison]. The pentaacetate derivative was also found to be identical with an authentic sample of glucose pentaacetate by the same procedures.

ACKNOWLEDGMENTS

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Antineoplastic agents. 168. Isolation and structure of axinohydantoin[1]

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Received July 4, 1989[5]

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Western (Palau) and Eastern (State of Truk) Caroline Islands and Papua New Guinea sponges of the genera *Axinella* and
Hymeniacidon were found to contain the cytostatic (PS ED_{50} 2.5 and 2.0 μg/mL) and antineoplastic (PS T/C 143 at 3.6 mg/kg
and T/C 138 at 3.6 mg/kg) pyrrologuanidines **1a** and **1b**. The related hydantoin **2**, designated axinohydantoin, was also isolated
from an *Axinella* sp. and its structure was assigned by X-ray crystallographic techniques. Present experience with sponges in the
Axinella and *Hymeniacidon* genera suggests that the previously known hymenialdisine (**1b**) and analogous imidazole derivatives
may be widely distributed among these and related orange colored Porifera.

Key words: axinohydantoin, hymenialdisine, *Axinella*, *Hymeniacidon*, cystostatic.

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On a trouvé que les éponges du genera *Axinella* et du *Hymeniacidon* des îles Caroline occidentale (Palau) et orientale (État
de Truk) ainsi que de la Nouvelle Guinée contiennent des pyrrologuanidines **1a** et **1b** qui sont des cytostatiques (PS ED_{50} 2,5 et
2,0 μg/mL) et des antinéoplasiques (PS T/C 143 à 3,6 mg/kg et T/C 138 à 3,6 mg/kg). À partir d'un *Axinella* sp., on a aussi
isolé l'hydantoïne apparentée **2**, appelée axinohydantoïne, et on a déterminé sa structure à l'aide de la diffraction des rayons-X.
L'expérience acquise avec les éponges de l'*Axinella* et de l'*Hymeniacidon* genera suggère que l'hyménialdisine (**1b**), qui était
connue antérieurement, ainsi que les dérivés imidazoles analogues sont peut-être très répandus dans ces éponges et dans les
Porifera apparentés de couleur orange.

*Mots clés* : axinohydantoïne, hyménialdisine, *Axinella*, *Hymeniacidon*, cytostatique.

[Traduit par la revue]

Introduction

Early (2) in our evaluation of marine animals as new sources
of potentially useful anticancer drugs, good leads were uncov-
ered among the Porifera, and this initial (1966–1968) promise is
now being amply realized (3, 4). In 1979 in Palau we collected a
Hymeniacidon species (at −40 m) and an *Axinella* sp. that
provided extracts with confirmed levels of activity against the
U.S. National Cancer Institute's (NCI) murine P388 lympho-
cytic leukemia (PS system). Other PS active sponge collections
were completed in 1981 (Papua New Guinea) and 1985 (Truk,
Federated States of Micronesia) that included *Axinella carteri*
(Dendy) and a *Hymeniacidon* species.

Initial extracts of each sponge were found to provide a
confirmed level of activity against the PS system. By means of
PS (*in vitro*) bioassay guided separation procedures, these
sponge species led to two cytostatic and antineoplastic alkaloids
(**1a,b**) accompanied in the case of *Axinella* sp. by a closely
related, but marginally (PS ED_{50} 18 μg/mL) inactive, com-
ponent (**2**). The PS active marine alkaloids proved to be
identical[6] with the known (5–7) hymenialdisine (**1b**, ref. 6. PS

ED_{50} 2.0 μg/mL and T/C 138 at 3.6 mg/kg)[7] and its debromo
derivative **1a** (refs. 5–7, PS ED_{50} 2.5 μg/mL and T/C 143 at
3.6 mg/kg).
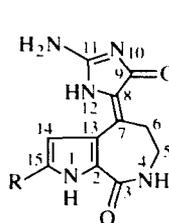
1a, R = H debromohymenialdisine

1b, R = Br hymenialdisine
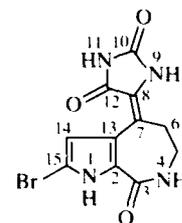
2

axinohydantoin

The unequivocal X-ray crystal structure of hymenialdisine
was nicely established by Cimino *et al.* (5) and reconfirmed in
the following year by the Kitagawa group (6). In turn, these
advances simplified characterization of the companion sub-
stance from *Axinella* sp., herein named axinohydantoin (**2**), as a
closely related compound. But establishing the exact geometri-
cal configuration for its hydantoin–lactam sp^2 bond required the
following crystal structure determination.

Axinohydantoin (**2**) crystallized from methanol as yellow
prisms, which corresponded to $C_{11}H_9BrN_4O_3$ (by hreims) and
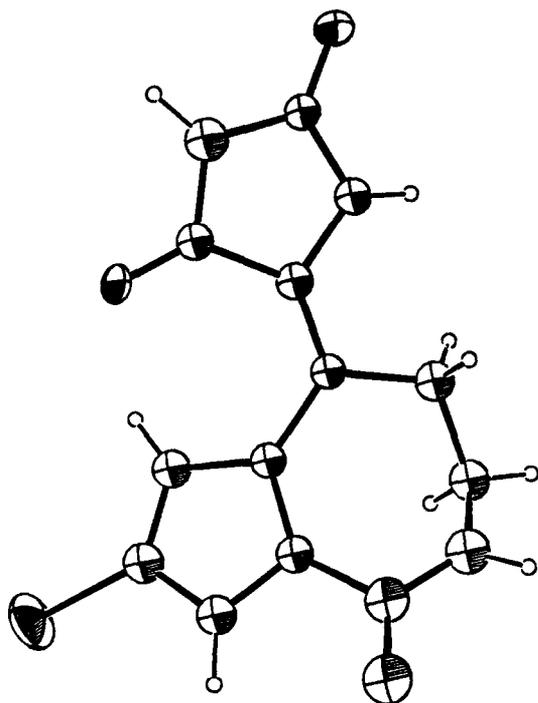with one mole of methanol. The structure was solved using

FIG. 1. ORTEP view of a single molecule of **2**, with 50% thermal ellipsoids.

MULTAN (8) and refined to $R = 0.054$ using anisotropic temperature factors for Br and oxygens other than O-3, and isotropic temperature factors for the other non-hydrogens and for hydrogens (unrefined) in calculated positions. HN-1, HN-11, and HN-9 were calculated to be 0.95 Å along a line to the respective oxygen. These positions differed very little from those calculated assuming bonding to trigonal atoms. HN-4 has been shown at the calculated position assuming a trigonal N-4 that is too far (2.4 Å) from the closest O-10 for significant hydrogen bonding; it may actually bend somewhat toward this O-10.

The structure deduced for axinohydantoin (**2**, Fig. 1) was found to be closely related to that of hymenialdisine (**1b**), with reversal of configuration at the C7—C8 double bond being the most interesting difference. In turn, this suggested that axinohydantoin was not simply a hydrolysis product of guanidine **1b**. The most prominent bond length difference between the two structures occurs at C10—O10, with 1.23 Å in hydantoin **2** compared to 1.33 Å for C11—N11 in **1b**. No significant differences in bond angles were observed. An angle of 36° was observed between the least-squares planes of the two nearly planar five-membered rings in hydantoin **2**, compared to 43.8° in guanidine **1b**. In both cases, the seven-membered ring has adopted a boat conformation with C-5 at the prow, and similar torsion angles except for C2—C3—N4—C5 expanding from −10.5° in **1b** to −15° in **2**, and C2—C13—C7—C6 contracting from 41.1° in **1b** to 31° in **2**. The twist angle C13—C7—C8—C12 about the carbon–carbon double bond increases from 0.5° in **1b** to 10° in **2**, presumably to relieve the steric interaction between O-12 and HC-14.

The arrangement of intermolecular hydrogen bonds govern-

ing the packing in hydantoin **2** (see supplementary material)[8] was found to be completely different than that in guanidine **1b** (6). The hydantoin ring in each molecule was found linked to the hydantoin rings of two other molecules via base-pairing interactions across centers of symmetry. The pyrrole NH proved to be hydrogen bonded to the methanol solvate oxygen and in turn to O-3. Only a few substances (9–12) with a hydantoin system have been isolated from sponges, and one of these, midpacamide, found by Scheuer and co-workers (9) in an unidentified Marshall Island sponge, may be biogenetically related to axinohydantoin. From evidence now in hand, pyrroles **1** and **2** and related substances may prove to be ubiquitous Porifera biosynthetic products.

Experimental

General methods

Marine sponge taxonomic identification was performed in the Smithsonian Institution where voucher specimens are deposited in the collections of the Department of Invertebrate Zoology, National Museum of Natural History. All solvents employed were redistilled. Size exclusion chromatography was accomplished with Sephadex LH-20 (particle size: 25–100 μm) supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Thin-layer chromatography was carried out with silica gel GHLF Uniplates (Analtech Inc.) and with RP-8 precoated plates (layer thickness: 0.25 mm) from E. Merck, Darmstadt, Germany. High-speed countercurrent chromatography was accomplished with an Ito multilayer coil extractor–separator (P.C. Inc., Potomac, MD) using 2.6 mm i.d. tubing, and an FMI Lab Pump.

Melting points are uncorrected and were determined on a Kofler-type hot-stage apparatus. Ultraviolet spectra were recorded employing a Hewlett–Packard model 8450 uv/vis spectrophotometer and ir spectra with a Nicolet ft-ir model MX-1 instrument. The nmr spectra were measured in DMSO-d_6 using a Bruker AM-400 instrument and are recorded in ppm downfield to TMS (assignments bearing the same superscript may be reversed). The ^{13}C nmr multiplicities were determined with APT experiments based on an average coupling constant of 135 Hz. The eims spectra were recorded with a Kratos AEI 5076 spectrometer at the NSF Regional Facility, University of Nebraska, Lincoln, Nebraska.

Palau Porifera (Axinella *sp. and* Hymeniacidon *sp.) collection and extraction*.

The initial collection of *Axinella* sp. (Demospongiae class, Axinellida order, Axinellidae family) in Palau, Western Caroline Islands, was conducted in May, 1979. The sponge displayed a brownish-yellow exterior of irregular mass. A 2-propanol–CHCl₃ extract gave confirmatory in vivo activity with PS T/C 201 at 100 mg/kg, PS ED₅₀ = 2.5 μg/mL. A scale-up re-collection (220 kg wet wt.) of this sponge was completed in March, 1985, and preserved in 2-propanol. The preserving solution was separated from the sponge, concentrated to an aqueous slurry, and extracted with CH₂Cl₂ (13). The remaining sponge material was re-extracted with 2-propanol–CH₂Cl₂ (1:1); the extract was separated, solvent removed, and the residue partitioned between CH₂Cl₂ and H₂O. At this early stage a solid precipitate appeared at the CH₂Cl₂–H₂O interface. The precipitate was separated and amounted to

1.8 kg (PS T/C 227 at 294 mg/kg, ED$_{50}$ 2.8 µg/mL). Analogous solid fractions were also obtained at this initial CH$_2$Cl$_2$ step during separations of the sponge extracts summarized below.

The orange sponge *Hymeniacidon* sp. (Demospongiae class, Halichondrida order, Hymeniacidonidae family) was collected (1979), re-collected (in 1985, 218 kg wet wt.), and extracted (2-propanol extract showed PS T/C 130 at 5.5 mg/kg and ED$_{50}$ 27 µg/mL) as summarized above. Removal of solvent from the initial 2-propanol extract led to an aqueous concentrate that contained 1.2 kg of a solid fraction with PS ED$_{50}$ = 3.7 µg/mL.

Isolation of hymenialdisine (1b) and axinohydantoin (2)

A 10 g aliquot from the 1.8 kg of solid precipitate noted above was dissolved in CH$_3$OH (400 mL) and separated by size exclusion chromatography on a column of Sephadex LH-20 (100 × 10 cm) to yield two major marine alkaloid fractions. When fraction 1 (elution volume: 12.0–12.6 L) was allowed to stand for 24 h at room temperature, axinohydantoin (2) slowly crystallized as yellow needles (30 mg): mp >350°C; tlc on silica gel R_f = 0.83, 1-BuOH–AcOH 50% (95:5); tlc on RP-8 R_f = 0.51, CH$_3$OH–AcOH 5% (1:1); uv (CH$_3$OH) λ_{max}: 264sh (log ε = 3.88), 345 (log ε = 4.16) nm; ir (KBr) ν_{max}: 1740, 1702, 1638, 1480, 1425, 1407 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ: 2.67 (2H, m, H-6), 3.22 (2H, m, H-5), 6.66 (1H, s, H-14), 7.89 (1H, t, HN-4), 9.83, 10.91 (2 × 1H, s, HN-9, HN-11), 12.35 (1H, s, HN-1); ^{13}C nmr (DMSO-d_6) δ: 36.2 (t, C-6), 38.5 (t, C-5), 101.6 (s, C-15), 113.9 (d, C-14), 120.0 (s, C-13), 121.2 (s, C-7), 125.5 (s, C-2), 126.5 (s, C-8), 153.8 (s, C-10), 162.7 (s, C-3), 163.3 (s, C-12); hreims *m/z*: 325.9842 and 323.9834 (C$_{11}$H$_9$N$_4$O$_3$Br requires 325.9836).

Fraction 2 (elution volume: 12.9–13.5 L) yielded a crystalline precipitate (100 mg), which was identified as hymenialdisine (1b) by comparison (uv, ir, ^{1}H nmr, eims) with an authentic sample (6).

Truk Porifera (Axinella carteri) collection and extraction

In May 1985, approximately 1 kg of an orange-yellow sponge subsequently identified as *Axinella carteri* (Dendy), was collected in the Truk Lagoon, Federated States of Micronesia, at −13 to −24 m. The preserving solution (2-propanol) was removed and this extract proved toxic down to 50 mg/kg against the PS leukemia. The 2-propanol extract was partitioned between CH$_2$Cl$_2$ and H$_2$O and the resulting CH$_2$Cl$_2$ extract was successively partitioned (13) between 9:1–4:1–1:1 MeOH:H$_2$O with hexane–CCl$_4$–CH$_2$Cl$_2$. The final CH$_2$Cl$_2$ extract showed PS T/C 135 at 100 mg/kg and PS cell line ED$_{50}$ = 1.2 µg/mL.

In October 1985, approximately 148 kg (wet wt.) of the sponge was recollected and preserved in MeOH. The MeOH solution was decanted, and the sponge was ground and extracted with MeOH:CH$_2$Cl$_2$ (1:1). The original MeOH solution was concentrated to an aqueous phase and extracted with CH$_2$Cl$_2$ (3×) followed by 1-BuOH. Study of this 1-BuOH fraction was discontinued when PS results showed minimal activity.

When the ambient temperature extraction of the sponge with MeOH:CH$_2$Cl$_2$ was completed, the aqueous MeOH phase was separated and concentrated to an aqueous phase, which was extracted with 1-BuOH (15 L). The 1-BuOH phase was concentrated, redissolved in MeOH (1.5 L), and dried to give a 232 g fraction (PS ED$_{50}$ 1.4 µg/mL). A 97 g aliquot of the MeOH soluble fraction was treated with 1-BuOH (800 mL, 50°C, 12 h) and the relatively insoluble part (50 g, PS ED$_{50}$ 1.5 µg/mL) was collected. The MeOH (600 mL) sparingly soluble portion weighed 4.26 g (PS ED$_{50}$ 0.11 µg/mL).

Papua New Guinea Porifera (Hymeniacidon sp.) collection and extraction

The collection (May 1981, near Motapure Island, Papua New Guinea) and recollection (October 1983, 44 kg wet wt.) of an orange *Hymeniacidon* sp. as well as the large scale extraction (crude extract PS T/C 136 at 100 mg/kg and ED$_{50}$ 24 µg/mL) and solvent partitioning were performed as described above for *A. carteri*. In this case, when the 934 g initial CH$_2$Cl$_2$ fraction was subjected to further separation by MeOH:H$_2$O with the hexane → CCl$_4$ → CH$_2$Cl$_2$ sequence, a total

TABLE 1. Positional and thermal parameters

Atom	x	y	z	B (Å^2)
Br	0.56111(6)	0.0097(2)	0.18172(7)	4.40(3)
O3	0.7915(4)	−0.210(1)	0.0865(4)	3.4(2)*
O12	0.7371(3)	−0.194(1)	0.4049(4)	3.0(2)
O10	0.9511(3)	−0.093(1)	0.5586(3)	3.3(2)
OM	0.6407(4)	−0.152(2)	0.0121(5)	7.3(3)
N1	0.6929(4)	−0.069(1)	0.1562(4)	2.3(2)*
N4	0.8760(4)	−0.055(1)	0.1624(5)	2.9(2)*
N11	0.8375(4)	−0.161(1)	0.4951(4)	2.5(2)*
N9	0.9106(4)	−0.056(1)	0.4357(4)	2.0(2)*
C15	0.6581(5)	−0.024(2)	0.2056(5)	2.5(2)*
C14	0.7046(4)	−0.000(2)	0.2706(5)	2.2(2)*
C13	0.7725(4)	−0.036(1)	0.2599(5)	1.8(2)*
C2	0.7630(5)	−0.073(1)	0.1875(5)	2.0(2)*
C3	0.8108(5)	−0.120(2)	0.1407(6)	2.8(2)*
C5	0.8961(5)	0.083(2)	0.2145(5)	2.6(2)*
C6	0.9053(4)	0.024(2)	0.2915(5)	2.4(2)*
C7	0.8393(4)	−0.031(1)	0.3144(5)	1.8(2)*
C8	0.8468(5)	−0.069(1)	0.3839(5)	1.9(2)*
C12	0.7982(5)	−0.147(1)	0.4248(5)	2.0(2)*
C10	0.9045(5)	−0.104(2)	0.5026(5)	1.9(2)*
CM	0.5809(8)	−0.093(2)	−0.0359(8)	6.3(4)*

*Starred atoms were refined isotropically.
Anisotropically refined atoms are given in the form of the isotropic equivalent thermal parameter, defined as $8\pi^2(U_{11} + U_{22} + U_{33})/3$.

of 135 g (PS ED$_{50}$ 14 µg/mL) of a solid interfacial fraction was collected and used to isolate hymenialdisines 1a and 1b.

Isolation of hymenialdisines 1a and 1b—Procedure A

An aliquot (250 mL) of the preceding *Axinella carteri* MeOH (600 mL) solution was applied to a column of Sephadex LH-20 (1.9 kg in MeOH). A total of 460 fractions of 20 mL each were collected and a fraction weighing 0.73 g (PS ED$_{50}$ 2.2 µg/mL) was further separated using high speed countercurrent distribution with an Ito coil. A 50 mg aliquot was applied (6 mL) in 1-BuOH:HOAc:H$_2$O (4:1:5) to the coil with the 1-BuOH phase as stationary (upper) and the aqueous part as mobile (lower) phase. Fractions (120) of 6.5 mL each were collected; fractionation was monitored with ultraviolet detection (254 nm). The fractions were neutralized (pH 7) with aqueous NaOH and refrigerated. Debromohymenialdisine 1a, 9 mg, PS ED$_{50}$ = 3.0 µg/mL, crystallized from fractions 28–33 and was identical (tlc, ms, nmr) with an authentic sample (6).

The MeOH less soluble fraction (4.26 g) described above was extracted with MeOH (5 × 25 mL) at 40°C and the solution filtered to give 3.73 g of residue. A 0.90 g portion was triturated with DMSO (10 mL). The soluble portion (0.25 g) was chromatographed on a column of Sephadex LH-20 in MeOH to provide 0.13 g of hymenialdisine (1b) as yellow crystals (PS ED$_{50}$ 0.62 µg/mL), identical (tlc and ms comparisons) with an authentic sample (6).

Procedure B

The 1.2 kg fraction (see above) from the Palau *Hymeniacidon* sp. was further separated by successive Soxhlet extraction (20 g aliquot) with CH$_2$Cl$_2$ (6 × 5 L), EtOH (6 × 5 L), and 1-BuOH (6 × 5 L) to give respectively 35 g (PS ED$_{50}$ 26 µg/mL), 500 g (PS ED$_{50}$ 8.6 µg/mL), and 106 g (PS ED$_{50}$ 2.6 µg/mL) fractions. A 10 g sample of the 1-BuOH fraction in MeOH was subjected to chromatography on a column of Sephadex LH-20 (500 g) to give 26 individual (by tlc comparisons) fractions using 4:1 CH$_2$Cl$_2$:MeOH. Of these, 56 mg proved to be largely debromohymenialdisine 1a (PS ED$_{50}$ 1.4 µg/mL) and hymenialdisine (5.5 mg, 1b, PS ED$_{50}$ 7.5 µg/mL) by comparison nmr and tlc.

Procedure C

The 135 g fraction from the Papua New Guinea *Hymeniacidon* sp.

was extracted (Soxhlet procedure with two stainless steel 1-gallon extractors) with EtOH to yield a 22 g alcohol soluble fraction. Treatment of this fraction with $CH_2Cl_2:CH_3OH$ (1:1) yielded a precipitate (4.3 g, PS ED_{50} 8.5 μg/mL), which was extracted with hot 1-BuOH. The 1.5 g 1-BuOH soluble fraction was preabsorbed onto silica gel and separated by chromatography on a column (3 × 62 cm) of silica gel (180 g). Gradient elution with 95:5 $CH_2Cl_2:CH_3OH$ with increments of MeOH provided fractions that yielded (0.17 g and 0.06 g respectively) debromohymenialdisine (1a, PS T/C 143 at 3.6 mg/kg and ED_{50} 2.5 μg/mL) and hymenialdisine (1b, PS T/C 138 at 3.6 mg/kg and ED_{50} 2.7 μg/mL). Both 1a and 1b were identified by direct comparison with authentic samples (6) employing tlc, ^{13}C and ^1H nmr, ms, uv, and ir spectral data.

X-ray structure of axinohydantoin (2)

$C_{11}H_8BrN_4O_3 \cdot CH_3OH$ fw = 357.17

Monoclinic, $C2/c$, a = 19.558(2), b = 7.505(1), c = 19.092(3) Å, β = 103.78(1)°, V = 2754.3 Å^3, Z = 8, ρ_c = 1.72; Nicolet P2$_1$ diffractometer, crystal 0.17 × 0.13 × 0.08 mm, 23°C, MoK$_{\alpha_1}$, λ = 0.71073 Å, 2θ/θ scans, 2θ$_{max}$ 50°; 836 of 2446 reflections with $F_o{}^2$ > 3σ ($F_o{}^2$) used; solved by direct methods; R = 0.053, R_w = 0.055 excluding unobserved reflections. Coordinates and isotropic temperature factors of non-hydrogens are given in Table 1.[8]

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ISOLATION AND STRUCTURE OF BRYOSTATINS 14 AND 15[1]

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SUMMARY: Further investigation of constituents from the marine bryozoan *Bugula neritina* employing new 1,000 Kg recollections from the Gulf of Mexico and Eastern Pacific Ocean (California) has led to isolation and structural determination of two previously undetected members of the bryostatin (1-13) series, bryostatins 14 (14) and 15 (15). Structural analyses were conducted primarily with high field (400 MHz)ʳ NMR and high resolution mass spectral techniques. Both new bryostatins significantly inhibited growth of the P388 lymphocytic leukemia.

Discovery[2] of bryostatins 1-13 (cf. 1-13) has made available a new class of important biochemical probes[3] with considerable clinical potential.[4] For example, with fresh samples of human myeloid leukemia, bryostatin 1 generally caused differentiation responses leading to macrophage-like morphology.[4a] Again, with peripheral blood cells from β-chronic lymphocytic leukemia patients, this substance triggered activation and differentiation[4b] and
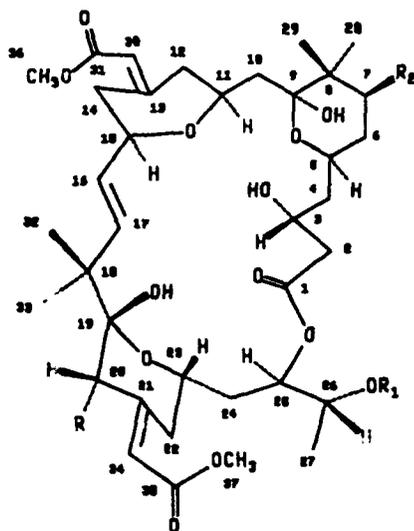
STRUCTURE	R	R_1	R_2	
1	B	H	A	Bryostatin 1
2	B	H	OH	Bryostatin 2
4	D	H	C	Bryostatin 4
5	A	H	C	Bryostatin 5
6	A	H	D	Bryostatin 6
7	A	H	A	Bryostatin 7
8	D	H	D	Bryostatin 8
9	D	H	A	Bryostatin 9
10	H	H	C	Bryostatin 10
11	H	H	A	Bryostatin 11
12	B	H	D	Bryostatin 12
13	H	H	D	Bryostatin 13
14	OH	H	C	Bryostatin 14
14a	A	A	C	
15	E	H	A	Bryostatin 15

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is undergoing clinical evaluation. In order to meet potential clinical supply requirements for the bryostatins, it became necessary to increase (to 1,000 kg, damp wt.) the size of *Bugula neritina* recollections from the Gulf of Mexico[2f] (Florida) and further explore such challenging quantities of biomass from the Eastern Pacific Ocean (California).[21] We now report the isolation and structural elucidation of bryostatin 14 (14) from the Gulf of Mexico specimens in 1.02×10^{-5}% yield and bryostatin 15 (15) in 8.6×10^{-7}% yield from the Pacific Ocean collection of this remarkable bryozoan.

Bryostatins 4-8 (4-8) and 10 (10) were again[2f] isolated from the Gulf of Mexico recollection (1986) and this allowed application of contemporary NMR techniques (HMBC and NOE) to make some refinements in position assignments (Table 1). In turn, these two-dimensional NMR correlations aided the characterization of bryostatin 14 (14), separated from the previously known bryostatins by chromatography.

Bryostatin 14 (14, P388 ED_{50} 0.33 μg/mL) exhibited a FAB mass spectral base peak at m/z 831 $[M+Li]^+$ corresponding to molecular formula $C_{42}H_{64}O_{16}$. The EIMS of bryostatin 14 typically[2a,2h] did not show a molecular ion. Fragments at m/z 806, 788 and 770 suggested loss of three hydroxyl groups. The ^{1}H-NMR spectra of bryostatin 14 indicated the presence of the bryopyran ring.[2b] Both ^{1}H-^{1}H COSY and ^{1}H-^{13}C chemical shift correlation spectra allowed assignment of most ^{1}H and ^{13}C signals (Tables 2 and 3). Exceptions involved several overlapped ^{1}H-NMR signals and ^{13}C-NMR signals for carbons without proton bonds. A doublet at δ 75.13 clearly indicated a free hydroxyl group at the C-20 position. A pivalate was evident from the strong signals in the ^{1}H (δ 1.16, 9 protons) and ^{13}C (δ 27.14)-NMR spectra. The shift of the H-7 signal downfield to δ 5.10 suggested attachment of the pivalate group at C-7. Other ^{1}H and ^{13}C NMR signals were consistent with the assigned structure. However, a methyl singlet at δ 1.24 assigned to H-33 was at lower field than expected (Table 2). Unequivocal support for the bryostatin 14 structural assignment (14) was obtained by acetylation (acetic anhydride/pyridine) to afford diacetate 14a. As one result, the H-33 signal shifted upfield (from δ 1.24 in bryostatin 14 to δ 1.04 or 0.93 in acetate derivative 14a). Acetylation of bryostatin 5 (5) yielded a single product identical with diacetate 14a. Thus, the paramagnetic shift of the H-33 signal of bryostatin 14 was due to the C-20 hydroxyl group.

Additional evidence for the structure previously assigned bryostatin 5 and thence bryostatin 14 was obtained by HMBC (^{1}H-detected multiple-bond heteronuclear multiple-quantum coherence)[5] NMR experiments that established attachment of the pivalate group at C-7, and unambiguous assignments for each of the carbonyl carbon atoms as well as C-8, C-9, C-13, C-18, C-19 and C-21 (Table 4). Finally, NOE difference spectroscopy was applied to assign the geminal dimethyl groups (C-28, 29 and C-32, 33). Irradiation of the H-28 signal at δ 0.91 enhanced the H-7 signal (at δ 5.10) and a broadened doublet signal at δ 1.64 (H-10α). Reciprocal irradiation of the H-7 signal enhanced the signal at δ 0.91. In contrast, irradiation of the signal at δ 0.98 enhanced only signals at δ 2.03 (doublet of doublets, H-10β) and 1.40 (H-6β). Therefore, the signal at δ 0.91 was assigned to the C-28 methyl and the signal at 0.98 to the C-29 methyl hydrogens. Irradiation of H-20 enhanced dramatically

Table 1. The ^{13}C-NMR chemical shift assignments for bryostatins 4, 5, 6, 8, and 10 recorded at 100.6 MHz, δ ppm in CDCl$_3$ solution.

	Bryostatin 4	Bryostatin 5	Bryostatin 6	Bryostatin 8	Bryostatin 10
C-1	172.29	172.37	172.48	172.24	172.66
2	42.14	42.19	42.17	42.30	42.14
3	68.46	68.53	68.48	68.47	68.15
4	39.91	39.96	39.85	39.87	39.78
5	65.48	65.56	65.62	65.72	65.63
6	33.19	33.24	33.38	33.38	33.20
7	72.64	72.66	72.71	72.54	72.61
8	41.20	41.26	41.04 _	41.02	41.27
9	101.73	101.79	101.85	101.82	101.79
10	41.86	41.92	41.91	41.96	42.05
11	71.45	71.51	71.54	71.51	71.35
12	44.13	44.17	44.16	44.14	44.19
13	157.23	157.18	157.06	156.71	157.07
14	36.48	36.46	36.44	36.37	36.57
15	78.91	78.98	79.04	79.08	78.95
16	129.66	129.73	129.68	129.56	130.45
17	138.97	138.96	138.96	139.13	137.90
18	44.75	44.80	44.80	44.82	44.75
19	98.84	98.85	98.83	98.89	100.94
20	74.24	74.45	74.42	74.24	39.78
21	151.83	151.73	151.68	151.81	157.03
22	31.20	31.23	31.20	31.22	36.11
23	64.70	64.74	64.72	64.71	64.62
24	35.81	35.85	35.85	35.89	35.73
25	73.59	73.67	73.76	73.69	73.84
26	70.03	70.09	70.13	70.18	70.21
27	19.83	19.70	19.66	19.78	19.66
28	21.00	21.06	21.08	21.06	21.06
29	16.92	16.98	16.92	16.88	17.06
30	113.99	114.10	114.14	114.31	114.19
31	166.73	166.79	166.81	166.72	166.82
32	19.78	19.86	19.71	19.78	20.39
33	24.57	24.63	24.60	24.57	24.45
34	119.59	119.73	119.72	119.63	115.73
35	166.98	167.00	166.99	166.99	167.00
36	51.08	51.16	51.13	51.07	51.07
37	51.01	51.07	51.07	51.01	50.84
R$_2$ 1'	178.32	178.32	173.60	173.40	178.14
2'	39.08	39.07	36.56	36.54	39.05
3'	27.09	27.15	18.55	18.54	27.16
4'	27.09	27.15	13.66	13.65	27.16
5'	27.09	27.15			27.16
R 1"	172.00	169.37	169.36	172.00	
2"	36.41	21.49	21.48	36.54	
3"	18.17			18.22	
4"	13.58			13.61	

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Table 2. The ^{1}H NMR data for compounds 14, 14a and 15 recorded at 400 MHz in CDCl$_3$ (some J values were measured with J-resolved 2D NMR).

	14	14a	15	15
H- 2	2.52 t(11)	2.39 dd(12.5,14)	2.45 brs	R
2	2.42 dd(2.2,11)	2.37 d(14)	2.45 brs	2" 5.93 d(15.4)
3	4.12 brd(11)	4.08^a	4.16 m	3" 7.24 m
4	1.97 brdd(11,15)	1.95^a	1.75 m	4" 6.39 dd(11,15.5)
4	1.54 brd(15)	1.53 dt(15,2)	1.60 m	5" 6.07 dd(7.5,15.5)
5	4.20 brt(11)	4.18 brt(11)	4.20 brt(10)	6" 4.38 q(7.5)
6α	1.67 brdd(5,12)	1.68 brdd(5,12)	1.77 m	7" 1.59 m
6β	1.40 dt(12,12)	1.38 dt(12,12)	1.48 m	1.72 m
7α	5.10 dd(5,12)	5.05 dd(5,12)	5.14 dd(5,12)	8" 0.94 t(7.5)
10α	1.64 brd(15)	1.62 brd(15)	1.65 m	
10β	2.03 dd(8,15)	2.03 dd(8,15)	2.07 m	
11	3.91 ddd(4,8,13)	3.78 brdd(8,13)	3.81 m	
12	2.15 brt(13)	2.15 brt(13)	2.21 brt(13)	
12	2.05 dd(8,14)	2.0^a	2.07^a	
14	3.65 brd(14)	3.58 brd(14)	3.68^a	
14	1.90 brdd(12,14)	1.85 brdd(12,14)	1.96 brt(12)	
15	4.02 brdd(8.5,12)	4.05 brdd(8.5,12)	4.07 m	
16	5.30 dd(8.5,16)	5.24 dd(8.5,16)	5.29 dd(8.5,16)	
17	5.75 d(16)	5.71 d(16)	5.78 d(16)	
20	3.89 d(7.5)	5.04 s	5.21 s	
22	3.68^a	3.62^a	3.68^a	
22	2.15 brdd(11,13)	1.98 brdd(11,13)	2.10 m	
23	3.98 brt(11)	3.93 brt(11)	4.01 m	
24	1.86^a	1.82^a	1.81 m	
24	1.80 brdd(11,14)	1.72 ddd(3,11,13)	1.81 m	
25	5.12 brm	5.25 brm	5.20 m	
26	3.74 dq(7,6.5)	4.95 dq(7,6.5)	3.81 m	
27	1.18 d(6.5)	1.16 d(6.5)	1.23 d(6.5)	
28	0.91 s	0.95^{b}s	0.94^{b}s	
29	0.98 s	0.88^{b}s	0.99^{b}s	
30	5.65 brs	5.62 brs	5.68 brs	
32	1.12 s	0.93^{b}s	0.99^{b}s	
33	1.24 s	1.04^{b}s	1.14^{b}s	
34	5.77 brs	5.91 d(1.8)	6.00 brs	
36	3.68 s	3.64 s	3.69 s	
37	3.66 s	3.61 s	3.66 s	
R$_2$ 3'	1.16 s	1.13 s		
4'	1.16 s	1.13 s		
5'	1.16 s	1.13 s		
20-OH	4.22 d(7.5)	2.01 s(R$_1$)	2.04 s(R$_2$)	
		2.09 s(R)		

a,　Couplings obscured due to overlapping.
b,　Assignments for these signals may be interchanged.

Table 3. The ^{13}C-NMR assignments for bryostatin 14 (14), derived diacetate (14a) and bryostatin 15 (15) recorded at 100.6 MHz, δ ppm in CDCl$_3$ solution; The n (negative, 3 or 1 protons) and p (positive, 2 or no protons) are APT results.

	14	14a	15		15
C-1	172.57p	170.82	172.24	R	
2	42.25p	42.21	41.95	1"	165.10
3	68.53n	68.60	68.48	2"	121.75
4	40.00p	39.98	39.87	3"	144.58
5	65.58n	65.83 —	65.73	4"	130.82
6	33.24p	33.20	33.34	5"	141.39
7	72.62n	71.47	72.88	6"	86.86
8	41.25p	41.18	40.98	7"	25.44
9	101.76p	101.76	101.83	8"	9.57
10	41.92p	41.96	42.28		
11	71.48n	72.14	71.49		
12	44.11p	44.05	44.14		
13	156.70p	156.06	156.67		
14	36.46p	36.33	36.36		
15	79.01n	79.03	79.08		
16	129.56n	129.54	129.58		
17	139.15n	139.21	139.09		
18	44.93p	44.77	44.88		
19	99.38p	98.88	98.95		
20	75.13n	71.47	74.33		
21	156.94p	151.63	151.73		
22	30.56p	31.13	31.30		
23	64.40n	64.60	64.72		
24	35.78p	35.90	35.84		
25	73.74n	74.32	73.63		
26	70.04n	70.35	67.00		
27	19.54n	19.88	19.80		
28	21.02n	21.01	21.14		
29	16.93n	16.85	16.82		
30	114.23n	114.63	114.32		
31	166.72p	167.00	166.72		
32	19.88n	16.49	24.65		
33	24.66n	24.50	29.69		
34	116.69n	119.68	119.74		
35	167.09p	166.64	166.98		
36	51.04n	51.08	51.05		
37	51.04n	51.05	51.05		
R$_2$ 1'	178.23p	177.93			
2'	39.03p	39.01			
3'-5'	27.14n	27.15			
R$_1$		170.30;21.47	170.99;21.06		
R		169.28;21.18			

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Table 4. Bryostatin 14 (14) [1]H- and [13]C multiple bond correlations (HMBC) recorded at 500 MHz in CDCl$_3$ solution.[a]

Proton position		Proton position	
H- 2	C-1, C-3, C-4	22	C-21, C-23, C-24, C-34
4	C-2	23	C-22
4	C-2	24	C-23
5	C-3	26	C-24, C-25, C27
6	C-5, C-8	27	C-25, C-26
6	C-4, C-5, C-8	28	C-7, C-8, C-9, C-29
7	C-1', C-6, C-8, C-28, C-29	29	C-7, C-8, C-9, C-28
10	C-8, C-9, C-11, C-12	30	C-12, C-13, C-14, C-31
10	C-9, C-11	32	C-17, C-18, C-19, C-33
12	C-11, C-13, C-14, C-30	33	C-17, C-18, C-19, C-32
12	C-11, C-13, C-14, C-30	34	C-20, C-21, C-22, C-35
14	C-13	36	C-31
14	C-13, C-15, C-16, C-30	37	C-35
15	C-17	R$_2$	
16	C-14, C-18	3'-5'	C-1', C-2'
17	C-15, C-18, C-19, C-32, C-33	C-9 OH	C-9
20	C-21, C-34	C-19 OH	C-18, C-19, C-20
22	C-21, C-20, C-34	C-20 OH	C-19, C-20

a, Underlined positions correspond to weak signals; H-3' correlated also with C-4', 5'; H-4' with C-3', 5' and H-5' with C-3', 4'.

the H-34 signal at δ 5.77 and to some extent the methyl signal at δ 1.12, but not at δ 1.24. In keeping with this result, irradiation of the signal at δ 1.12 (C-32 hydrogen) enhanced the signals for H-20 (δ 3.89) and H-16 (δ 5.30) whereas irradiation of the H-33 methyl signal (at δ 1.24) increased the H-17 signal (δ 5.75). Thus, the methyl hydrogen signals at δ 1.12 and 1.24 were assigned respectively to C-32 and C-33.

For reisolation of bryostatins 1 (1) and 2 (2) from a more recent (1987) recollection (~1,000 kg, damp wt) of California *Bugula neritina* we initiated separation of the crude extract as previously described[21] and then devised a very useful high speed countercurrent distribution (HSCCD)[6] technique followed by further separation using HPLC and recrystallization to yield 8.6 mg (8.6 x 10^{-7}% yield) of bryostatin 15 (15, P388 ED_{50} 1.4 μg/mL).

The FAB mass spectrum of bryostatin 15 gave $[M+Li]^+$ at m/z 927 corresponding to molecular formula $C_{47}H_{68}O_{18}$ (16 mass units more than bryostatin 1 at mol. wt. 904). The ^{1}H NMR (400 MHz) spectrum revealed a macrocyclic lactone possessing an octadienoate side chain similar to bryostatin 1 (1). But chemical shifts of hydrogen signals in the olefinic region suggested a substitution change in the octadienoate side chain. The C-4" hydrogen signal of this ester appeared at δ 6.39 (dd, J 15.5, 11 Hz) and the C-5" at δ 6.07 (dd, J 15.5, 7.5 Hz) downfield compared to their counterparts in bryostatin 1 at δ 6.16 (dd, J 8.5, 2.4 and 4.8, 1.5 Hz respectively). At other positions in the C-20 ester the 2" hydrogen signal showed a doublet at δ 5.93 (J 15.4 Hz). The 3" hydrogen appeared slightly downfield at δ 7.28 (multiplet) compared to that of bryostatin 1 at δ 7.25 (multiplet). These observations were further supported and confirmed by 2D COSY and ^{13}C NMR experiments which led to a firm structure assignment for bryostatin 15 (15).

The biosynthetic processes orchestrated by *Bugula neritina* have produced a very useful series of bryostatins for detailed structure/activity studies. Whether by endogenous and/or exogenous biosyntheses, we now have a number of subtle structural modifications in hand that would be very difficult to realize by total[7] or semisyntheses. In turn, further biological evaluation of these substances should provide important insights for future anticancer drug design.

EXPERIMENTAL

GENERAL PROCEDURES. Solvents used for column chromatography were freshly distilled. Sephadex LH-20, particle size 25-100 μm, used in gel permeation and partition column chromatographic separations was obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. The P.C. Inc. Ito-Multilayer Coil Separator-Extractor Model 1 was employed for high speed countercurrent distribution (HSCCD). An FMI lab pump Model RP SYX (Fluid Metering Inc., Oyster Bay, N.Y.) delivered the mobile phase and fractions were collected using Gilson FC-220 race track and FC-80 microfractionators. Thin layer chromatography silica gel plates were obtained from Analtech, Inc. The TLC plates were viewed under shortwave UV-light and then developed by 20% sulfuric acid and/or anisaldehyde-acetic acid spray reagent followed by heating at approximately 150°C. Uncorrected melting points were observed with a Kofler-type mp apparatus. Optical rotations were determined employing a Perkin-Elmer Model 241

polarimeter. IR spectra were recorded with a Nicolet MX-1 FT-IR Spectrometer and UV spectra were obtained by using a Hewlett-Packard 8450 UV-VIS spectrometer. In high pressure liquid chromatography separations Phenomenex Prepex (particle size 5-20 μ, ϕ 10.0 mm x 25 cm) C-8 was used in reversed phase mode and Phenomenex Prepex (particle size 5-20 μ, ϕ 10.0 mm x 25 cm) silica gel was used in normal phase mode using Altex (Model 110A) solvent metering pumps and Gilson HM UV detection at 254 nm. The ^{1}H NMR, ^{13}C NMR, 2D COSY, ^{1}H-^{13}C correlation, and NOE were recorded with a Bruker AM-400 instrument equipped with cryomagnet and ASPECT-3000 computer. The HMBC data were recorded using a Varian 500 NMR spectrometer. Mass spectra (70 eV and FAB) were obtained employing a Kratos MS-50 spectrometer.

Bryostatin 14 (14). Approximately 1,000 kg (damp wt.) of *Bugula neritina* was recollected in May, 1986 in the Gulf of Mexico near Florida, USA. The animal was preserved in 2-propanol and subjected to separation as previously discussed for a 50 kg, 1984 collection.[2f] The methylene chloride fraction from the solvent partition sequence was diluted with a mixture of ethyl acetate-2-propanol-water (12:1:6, 55 L). Separation of the organic phase (33.6 L) yielded 906.5 g of P388 lymphocytic leukemia cell line active fraction which was subjected to a series of steric exclusion and partition column chromatographic steps using Sephadex LH-20 and silica gel, similar to those previously described.[2f] Further purification was performed with high speed countercurrent distribution employing hexane-ethyl acetate-methanol-water (3:7:5:5), with the upper layer as mobile phase and lower layer as stationary phase (detailed below for bryostatin 15) followed by HPLC using hexane-isopropanol (9:1) in normal phase and methanol-water (4:1) in reversed phase. By these techniques the known bryostatin 4 (306 mg, 3.06 x10^{-5}% yield),[2d] bryostatin 5 (187 mg, 1.87 x 10^{-5}% yield),[2e] bryostatin 6 (32.5 mg, 3.25 x 10^{-6}% yield),[2f] bryostatin 7 (3.1 mg, 3.1 x 10^{-7}% yield),[2f] bryostatin 8 (23.5 mg, 2.35 x 10^{-6}% yield),[2f] and bryostatin 10 (39.0 mg, 3.9 x 10^{-6}% yield)[2h] were obtained accompanied by 102 mg (1.02 x 10^{-5}% yield) of the new bryostatin 14 (14) as an amorphous powder: mp 174-176°C, $[\alpha]^{22}_D$ = +41.3°, (c 0.92, CH_2Cl_2); HRFABMS (3-NBA/LiI as matrix), found, 831.4346 (calc. for $C_{42}H_{64}O_{16}Li$ 831.4354); EIMS 70 eV, m/z, 806[M-H_2O]$^+$ (19%), 788[806-H_2O^+] (100%), 774[806-CH_3OH]$^+$ (80%), 770[788-H_2O)]$^+$ (33%); FABMS, m/z, 831[M+Li]$^+$ for mol. wt. 824 corresponding to $C_{42}H_{64}O_{16}$; IR (thin film) ν_{max} cm^{-1}: 3460 (OH), 1730 (COO), 1650 (C=C), 1170 (COOR); NMR (^{1}H and ^{13}C) appear in Tables 2-4; and P388 ED$_{50}$ = 0.33 μg/ml.

Acetylation of bryostatin 5 (5). To bryostatin 5 (5, 0.8 mg) in acetic anhydride (100 μl) was added 50 μl of pyridine. The reaction course was monitored by TLC. After 10 hr, reaction was complete and afforded 0.8 mg of bryostatin 5 26-acetate (14a); R$_f$=0.61 (in 5:4 hexane:ethyl acetate), 0.90 (in 3:1:1 toluene:ethyl acetate:methanol), 0.40 (in 9:1 methanol-water, RP C-8 plate).

Acetylation of bryostatin 14 (14) A sample (0.8 mg) of bryostatin 14 (14) was acetylated as summarized above for bryostatin 5 (5), except for a 20 hr reaction period. HPLC yielded 0.5 mg of pure bryostatin 14 20,26-diacetate identical (by TLC and 400 MHz ^{1}H-NMR) with bryostatin 5 26-diacetate and exhibiting $[\alpha]^{22}_D$ + 73.2° (c 0.59, CH_2Cl_2).

Bryostatin 15 (15). *Bugula neritina* (1,000 kg, damp wt) was recollected from the U.S. Southern California Coast in 1987. The animal was preserved in 2-propanol and this solution

was partitioned with methylene chloride to produce a fraction (6.13 kg) that was partitioned between hexane and 9:1 methanol-water. The hexane fraction was evaporated under reduced pressure to produce 4.3 kg of hexane extract. The methanol water portion was adjusted to a concentration of 3:2 and extracted with methylene chloride. Removal of solvent from the methylene chloride fraction afforded 1.25 kg of a P388 cell line active fraction, which was subjected to a series of steric exclusion and partition column chromatographic steps using Sephadex LH-20.[21] Typically, 40 to 45 g aliquots of active methylene chloride fraction were applied to Sephadex LH-20 in 1:1 methylene chloride-methanol. Fractions containing bryostatins 1 and 2 were located by TLC (95:5 methylene chloride-methanol) giving a combined weight of 348.7 g. Partition chromatography of 26 g aliquots on Sephadex LH-20 using hexane:toluene:methanol (3:1:1) provided separate fractions enriched in bryostatin 1 (37.51 g) and bryostatin 2 (14.11 g). High speed countercurrent distribution allowed further purification of bryostatins 1 and 2. A biphasic solvent system was prepared from hexane-ethyl acetate-methanol-water (4.5:1.5:1:0.3). HSCCD was accomplished with the Ito horizontal flow-through coil planet centrifuge using the planet gear drive at ∼ 800 rpm. The column consisted of 2.6 mm PTFE tubing (approx. volume 375 ml). The column was filled with the lower (stationary) phase of the two phase solvent system and counter-balanced. An 0.80 g aliquot (from 37.51 g of bryostatin 1 enriched fraction) dissolved in 3-4 ml of stationary phase was pumped into the column. The upper phase of the solvent system (the mobile phase) was pumped into the column from a tail to head direction with planetary motion of the column. An FMI lab pump maintained pressure at 25-30 psi. Fractions were collected and monitored by TLC. From 140 fractions (18 ml each) collected (2.5 L mobile phase) over a 5 hr period, fractions 50-120 contained bryostatin 1. The resulting bryostatin 1 fraction weighed 0.18 g. The HSCCD was repeated (45x) to give 4.4 g total of nearly pure bryostatin 1. Similar experimental procedures were followed for purification of the bryostatin 2 containing fraction. The solvent system was prepared using hexane-ethyl acetate-methanol-water (4:2.5:1.2:0.5). The lower phase was stationary. After HSCCD (14x) a combined fraction (3.36 g) enriched in bryostatin 2 was obtained. Subsequent flash column chromatography (silica gel) with 1:1 hexane-ethyl acetate for bryostatin 1 separation and 9:1 ethyl acetate-hexane for bryostatin 2 separation, and crystallization from ethyl acetate-hexane produced bryostatin 1 as an amorphous solid, mp 226-30°C (1.5 g, 1.5 x 10^{-4}% yield) and bryostatin 2, mp 186-187°C, (2.0 g, 2.0 x 10^{-4}% yield). The mother liquor from the bryostatin 1 crystallization was separated by HPLC with a Prepex 5-20 silica column (10 mm x 25 cm). Elution with hexane-methylene chloride-methanol (14:8:1) at a flow rate of 0.8 ml/min gave bryostatin 15 as an amorphous solid, mp 140-141°C (8.6 mg, 8.6 x 10^{-7}% yield) and bryostatin 8 (5.6 mg, 5.6 x 10^{-7}% yield). The known bryostatins 1, 2 and 8 were identified by direct comparison (principally by 400 MHz ^{1}H NMR and TLC) with authentic samples.

Bryostatin 15 exhibited FABMS m/z 927 [M+Li]$^+$ for mol. wt. 920 corresponding to $C_{47}H_{68}O_{18}$. $[\alpha]^{25}_D$ + 26° (c 0.27, CH$_3$OH); UV (CH$_3$OH) λ_{max} 227 nm (ϵ 25,995); IR (thin film) ν_{max} 3464, 2923, 2845, 1735, 1470, 1375, 1360, 1240, 1135, 1090 cm^{-1}. For the ^{1}H and ^{13}C NMR see Tables 2 and 3.
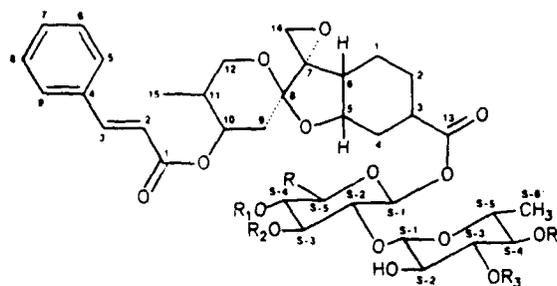
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ANTINEOPLASTIC AGENTS, 177.[1] ISOLATION AND STRUCTURE OF PHYLLANTHOSTATIN 6

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ABSTRACT.—The isolation and structural elucidation of a new *Phyllanthus* glycoside, phyllanthostatin 6 [7], was summarized. Phyllanthostatin 6 [7] was isolated from the roots of *Phyllanthus acuminatus* (Euphorbiaceae) and was found to inhibit ($ED_{50} = 0.35$ μg/ml) growth of the murine P-388 lymphocytic leukemia cell line. Two other new constituents were shown to be didesacetylphyllanthostatin 3 [9] and descinnamoylphyllanthocindiol [10]. Structure determinations were achieved employing hrfabms and 2D-nmr spectroscopy. Application of an hplc separation technique to the *Phyllanthus* glycosides and development of a new isolation procedure for the major antineoplastic constituent, phyllanthoside [1], are also described.

The Central American tree *Phyllanthus acuminatus* Vahl (Euphorbiaceae) has been found to produce a new series of potentially useful antineoplastic glycosides. From 1978 to 1986, Costa Rican collections of the roots and stems of this tree were investigated; these investigations led to the isolation and structural elucidation of phyllanthoside [1], phyllanthostatin 1 [2], the related phyllanthostatins 2 [6], 3 [8], 4 [3], and 5 [4] (2,3), and two cytostatic lignans (4). Recently, three new lignans were isolated from the Indian medicinal plant *Phyllanthus niruri* (5). Because of strong activity against human neoplastic cell lines representing breast, CNS (TE671), colon (Colo 205), lung, ovary, and melanoma (Lox) cancers combined with curative levels of activity against the U.S. National Cancer Institute's (NCI) murine B16 melanoma, the phyllanthoside-phyllanthostatin 1 ortho acid equilibrium product has been undergoing preclinical development by the NCi Division of Cancer Treatment and is now in phase 1 clinical trial. Subsequently, Smith and colleagues completed the first total synthesis of phyllanthoside (6), phyllanthostatin 1 (7) and phyllanthostatin 2 (8). A variety of syntheses are now available for the aglycone, phyllanthocin (9).

	R	R_1	R_2	R_3	R_4
1	Me	H	Ac	Ac	H
2	Me	Ac	H	Ac	H
3	Me	H	Ac	H	Ac
4	Me	Ac	H	H	Ac
5	Me	H	H	H	H
6	CH_2OH	H	Ac	Ac	H
7	CH_2OH	H	H	H	H

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[1]For Part 176, see Kamano *et al.* (1).

The present report summarizes a procedure for improving the yield of phyllanthoside [1]. In addition, a sixth member [7] of the cytostatic phyllanthostatin series and two inactive (NCI murine P-388 lymphocytic leukemia cell line, PS system) transformation products have been discovered. Earlier (2) phyllanthoside was isolated in yields ranging from $7.4 \times 10^{-4}\%$ to $1.4 \times 10^{-2}\%$. In the present study we found that extraction of the dry root with CH_2Cl_2 is an efficient and selective way to obtain a phyllanthoside-rich (corresponding to ~1% of the root) crude extract. The extract was efficiently separated by size exclusion chromatography on Sephadex LH-20 [elution with *n*-hexane–CH_2Cl_2 (1:3) and *n*-hexane–CH_2Cl_2–Me_2CO (1:3:1)] followed by high-speed countercurrent distribution (hsccd) with *n*-hexane–CH_2Cl_2–MeOH–H_2O (2:4:5:2) as solvent system. By this means phyllanthoside [1] was quickly isolated (only 4 steps) in yields as high as 0.2%, and rearrangement and degradation of glycoside 1 were minimized.

The hplc technique we previously developed for detection of phyllanthostatin A (4) was very helpful in developing the new isolation procedure for phyllanthoside. Inspection of the hplc analyses corresponding to crude CH_2Cl_2 (Figure 1a) and MeOH (Figure 1b) extracts of *P. acuminatus* illustrates this point. Both extracts displayed a large peak assigned to phyllanthoside [1], the most dominant phyllanthostatin constituent in crude extracts of *P. acuminatus*.
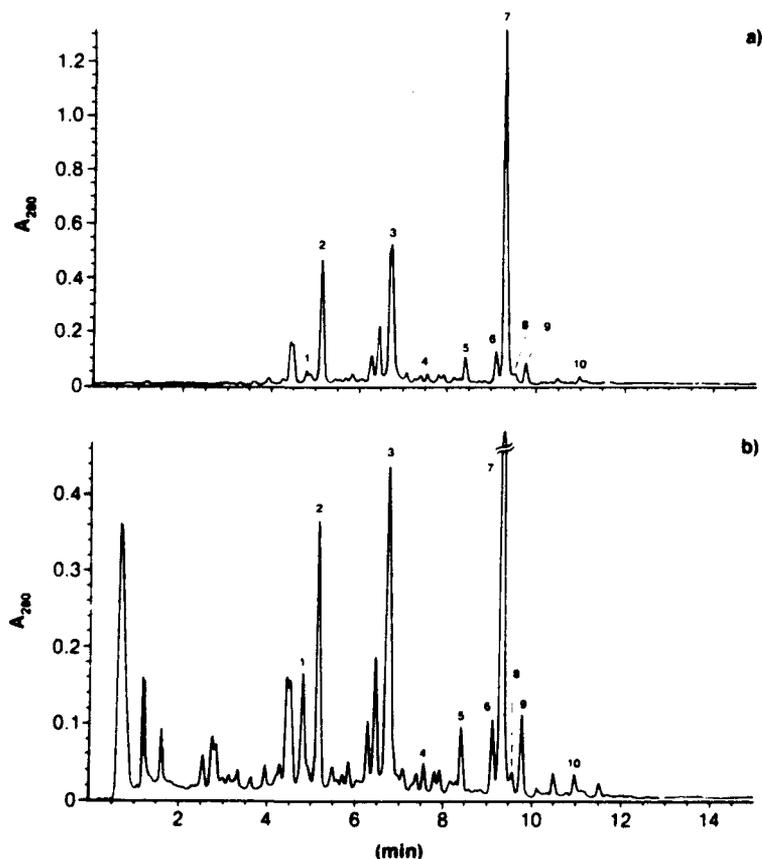
FIGURE 1.   Hplc separation of a *Phyllanthus acuminatus* CH_2Cl_2 extract (a) and an MeOH extract (b) using RP-18 Si gel with a linear gradient of MeCN-H_2O (3:7→7:3) (photodiode array detector). Identified peaks are noted with corresponding structure numbers.

Figure 2 illustrates the separation of a mixture of phyllanthoside [1] and its isomers 2–4. *Phyllanthus* glycosides 7/9 and 6/8, which coeluted (Figure 1) on an RP-18 hplc column, were easily separated on RP-8 (aqueous MeOH). However, the latter system was less powerful for the separation of complex samples such as total extracts. Finally, a freshly prepared CH_2Cl_2 extract of the original *P. acuminatus* roots (collected in 1978) was analyzed as just described. Again, large amounts of phyllanthoside and only traces of phyllanthostatin 1 were detected. Compared to a 1986 sample, the chr. matogram (not shown) obtained from the 1978 sample displayed a much larger peak at Rt 6.7 min, assigned to phyllanthostatin 3 [8], as well as additional peaks between Rt 4 and 6 min (nonidentified degradation products).
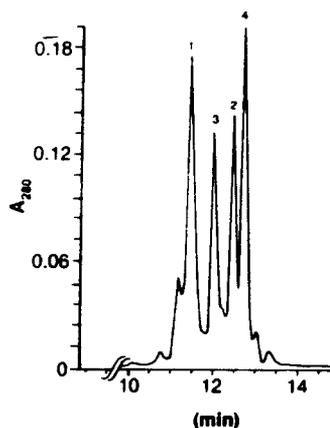
FIGURE 2.   Hplc separation of phyllanthoside [1] isomers on a column of RP-8 Si gel using a $MeOH-H_2O$ (2:3→9:1) gradient.

Phyllanthostatin 6 [7] was isolated from a fresh 1986 MeOH extract of *P. acuminatus*. The MeOH extract (22 g) was separated by size exclusion chromatography (Sephadex LH-20; MeOH), affording a phyllanthostatin-6-rich fraction (4.95 g), which was further purified by hsccd (4, 10–12) using a $CH_2Cl_2-MeOH-H_2O$ (5:5:3) solvent system. Semi-preparative reversed-phase hplc finally afforded 12 mg of phyllanthostatin 6 [7] (3.7×10^{-3}% yield, PS ED_{50} 0.35 μg/ml). Didesacetylphyllanthostatin 3 [9] and descinnamoylphyllanthocindiol [10] were both isolated from a fraction prepared during an earlier (1983) large-scale isolation of the phyllanthostatins (2). Separation of these relatively polar *Phyllanthus* constituents was accomplished by a size exclusion (Sephadex LH-20), hsccd, and reversed-phase liquid chromatographic sequence. The pure compounds 9 (105 mg) and 10 (0.75 g) were found to be inactive against the PS cell line. Structural determinations were conducted as follows.

Phyllanthostatin 6 [7] showed spectroscopic (uv, ir, nmr) properties similar to those of the phyllanthostatins. Acid hydrolysis of the glycoside 7 afforded two hexoses with the same tlc mobility as glucose and 6-deoxyglucose. By hrfabms the molecular formula was established as $C_{36}H_{48}O_{16}$. The ion observed at m/z 613 $[(M + Na) - 146]^+$ indicated that deoxyglucose was the terminal hexose of the disaccharide moiety of phyllanthostatin 6. Both the ^1H- and ^{13}C-nmr chemical shifts were assigned based on 2D nmr experiments (^1H, ^1H-COSY and ^1H, ^{13}C-COSY). Chemical shifts of the cinnamoyl-sesquiterpene moiety were identical to those previously assigned to the phyllanthostatin aglycone (2). For example, the epoxide was confirmed by carbon resonances at δ 49.80 (C-14), 71.02 (C-7), and 102.08 (C-8) and by the C-14 protons at δ 2.93 ppm.
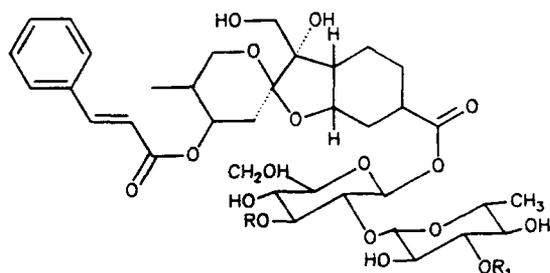
R   R₁

8   Ac   Ac
9   H   H
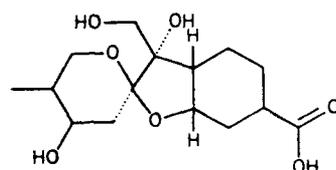
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From nmr and ms spectra it became apparent that the phyllanthostatin 6 disaccharide was not acetylated. The disaccharide proton resonances were fully assigned by ^{1}H,^{1}H-COSY and double-quantum filtered phase-sensitive COSY experiments (13,14). Figure 3 shows the sugar resonances between δ 2.9 and 4.2 ppm with the corresponding correlation peaks (double-quantum filtered phase-sensitive COSY spectrum). Interpretation of the latter spectrum compared to a normal ^{1}H,^{1}H-COSY was simplified by less overlapping of the correlation peaks on and close to the diagonal. The C-14 protons, for example, appeared as a very weak signal, whereas the normal ^{1}H,^{1}H-COSY spectrum showed a prominent signal at δ 2.93 ppm. Complete correlation between sugar protons was observed from S-1 through S-6 and from S-1′ through S-6′, respectively. The ^{1}H,^{13}C-COSY spectrum showed that the S-2 proton was correlated to the carbon resonance at δ 82.15 ppm typical of glycosylation at this position and confirming glucose as the inner sugar. Chemical shifts assigned to the terminal sugar were typical of 6-deoxy-D-glucose (2). The coupling constants ($J = 8$ Hz) of both anomeric
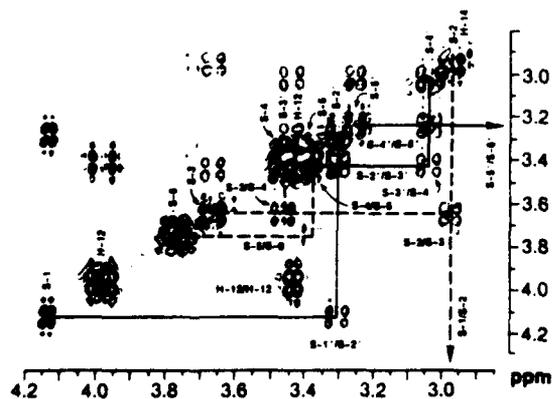
FIGURE 3.   Double-quantum-filtered phase-sensitive COSY spectrum of phyllanthostatin 6 [7] carbohydrate moiety (400 MHz, CDCl₃).

protons confirmed the β linkage of the 2-O-(6-deoxy-D-glucopyranosyl)-D-glucopy-ranosyl unit. The anomeric proton and carbon of the inner glucose unit displayed chemical shifts (δ 5.48/92.40 ppm) identical to those observed with phyllanthostatin 3 [8]. Overall assignment of the phyllanthostatin 6 [7] chemical shifts were in agreement with those reported (2) for the other phyllanthostatins. Thus, structure 7 was assigned to this new member of the series.

The molecular formula of didesacetylphyllanthostatin 3 [9] was determined by hrfabms to be $C_{36}H_{50}O_{16}$. Except for ^{13}C-nmr chemical shifts recorded for C-7 (85.33 ppm), C-8 (106.31 ppm), and C-14 (66.61 ppm), indicating a diol unit at C-7–C-14 (2), glycoside 9 displayed spectroscopic properties similar to those of phyllanthostatin 6 [7]. The ^1H- and ^{13}C-nmr resonances assigned to positions 1–15 and 1'–9' were in accord with those of phyllanthostatin 3, but chemical shifts of the sugar moiety were more typical of a β-linked phyllanthose unit and indeed agreed with the corresponding data for didesacetylphyllanthoside [5] (2). Hence, this component was assigned to didesacetylphyllanthostatin 3 [9].

Descinnamoylphyllanthocindiol [10] gave the same tlc color reaction (brown-gray↦pink after 24 h) upon development with anisaldehyde, as observed with the phyllanthostatins. Lack of uv absorption suggested absence of the cinnamoyl ester, and the molecular formula (by hrfabms), $C_{15}H_{24}O_7$, suggested lack of a disaccharide unit (confirmed by nmr analyses). Assignments for the ^1H and ^{13}C chemical shifts were achieved by 2D nmr techniques and indicated a phyllanthocindiol (2) analogue with a hydroxyl at C-10 and a carboxyl group at C-3. Compared to glycoside 9, carbon resonances C-9 to C-11 of glycoside 10 appeared at higher (C-11 at δ 34.22, C-9 at δ 36.76 ppm) and lower (C-10 at δ 68.22 ppm) fields in agreement with a hydroxyl group at C-10. Structure 10 was thereby identified as descinnamoylphyllanthocindiol.

Both the improved procedure herein summarized for isolation of phyllanthoside and its useful total synthesis (6,7) have diminished the problem of future supplies of this substance and the isomeric phyllanthostatin 1. Isolation of phyllanthostatin 6 appears to complete the series of principal antineoplastic and/or cytostatic glycosides produced by *P. acuminatus*.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All hplc-grade solvents (Omnisolv) were obtained from EM Science, and all other solvents were redistilled. Adsorption cc was performed with Si gel 60 (70–230 mesh, E. Merck, Darmstadt, Germany). Reversed-phase Si gel chromatography was accomplished with RP-8 Lobar columns (size B, 40–63 μm, E. Merck) and size exclusion chromatography with Sephadex LH-20 (particle size: 25–100 μm) supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Tlc was carried out with Si gel GHLF Uniplates (Analtech Inc.). The tlc plates were examined under uv light and developed with an anisaldehyde spray reagent. High-speed countercurrent distribution (hsccd) was performed with an Ito Multilayer Coil Extractor-Separator (P.C. Inc., Potomac, Maryland) using 2.6 mm i.d. tubing, FMI Lab Pump, Linear recorder, and Gilson Model Holochrome uv/vis detector (2.5 mm/3.2 μl cell) with a Micro Fractionator. The hplc-uv/vis separations were accomplished with Ultremex 3 μm RP-8 and RP-18 columns (100 × 4.6 mm i.d.; Phenomenex, Rancho Palos Verde, California). The mobile phase was delivered by two Gilson Model 302 pumps using an Apple II programmer and a Rheodyne 7161 injector with a 0.5 μm in-line precolumn filter (Rainin). Linear gradient elution was carried out with MeCN-H_2O (3:7↦7:3) (RP-18) and MeOH-H_2O (2:3↦9:1) (RP-8) within 15 min at a flow rate of 1 ml/min. All pure compounds for hplc analyses were dissolved in MeOH (~0.1 mg/ml). Roots of *P. acuminatus* (1 g powder) were extracted at room temperature with MeOH or CH_2Cl_2 (3 × 30 ml solvent). The MeOH extract (78 mg) and the CH_2Cl_2 extracts (16 mg of 1978 samples and 8 mg of 1986 specimens) were dissolved or suspended in 2 and 1 ml of MeOH, respectively. These solutions were each passed through a Sep-Pak C-18 cartridge (Waters). The cartridges were washed with MeOH until 4 ml (MeOH extract) and 2 ml (CH_2Cl_2) eluates were collected. These solutions (10 μl) were injected for hplc analyses. Signals were identified by comparison of retention times with those of authentic samples by co-injection. More than 20 non-identified minor peaks with uv spectra typical of the phyllanthostatins were observed. The same results were obtained when crude extracts were separated on RP-8 Si gel with MeOH-H_2O (2:3↦9:1) as eluent.

Melting points are uncorrected and were determined using a Kofler-type hot-stage apparatus. Optical rotations were measured with a Perkin-Elmer Model 241 Automatic Polarimeter. The uv spectra were recorded employing a Hewlett-Packard Model 8450A uv/vis spectrophotometer and ir spectra with a Nicolet Ft-ir Model MX-1 instrument. Nmr spectra were measured using a Bruker AM-400 instrument and are recorded in ppm downfield to TMS. Assignments bearing the same superscript may be reversed. The ^{13}C-nmr multiplicities were determined with APT experiments based on an average coupling constant of 135 Hz. Normal 2D homonuclear and heteronuclear shift correlated spectra were recorded using standard pulse sequences (15–17). The double-quantum filtered phase-sensitive COSY experiment was pursued following the procedure of Wuethrich and co-workers (13,14). Eims spectra were obtained using a Varian MAT 312 spectrometer. Fabms spectra were recorded with a MS-50 instrument at the NSF Regional Facility, University of Nebraska, Lincoln.

PLANT MATERIAL AND EXTRACTION.—The 1986 collection of *P. acuminatus* roots was obtained in Costa Rica, and a voucher specimen is preserved in our Institute. The dry powdered roots (345 g) were extracted at room temperature successively with *n*-hexane, CH_2Cl_2, and MeOH (3 × 6 liters each solvent), yielding 0.6, 3.6, and 25.0 g extracts, respectively. Extraction, solvent partitioning, and chromatographic separation of *P. acuminatus* (1978 collection) were performed as described by Pettit *et al.* (2).

ISOLATION OF PHYLLANTHOSIDE [1].—The 1986 CH_2Cl_2 extract (3.6 g) was separated on a column of Sephadex LH-20 (60 × 4 cm i.d.) with *n*-hexane–CH_2Cl_2 (1:3) as initial solvent system. After eluting with 2.5 liters, the solvent was changed to *n*-hexane–CH_2Cl_2–Me_2CO (1:3:1), and a phyllanthoside-rich (0.833 g) fraction was eluted. An aliquot of this fraction (106 mg) was purified by hsccd with the solvent system *n*-hexane–CH_2Cl_2–MeOH–H_2O (2:4:5:2). The sample was dissolved in a mixture (4 ml) of stationary and mobile phase (6:1) and introduced in the coil through the head inlet. The coil was rotated at 800 rpm, and the mobile phase (lower layer) was pumped at a flow rate of 200 ml/h. Detection (uv) was set at 280 nm and fractions collected every 1.5 min. Retention of the stationary phase was 45%. Pure (by hplc) phyllanthoside (93 mg) was obtained (elution volume 265–335 ml) and its identity confirmed by comparison with an authentic sample (ir, ^{1}H nmr, and ^{13}C nmr).

ISOLATION OF PHYLLANTHOSTATIN 6 [7].—The MeOH extract (22 g) of *P. acuminatus* (1986 collection) was separated by size exclusion chromatography on Sephadex LH-20 (100 × 10 cm i.d., MeOH), and 7 fractions were collected. Fraction 5 (4.95 g, elution volume 6150–6700 ml) was further separated by hsccd with the solvent system CH_2Cl_2-MeOH-H_2O (5:5:3). Samples (2 g) were dissolved in a mixture of upper (13 ml) and lower (2 ml) phas   The hsccd was conducted with the lower, organic phase and a flow rate of 200 ml/h. A uv detector was set at 280 nm and fractions collected every 1.5 min. Fractions eluted between 120 and 150 min after sample introduction were combined and afforded 38 mg of almost pure phyllanthostatin 6 [7]. Combined fractions were purified by semi-preparative hplc (RP-8, Prepex 5-20 µm, 250 × 10 mm, Phenomenex) with aqueous MeOH-H_2O (3:7) at a flow rate of 2 ml/min, yielding 12 mg (3.7 × 10^{-3}% yield) of phyllanthostatin 6 [7]: amorphous solid; mp 136–139°; tlc on Si gel R_f 0.12 [CH_2Cl_2-MeOH (9:1)], R_f 0.25 [CH_2Cl_2-MeOH-H_2O (5:5:3) (lower phase)]; [α]^{25}D + 12.0° (*c* = 0.25, CH_2Cl_2); hrfabms *m/z* [M + Na]$^+$ 759.2839 (calcd for $C_{36}H_{48}O_{16}Na$, 759.2840) with Δ = 0.1 ppm, [(M + Na) − 146]$^+$ 613; uv λ max (MeOH) 277 nm; ir (KBr) ν max 3422, 2940, 1745, 1707, 1635, 1450, 1315, 1281, 1169, 1123, 1075, 1021 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.83 (3H, d, *J* = 6 Hz, H-15), 1.20 (3H, d, *J* = 6 Hz, S-6'), 1.27 (2H, m, H-2), 1.57 (H-1), 1.63 (H-9), 1.76 (H-4), 1.91 (H-9), 1.94 (H-11), 1.98 (H-1, H-6), 2.32 (H-4), 2.50 (H-3), 2.93 (2H, br s, H-14), 2.97 (S-2), 3.04 (S-4'), 3.25 (S-5'), 3.30 (S-2'), 3.37 (S-5), 3.43 (H-12), 3.44 (S-3'), 3.46 (S-4), 3.66 (S-3), 3.75 (S-6), 3.98 (1H, dd, *J* = 11.5 Hz, H-12), 4.13 (1H, d, *J* = 8 Hz, S-1'), 4.42 (H-5), 5.14 (H-10), 5.48 (1H, d, *J* = 7.7 Hz, S-1), 6.56 (1H, d, *J* = 16.1 Hz, H-2'), 7.39 (3H, br s, H-5', H-7', H-9'), 7.56 (2H, br s, H-6', H-8'), 7.78 (1H, d, *J* = 16.1 Hz, H-3'); ^{13}C nmr ($CDCl_3$) δ 12.73 (q, C-15), 17.76 (q, S-6'), 21.84 (t, C-1), 25.62 (t, C-2), 29.47 (t, C-4), 33.14 (d, C-11), 34.31 (t, C-9), 37.04 (d, C-3), 38.21 (d, C-6), 49.80 (t, C-14), 61.64 (t, S-6), 62.79 (t, C-12), 69.15 (d, S-4), 69.70 (d, C-10), 71.02 (s, C-7), 72.16 (d, S-5')ᵃ, 72.61 (d, C-5), 74.87 (d, S-2')ᵃ, 75.15 (d, S-4'), 76.03 (d, S-3')ᵇ, 76.10 (d, S-3), 76.30 (d, S-5)ᵇ, 82.15 (d, S-2), 92.40 (d, S-1), 102.08 (s, C-8), 104.50 (d, S-1'), 118.57 (d, C-2'), 128.29 (2 × d, C-6', C-8'), 129.24 (2 × d, C-5', C-9'), 130.61 (d, C-7'), 134.34 (s, C-4'), 145.01 (d, C-3'), 166.99 (s, C-1'), 174.38 (s, C-13).

HYDROLYSIS.—A solution of phyllanthostatin 6 [7] (2 mg) in MeOH (2 ml) and 2 N HCl (10 ml) was heated at reflux for 30 min, diluted with H_2O, and extracted with $CHCl_3$. The aqueous phase was neutralized (NaHCO$_3$), the solvent was evaporated, and the sugars were extracted with pyridine. Glucose and 6-deoxyglucose were detected in the extract by tlc on Si gel using the solvent system EtOAc-MeOH-H_2O-HOAc (65:15:15:30) followed by spraying with anisaldehyde reagent and heating to reveal spots at R_f 0.58 and R_f 0.70 characteristic of D-glucose and 6-deoxy-D-glucose, respectively.

ISOLATION OF DIDESACETYLPHYLLANTHOSTATIN 3 [9] AND DESCINNAMOYLPHYLLANTHOCIN-DIOL [10].—A fraction obtained from an earlier large-scale isolation of phyllanthoside (2) was separated by size exclusion chromatography on Sephadex LH-20 in MeOH (100×10 cm i.d.; 100 g and 92 g samples) yielding 11 fractions. Part (6 g) of the major fraction (111 g; elution volume 4550–5925 ml) was further separated by hsccd with the solvent system CH_2Cl_2-MeOH-H_2O (5:5:3). The organic layer was used as mobile phase and was passed at a flow rate of 400 ml/h. Retention of the stationary phase was about 50%. Samples (3×2 g) were dissolved in a 20 ml mixture of both phases, and fractions were collected every minute. Fractions eluted between volumes 150 and 250 ml were combined (0.36 g) and further purified by reversed-phase liquid chromatography with MeOH-H_2O (3:2$\mapsto$7:3) (Lobar RP-8, size B) to afford 105 mg of didesacetylphyllanthostatin 3 (7×10^{-6}% yield). Another aliquot (6.3 g) of the main fraction was separated by reversed-phase liquid chromatography with MeOH-H_2O (1:3) (Lobar RP-8, size B, 3×2.1 g samples). Fractions containing diol 10 were combined in MeOH solution and further purified on a column of Sephadex LH-20, yielding 0.75 g of descinnamoylphyllanthocindiol [10] (5×10^{-5}% yield).

Didesacetylphyllanthostatin 3 [9] was isolated as an amorphous solid: mp 135–139°; tlc on Si gel R_f 0.08 [CH_2Cl_2-MeOH (9:1)]; $[\alpha]^{25}D$ +9.1° ($c = 0.11$, CH_2Cl_2); hrfabms m/z $[M + Li]^+$ 745.3236 (calcd for $C_{36}H_{50}O_{16}Li$, 745.3260), $\Delta = 3.3$ ppm; uv λ max (MeOH) 277 nm; ir (KBr) ν max 3433, 2940, 1745, 1707, 1635, 1445, 1309, 1281, 1233, 1169, 1117, 1074 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.85 (3H, d, $J = 5.6$ Hz), 1.21 (3H, d, $J = 5.5$ Hz, S-6'),[a] 1.25 (3H, d, $J = 5.5$ Hz, S-6)[a], 1.32 (H-2), 1.38 (H-1), 1.59 (H-1), 1.73 (H-4), 1.82 (H-6), 1.94 (H-9, H-11), 2.02 (H-2), 2.14 (H-9), 2.17 (H-4), 2.51 (H-3), 3.00 (S-2), 3.04 (S-4), 3.05 (S-4'), 3.23 (S-5')[b], 3.25 (S-2'), 3.38 (S-5)[b], 3.42 (S-3'), 3.49 (H-12, H-14), 3.57 (S-3), 3.93 (H-14), 4.01 (H-12), 4.16 (1H, d, $J = 7.7$ Hz in C_5D_5N, S-1'), 4.18 (H-5), 5.13 (H-10), 5.45 (1H, d, $J = 8.1$ Hz in C_5D_5N, S-1), 6.50 (1H, d, $J = 15.8$ Hz, H-2'), 7.46 (H-5', H-7'), 7.55 (H-6', H-8'), 7.75 (1H, d, $J = 15.8$ Hz, H-3'); ^{13}C nmr ($CDCl_3$) δ 12.67 (q, C-15), 17.61 (q, S-6')[a], 17.82 (q, S-6)[a], 20.47 (t, C-1), 26.11 (t, C-2), 29.47 (t, C-4), 33.21 (d, C-11), 35.32 (t, C-9), 36.87 (d, C-3), 43.22 (d, C-6), 62.77 (t, C-12), 66.21 (t, C-14), 70.06 (d, C-10), 72.10 (d, S-2'), 72.70 (d, S-5)[b], 72.83 (d, C-5), 74.58 (d, S-5')[b], 75.07 (2d, S-4, S-4'), 75.88 (d, S-3'), 76.23 (d, S-3), 81.70 (d, S-2), 85.33 (s, C-7), 92.28 (d, S-1), 104.15 (d, S-1'), 106.31 (s, C-8), 118.56 (d, C-2'), 128.32 ($2 \times$ d, C-6', C-8'), 129.22 ($2 \times$ d, C-5', C-9'), 130.52 (d, C-7'), 134.35 (d, C-4'), 145.15 (d, C-3'), 167.22 (s, C-1'), 174.72 (s, C-13).

Descinnamoylphyllanthocindiol [10] was obtained as an amorphous solid: mp 60–65°; tlc on Si gel R_f 0.18 [CH_2Cl_2-Me_2CO-H_2O (20:80:5)]; $[\alpha]^{25}D$ +92° ($c = 0.25$, MeOH); hrfabms m/z 323.1688 $[M + Li]^+$ (calcd for $C_{15}H_{24}O_7Li$, 323.1683), $\Delta = 1.5$ ppm; ir (KBr) ν max 3456, 2954, 1707, 1455, 1417, 1390, 1121, 1082, 1040, 1022, 985 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 0.90 (3H, d, $J = 6.8$ Hz, H-15), 1.30–1.39 (H-2), 1.51–1.60 (H-1), 1.72–1.82 (H-1, H-4, H-6, H-9, H-11), 2.05–2.13 (H-2, H-9), 2.18–2.22 (H-4), 2.59–2.65 (H-3), 3.44 (1H, d, $J = 11.5$ Hz, H-14), 3.51 (H-12), 3.79 (H-12), 3.86 (H-10), 4.00 (1H, d, $J = 11.5$ Hz, H-14), 4.20 (H-5); ^{13}C nmr [$CDCl_3$-MeOD (9:1)] δ 12.56 (q, C-15), 20.21 (t, C-1), 25.74 (t, C-2), 29.33 (t, C-4), 34.22 (d, C-11), 36.76 (t, C-9), 36.84 (d, C-3), 42.71 (d, C-6), 62.03 (t, C-12), 65.19 (t, C-14), 68.22 (d, C-10), 74.01 (d, C-5), 84.11 (s, C-7), 107.52 (s, C-8), 180.00 (s, C-13, in MeOD).

ACKNOWLEDGMENTS

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A P P E N D I X   A

Table I

Prescreen Submissions for Grant Period

All plants and marine animals listed represent crude extracts unless otherwise noted.

Date	Total	Plants	Marines	Mycelliums	Synthetics
In BRFF Repository	1770	81	1401	288	
" "	161		19* 142		
5/22/90	144		144		
6/26/90	183	21	162		
8/20/90	152	32	120		
8/23/90	111		111		
9/21/90	200	3	197		
10/11/90	200	27	173		
11/13/90	200	196	4		
11/29/90	189	69	120		
12/13/90	161		161		
1/15/91	15				15
1/23/91	195		195		
2/4/91	30	30*			
2/19/91	193	54	139		
2/28/91	191	191			
3/13/91	298	255	43		
3/27/91	200		200		
4/10/91	198	3	195		

Date	Total	Plants	Marines	Mycelliums	Synthetics
4/24/91	197	37	160		
5/8/91	211	103	9*		
			99		
5/22/91	200	200			
6/6/91	200	140	60		
6/21/91	200	200			
	————	————	————	——	——
	5,799	1,642	3,854	288	15

* - fractions

Summary: 1,612 Plant Crudes
            30 Plant Fractions
         3,826 Marine Crudes
            28 Marine Fractions
           288 Mycelliums
            15 Synthetics

1420 of the total submissions showed activity in one or more of the viruses and have been submitted for full screen testing. This figure equals 24% of the total number of samples submitted for prescreen testing during the grant period.

Table II

Prescreen Actives (2/6/89 - 10/31/90)

Drug_Num	AVS_Num	Virus
B23-11C	4855	PT,YF
B720914	7332	YF
B720916	7099	PT
B720933	8513	VEE
B720937	7100	PT
B720940	7101	PT
B720941	7102	PT
B720951	7103	PT
B720952	7104	PT
B720958	7105	PT
B720963	7106	PT
B720979	7107	PT
B720998	7349	PT
B721000	7350	PT
B721011	7298	PT
B721021	7299	PT
B721031	7368	PT
B721045	7373	PT
B721045	7373	YF
B721051	7375	PT,YF
B721053	7377	YF
B721054	7378	YF
B721055	7379	PT
B721060	7300	PT
B721061	7301	PT
B721062	7302	PT
B721063	7383	PT
B721064	7303	PT
B721092	1312	PT
B721095	1315	PT
B721166	1908	PT,YF
B721173	7108	YF
B721177	7385	PT
B721178	7386	PT
B721257	7390	YF
B721260	7391	PT
B721295	8309	PT
B721348	8311	PT
B721374	7304	PT
B721377	7305	PT
B721378	7306	PT
B721392	7307	PT
B721511	1801	PT,YF
B721532	1818	PT
B721568	1850	PT,YF
B721592	7308	PT
B721595	7309	PT
B721596	7310	PT
B721604	8323	PT
B721611	7311	PT
B721616	7399	PT
B721628	7403	PT

Drug_Num	AVS_Num	Virus
B721743		PT
B721749	6584	PT
B721787	6585	PT
B721818	6586	PT
B721823	6248	PT,YF
B721826	6587	PT
B721838	6588	PT
B721880	6589	PT
B721892	6590	PT
B721899	6591	PT,YF
B721905	6592	PT
B721908	6593	PT
B721910	6594	PT
B721917	6595	PT
B721925	6596	PT
B721953	6597	PT,YF
B721958	6598	PT
B721979	6599	PT
B722006	6600	PT
B722048	6601	PT
B722052	6602	PT
B722054	6603	PT
B722060	9180	PT
B722076	6604	PT
B722077	6605	PT
B722078	6606	PT
B722080	6607	PT
B722081	6608	PT
B722087	6609	PT,YF
B722089	6610	PT
B722091	6611	PT
B722094	6612	PT
B722109	6613	YF
B722111	6614	YF
B722116	9183	PT,YF
B722117	9184	PT
B722141	6615	PT
B722162	6616	PT
B722165	6617	PT,YF
B722168	6618	PT
B722181	6619	YF
B722182	6620	PT,YF
B722183	6621	PT,YF
B722222	6622	PT
B722224	6623	PT
B722228	6624	PT
B722230	6625	PT
B722239	6626	PT,YF
B722241	6627	PT,VF
B722246	6628	PT,YF
B722247	6629	PT,YF
B722279	8238	PT
B722280	8239	PT

Drug_Num	AVS_Num	Virus
B722518	7109	YF
B722525	7110	PT
B722559	8240	PT
B722591	8241	PT
B722607	8242	PT
B722628	8244	PT
B722632	8245	PT
B722634	8246	PT
B722689	8228	PT
B722743	8250	PT
B722745	8251	PT
B722752	7405	PT
B722805	7111	PT
B722808	7112	PT
B722811	7113	PT
B722823	8271	PT
B722824	7114	YF
B722849	7115	PT
B722854	7406	PT
B722867	7312	PT
B722871	7313	PT
B722874	7314	PT
B722883	7315	PT
B722886	7316	PT
B722889	7317	PT
B722904	7318	PT
B723037	7116	PT
B723044	7117	PT
B723061	7443	VEE
B723062	7445	PT
B723096	7439	VEE
B723103	7442	VEE
B723106	7408	PT
B723110	7410	PT
B723123	7438	YF
B723136	7446	VEE
B723141	7440	YF,VEE
B723148	7441	VEE
B723203	7444	VEE
B723247	8201	VEE
B723250	8212	VEE
B723268	8216	PT
B723275	8217	VEE
B723278	8218	PT
B723280		PT
B723286	8220	VEE
B723289	8221	VEE
B723290	8222	PT,VEE
B723297	8223	PT
B723315	8226	VEE
B723318	8227	VEE
B723322	8210	VEE

Drug_Num	AVS_Num	Virus
B723409	8204	VEE
B723412	8206	YF
B723414	8208	VEE
B724373	6630	PT
B724379	6631	PT
B724382	6632	PT
B724384	6633	PT
B724385	6634	PT
B724387	6635	PT
B724394	6636	PT
B724396	6637	PT
B724405	6638	PT
B724406	6639	PT
B724411	6640	PT
B724413	6641	PT
B724415	6642	PT
B724416	6643	PT
B724417	6644	PT
B724418	6645	PT
B724420	6646	PT
B724423	6647	PT
B724433	6648	PT
B724434	6649	PT
B724436	6650	PT
B724439	6651	PT
B724442	6652	PT,YF
B724447	6653	PT
B724453	6654	PT
B724455	6655	PT
B724456	6656	PT
B724457	6657	PT
B724458	6658	PT
B724466	6659	PT,YF
B724468	6660	YF
B724508	6661	PT
B724509	6662	PT
B724512	6663	PT
B724517	6664	PT
B724519	6665	PT
B724521	6666	PT
B724525	6667	PT
B724526	6668	PT
B724527	6669	PT
B724530	6670	PT,YF
B724535	6671	PT
B724544	6672	PT
B724549	6673	PT
B724553	6674	PT
B724558	6675	PT
B724566	6676	PT
B724586	6677	PT
B724590	6678	PT
B724592	6679	PT,YF

Drug_Num	AVS_Num	Virus
B724596	6680	PT
B724607	6681	PT
B724610	6682	YF
B724618	6683	YF
B724627	6684	PT
B724633	6685	PT
B724642	6686	PT
B724644	6687	PT
B724652	6688	PT
B724654	6689	PT
B724657	6690	PT
B724661	6691	PT
B724664	6692	PT
B724667	6693	PT
B724670	6250	PT
B724697	6251	PT
B724698	6252	PT
B724701	6253	YF
B724712	6254	PT
B724714	6255	PT
B724716	6256	PT
B724719	6257	PT
B724720	6258	PT
B724722	6259	PT
B724724	6694	PT
B724728	6695	PT
B724729	6696	PT
B724732	6697	PT
B724740	6261	PT
B724762	6262	PT
B724764	6263	PT
B724769	6264	PT
B724772	6698	YF
B724781	6265	PT
B724783	6699	PT
B724785	6700	PT
B724797	6266	PT
B724812	6701	YF
B724820	6268	PT
B724825	6269	PT
B724832	6702	PT
B724844	6270	YF
B724852	6272	YF
B724855	6703	PT
B724860	6274	PT
B724863	6275	PT,YF
B724866	6704	PT
B724885	6705	PT
B724886	6277	PT
B724898	6706	YF
B848989	8258	PT
B848990	8259	PT
B849035	8234	PT

Drug_Num	AVS_Num	Virus
B849179	8236	PT
B849180	8237	PT
B849286	4276	YF
GRP19380	4279	PT,YF
GRP19381	4280	PT,YF
GRP19386	7320	PT
GRP19396	7321	YF
GRP19416	7414	PT
GRP19418	7415	PT
GRP19423	7417	PT
GRP19424	7418	PT
GRP19435	7424	PT

Table III

Prescreen Actives (11/1/90 - 7/5/91)

Virus	Ctrl. B No.	AVS No.
PT	604736-F046	11029
PT,YF	604736-F047	11030
PT	604736-F056	
PT,YF	604736-F057	11031
PT	604736-F058	
PT	631963-F010	
PT	631963-F015	11032
PT,VEE	631963-F016	11033
PT	634131-F028	
YF,VEE	634131-F031	11034
YF,VEE	634131-F032	11035
PT	634131-F064	
PT,YF,VEE	634131-F065	11036
PT,YF,VEE	634131-F068	11037
PT,YF	642761-F016	11038
PT	642761-F017	11039
PT	642761-F018	11040
PT	644263-F106	11041
PT,VEE	644263-F107	11042
PT	678018-F243	11043
PT,YF	678018-F244	11044
PT,YF	678018-F245	11045
VEE	678018-F246	11046
PT,YF	706269	9213
PT	706308	
PT	709724	
PT,VEE	709752	9206/9399
VEE	709761	9400
PT	709763	
PT,YF,VEE	710030	9207/9214/9407
PT,YF,VEE	710041	9237/9401
PT	710043	
PT	710046	
PT	710057	
VEE	710064	9402
VEE	710068	9403
VEE	710072	9404
PT	710100	9211
PT	710110	
PT	710124	
VEE	710126	9405

PT,VEE	710128	9406
PT	710130	
PT	710131	
PT	710137	
PT	710138	
PT	710151	
PT	710152	
PT	710154	
VEE	710157	9408
PT	710161	
PT,VEE	710162	9409
PT	710174	
PT	710176	
PT	710179	
PT	710183	
PT	710210	
PT,VEE	711707	9410
VEE	711710	9411
PT,VEE	711711	9412
PT,VEE	711712	9413
YF	711714	
PT,VEE	711715	
YF,VEE	711716	
PT,VEE	711717	
YF	711718	
YF,VEE	711719	
YF	711720	
YF	711722	
YF	712291	
YF	712292	
YF	712295	
YF	712296	
YF	712299	
YF	712300	
PT	714994	9121
PT	714997	9122
YF	715001	9123
VEE	715010	9124
VEE	715011	9125
VEE	715012	9126
PT	715022	9127
PT	715023	9128
PT	715026	9129
PT,VEE	715060	9130

YF	715062	
YF	715068	
PT,YF,VEE	715070	
YF	715074	
YF	715075	
YF	715078	
YF	715082	
YF	715089	
PT	715091	
PT,VEE	715093	
VEE	715095	
PT	715101	
VEE	715111	
PT,VEE	715112	
PT	715113	
YF	715122	
PT,YF,VEE	715141	11047
VEE	715160	
YF	715180	
PT	715186	
PT	715234	
VEE	715284	
VEE	715627	
PT	718257	
VEE	718546	
PT	718548	
PT	718550	
PT,YF,VEE	718551	
PT	718553	
PT,VEE	718574	11048
PT,YF	718577	11049
PT	718580	11050
PT	718582	11051
PT	718583	11052
PT	718586	11053
PT	718587	11054
VEE	718588	
PT	718595	11055
PT	718598	11056
PT,VEE	718599	11057
PT	718603	11058
YF,VEE	718636	11059
VEE	718640	
VEE	718642	
VEE	718645	
PT,YF	718648	11060
YF	718649	11061
PT,YF	718656	11062
YF,VEE	718789	11063
YF,VEE	718799	11064

PT,VEE	718807	11065
VEE	718813	11066
PT,VEE	718819	11067
PT	718821	11068
VEE	718825	
PT,VEE	718838	11069
VEE	718840	11070
PT,VEE	718841	11071
PT	718842	11072
YF	718843	11073
PT	718849	11074
YF	718860	11075
PT	718862	11076
YF	718876	11077
VEE	718882	11078
VEE	718889	11079
VEE	718917	11080
VEE	718924	11081
PT,YF,VEE	718934	11082
PT	718935	11083
VEE	718942	11084
VEE	718954	11085
VEE	718969	11086
VEE	718978	11087
PT,VEE	718979	11088
PT,VEE	718982	11089
PT	718983	11090
PT	718990	11091
PT	718996	11092
VEE	719004	11093
PT,VEE	719006	11094
PT,YF,VEE	719007	11095
YF	719008	11096
YF	719215	9131
YF	719216	9132
YF	719217	9133
YF,VEE	719218	9134
YF	719219	9135
YF	719220	9136
YF,VEE	719221	9137
PT,YF	719222	9138
YF	719224	9139
YF	719225	9140
YF	719226	9141
YF	719228	9142
VEE	719229	9143
VEE	719230	9144
YF	719231	
PT	719232	9145
PT	719238	9146
YF,VEE	719240	9147

PT,YF	719241	9148
PT,YF,VEE	719242	9149
YF,VEE	719244	9150
PT,YF	719245	9151
YF	719246	9152
PT,YF	719247	9153
PT	719255	9154
PT	719256	9155
YF	719257	9156
YF	719258	9157
YF	719259	9158
PT,YF	719260	9159
VEE	719265	9160
VEE	719266	9161
PT,VEE	719267	9162
VEE	719272	9163
YF	719274	9164
VEE	719278	9165
PT,VEE	720392	11097
YF	720394	11098
VEE	720396	11099
PT,VEE	720398	11100
VEE	720399	11101
YF	720400	11102
VEE	720405	11103
PT,YF	720409	11104
YF	720410	11105
YF	720412	11106
YF	720415	11107
PT	720418	11108
YF	720419	11109
PT	720424	11110
YF,VEE	720425	11111/11112
YF,VEE	720427	11113/11114
PT,YF	720430	11115/11116
YF	720431	11117
YF	720433	11118
VEE	720434	11119
VEE	720435	11120
YF	720436	11121
YF	720443	11122
PT,YF	720445	11123
VEE	720820	9166
YF	720821	9167
PT,VEE	720825	9168
YF	720828	9169
PT	720832	9170
PT,VEE	720834	9171
YF,VEE	721643	9172

YF	721746	9173
VEE	721777	9174
YF	721778	
PT,YF,VEE	721781	
VEE	721786	9175
YF	722117	9184
PT,YF	722161	9185
YF,VEE	722174	9187
YF	722179	9188
VEE	722184	
YF	722186	9189
YF	722190	9190
YF	722194	9191
PT	722210	
YF	722215	9194
YF	722229	9195
VEE	722230	6625
PT	722231	
YF	722233	9196
YF	722236	9198
PT,YF	722240	
YF	722242	9199
YF	722245	9201
PT	722246	6628
YF	722248	9202
YF	722278	
VEE	722504	9205
YF,VEE	722506	
PT,YF,VEE	722508	
YF,VEE	722510	
PT,VEE	722872	9208
PT,YF	722873	9209
YF	722876	9210
PT	722883	7315/9211
YF	722884	9212
PT	722886	7316/9213
PT,VEE	722889	7317/9214
VEE	722899	9216
YF,VEE	722890	9215
PT	722902	8374/9217
VEE	722905	9218
VEE	722908	9219
YF	722909	
VEE	722911	9220
VEE	722914	9221
PT,VEE	722915	9222
VEE	722917	9223
PT,VEE	722920	
PT,YF	722921	
VEE	722922	9224

VEE	722926	9225
PT,VEE	722929	
VEE	722931	9226
VEE	722932	9227
YF	722933	
VEE	722935	9228
PT,VEE	722936	9229
PT,YF	722937	
VEE	722938	9230
YF,VEE	722942	
VEE	722944	9231
VEE	722950	9232
PT,YF,VEE	722965	9233
VEE	722976	9234
VEE	722987	9235
VEE	722992	9236
YF	723003	
PT	723004	
YF	723006	
PT,VEE	723035	
PT,YF,VEE	723047	
PT,YF	723169	9238
PT	723172	9239
VEE	723420	9240
VEE	723424	9241
VEE	723425	9242
VEE	723426	9243
YF,VEE	723427	
PT,YF	723428	
VEE	723429	9244
VEE	723430	9245
PT,VEE	723431	
VEE	723432	9246
YF	723435	9247
YF	723436	9248
YF	723438	9249
YF	723441	9250
YF	723444	9251
VEE	723448	
VEE	723449	9252
YF	723452	9253
VEE	723455	9254
VEE	723457	
PT,YF	723459	9255
VEE	723460	
VEE	723463	
VEE	723465	
PT	723466	9256
PT,VEE	723467	9257
VEE	723468	

PT	723471	9258
PT,VEE	723472	
PT,VEE	723473	9259
VEE	723474	
VEE	723475	
PT	723477	9260
VEE	723482	
PT	723483	9261
YF	723484	9262
YF	723485	9263
VEE	723486	9264
VEE	723487	9265
PT	723493	9266
YF	723801	9267
VEE	723802	9268
PT,VEE	723807	9269
PT,YF	723816	
VEE	723821	
YF	723822	
YF,VEE	723826	
VEE	723827	
PT,YF,VEE	723828	
PT,VEE	723829	
PT	723830	
VEE	723831	
PT,YF,VEE	723833	
PT,YF	723835	
PT	723837	
VEE	723838	
VEE	723839	
VEE	723840	
VEE	723842	
PT	723846	
VEE	723849	
PT,YF	723864	
YF	723868	
YF	723871	
PT	723872	
YF	723875	
PT	723876	
PT	723877	
PT,YF	723879	
YF	723881	
PT,VEE	723882	
PT,YF,VEE	723883	
VEE	723885	
VEE	723886	
YF	723887	
PT	723898	
PT,YF	723900	
YF	723903	
VEE	723904	

PT,VEE	723908
PT,VEE	723909
PT,VEE	723910
YF,VEE	723911
YF,VEE	723912
VEE	723915
YF,VEE	723917
YF	723918
VEE	723922
YF	723932
PT	723933
PT	723934
PT,VEE	723937
VEE	723939
YF	723944
PT,VEE	723946
VEE	723948
PT,YF	723949
YF	723950
VEE	723951
PT,YF	723954
PT,YF,VEE	723957
PT,YF	723958
VEE	723959
VEE	723960
VEE	723962
VEE	723963
VEE	723964
VEE	723965
VEE	723970
YF	723972
PT	723973
PT,VEE	723975
PT	723976
PT	723977
PT	723979
VEE	723980
VEE	723983
VEE	723985
PT	723989
VEE	723992
YF	723993
VEE	723994
VEE	723995
VEE	723996
VEE	723997
PT	723998
VEE	723999
PT,VEE	724001
PT	724004

VEE	724005	
VEE	724009	
VEE	724010	
VEE	724011	
VEE	724012	
VEE	724015	
VEE	724019	
VEE	724021	
PT,YF,VEE	724025	
VEE	724029	
VEE	724030	
VEE	724033	
VEE	724035	
VEE	724040	
YF	724042	
PT	724045	
VEE	724046	
?	724055	11589
PT	724058	11590
?	724060	11591
YF,VEE	724062	11592
YF	724063	11593
YF	724067	11594
?	724069	11595
VEE	724070	11596
PT,YF	724073	11597
PT	724075	11598
PT	724076	11599
VEE	724078	11600
PT	724081	11601
YF	724086	11602
VEE	724087	11603
YF	724091	11604
VEE	724093	11605
YF	724100	11606
PT,YF	724108	11607
YF	724109	11608
PT	724110	11609
YF,VEE	724111	11610
YF	724112	11611
PT,VEE	724113	11612
VEE	724114	11613
YF	724124	11614
YF	724127	11615
YF	724130	11616
PT	724131	11617
VEE	724135	11618
PT	724136	11619
YF	724137	11620
PT	724139	11621
PT,YF	724140	11622
PT	724141	11623

PT	724143	11624
YF	724145	11625
PT,VEE	724148	11626
PT	724151	11627
PT	724155	11628
VEE	724163	11629
PT	724164	11630
PT,VEE	724165	11631
PT,VEE	724170	11632
PT	724172	11633
VEE	724173	11634
PT,VEE	724181	11635
PT	724182	11636
PT,VEE	724184	11637
PT,YF	724186	11638
PT,YF	724187	11639
PT	724192	11640
VEE	724194	11641
VEE	724198	11642
PT	724199	11643
PT	724201	11644
PT,VEE	724202	11645
VEE	724203	11646
VEE	724205	11647
VEE	724207	11648
PT	724209	11649
PT,VEE	724210	11650
VEE	724212	11651
YF,VEE	724214	11652
YF,VEE	724215	11653
PT	724216	11654
VEE	724217	11655
PT,VEE	724219	11656
YF,VEE	724220	11657
VEE	724221	11658
VEE	724223	11659
VEE	724224	11660
VEE	724225	11661
PT,YF	724226	11662
PT	724227	11663
VEE	724228	11664
PT,YF,VEE	724230	11665
VEE	724231	11666
VEE	724232	11667
VEE	724233	11668
YF	724238	
PT,VEE	724240	
VEE	724242	
YF,VEE	724243	
PT	724244	
PT	724247	
YF	724248	

VEE	724249
YF	724251
PT,VEE	724252
PT,VEE	724253
VEE	724254
YF	724256
VEE	724257
VEE	724258
VEE	724260
YF	724261
PT,YF	724266
PT,YF	724268
YF	724270
YF	724271
PT,YF	724275
YF	724279
PT	724280
YF	724281
PT	724283
YF	724287
VEE	724288
PT,VEE	724289
YF	724290
PT	724291
PT	724293
YF	724294
PT	724296
YF	724297
YF	724298
YF	724299
YF	724300
PT	724301
PT	724303
PT,YF	724308
PT	724310
YF	724312
YF	724313
YF	724316
VEE	724320
PT,YF	724323
YF,VEE	724324
YF,VEE	724327
VEE	724335
PT,VEE	724337
YF	724338
VEE	724339
PT,VEE	724340
PT	724348
PT,YF	724354
YF	724355
PT	724356
YF	724358

VEE	724361	
VEE	724363	
VEE	724365	
VEE	724366	
VEE	724367	
VEE	724368	
YF	725005	
YF,VEE	725006	11323
YF,VEE	725007	11324
YF	725008	11325
PT,VEE	848631	9270
PT	848633	9271
PT	848634	9272
PT	848635	9273
PT,VEE	848649	9274
PT	848650	9275
PT,YF,VEE	848653	9276
PT,VEE	848654	9277
PT,VEE	848656	9278
PT	848657	9279
PT	848659	9280
PT	848660	9281
PT	848662	9282
PT	848663	9283
PT,YF	848669	9284
PT,VEE	848670	9285
PT,VEE	848671	9286
PT	848673	9287
PT	848676	9288
PT	848678	9289
PT	848679	9290
PT	848681	9291
PT,VEE	848691	9292
PT,VEE	848699	9293
PT	848700	9294
PT	848703	9295
PT	848706	9296
VEE	848709	9297
PT	848711	9298
PT	848716	9299
PT	848717	9300
PT	848720	9301
VEE	848722	9302
PT	848724	9303
PT,VEE	848725	9304
VEE	848727	9305
VEE	848728	9306
PT	848729	9307
VEE	848730	9308

VEE	848731	9309
PT	848732	9310
VEE	848733	9311
PT,VEE	848734	9312
VEE	848735	9313
VEE	848736	9314
VEE	848737	9315
PT,VEE	848738	9316
PT	848739	9317
PT,VEE	848740	9318
PT	848741	9319
YF,VEE	848742	9320
PT	848745	9321
PT	848747	9322
PT	848748	9323
PT	848749	9324
PT	848750	9325
PT	848751	9326
PT,YF,VEE	848752	9327
PT,VEE	848753	9328
PT	848755	9329
VEE	848757	9330
PT	848767	9331
YF	848771	9332
YF	848774	9333
PT	848782	9334
PT	848788	9335
PT	848790	9336
PT	848792	9337
PT,VEE	848793	9338
PT,YF	848794	9339
PT,YF,VEE	848795	9340
YF,VEE	848796	9341
PT,VEE	848797	9342
VEE	848798	9343
YF	848800	9344
VEE	848801	9345
PT,VEE	848804	9346
VEE	848805	9347
VEE	848808	9348
YF,VEE	848809	9349
VEE	848810	9350
PT,VEE	848811	9351
PT,VEE	848812	
PT,YF,VEE	848814	9352
PT	848816	9353
PT	848838	9355
PT,YF,VEE	848839	9356
PT,VEE	848841	9357
PT,VEE	848843	9358
PT,VEE	848845	9359
PT,VEE	848848	9360

PT	848861	9361
PT,VEE	848864	9362
PT	848867	9363
VEE	848869	9364
PT,VEE	848870	9365
PT	848873	9366
PT	848874	9367
PT	848876	9368
PT	848879	9369
PT	848880	9370
PT	848881	9371
PT	848882	9372
PT	848883	9373
VEE	848892	9374
PT,VEE	848893	9375
PT,VEE	848895	9376
PT	848896	9377
PT	848897	9378
PT	848899	9379
PT	848900	9380
PT	848901	9381
PT	848903	9382
PT	848904	9383
PT,YF	848905	9384
PT	848906	9385
PT,VEE	848907	9386
PT	848909	9387
PT,VEE	848911	9388
PT	848913	9389
PT,YF	848914	9390
YF	848916	9391
YF	848917	9392
PT	848920	9393
PT	848921	9394
YF	848924	9395
PT	848926	9396

YF,VEE	849188
PT	849190
PT,YF,VEE	849192
VEE	849195
YF,VEE	849196
VEE	849197
PT	849199
VEE	849200
VEE	849201
VEE	849202
VEE	849204
VEE	849206
VEE	849216

Virus	Ctrl GRP No.	AVS No.
VEE	849219	
VEE	849220	
PT	849239	9397
VEE	849240	9398
PT,YF	849259	
PT	849268	11387
VEE	849269	11388
PT,VEE	849271	11389
PT,VEE	849272	11390
PT	849273	
PT	849275	11391
PT	849277	11392
?	849278	11393
?	849280	11394
PT	849281	11395
PT	849283	11396
PT	849284	11397
PT	849285	11398
PT	849289	11399
YF	849291	
PT	849292	11400
VEE	849295	11401
PT	849296	
PT,VEE	849297	11402
PT,YF,VEE	849298	11403
PT	849299	11404
YF,VEE	849300	11405
VEE	849302	11406
PT	849305	
VEE	849307	11407
VEE	849308	14408

Virus	Ctrl GRP No.	AVS No.
PT	18058	11124
PT	18060	11125
PT	18061	11126
YF	18062	11127
PT	18063	11128
YF	18066	11129
YF	18068	11130
PT,VEE	19457	11131
PT,YF,VEE	19458	11132
PT,YF	19459	11133
PT,VEE	19462	11134
PT,YF	19463	11135
YF,VEE	19464	11136
VEE	19465	11137
YF	19467	11138

PT	19469	11139
PT,VEE	19470	11140
PT,VEE	19471	11141
PT	19472	11142
PT,YF,VEE	19473	11143
PT	19474	11144
YF	19479	11145
PT	19480	11146
PT,VEE	19481	11147/11148
PT,VEE	19486	11149
PT,VEE	19490	11150
PT,VEE	19491	11151
PT	19492	11152
PT	19493	11153
PT,VEE	19501	11154
PT,YF,VEE	19502	11155
PT	19509	11156
PT	19510	11157
PT	19511	11158
PT	19512	11159
YF	19514	11160
PT	19516	11326
PT	19521	11327
?	19522	11328
?	19523	11329
VEE	19525	11330
PT	19528	
PT	19529	11331
PT	19533	11332
PT	19538	11333
PT	19539	11334
PT	19541	11335
PT	19544	11336
PT	19545	11337
?	19550	11338
PT	19573	11339
PT	19574	11340
PT	19576	11341
PT	19578	11342
PT	19579	11343
PT	19583	11344
VEE	19584	11345
VEE	19594	11346
VEE	19598	11347
?	19599	11348
PT	19601	11349
PT,VEE	19602	11350
PT	19603	11351

PT	19605	11352
PT	19608	11353
PT	19610	11354
PT	19611	11355
PT	19612	11356
?	19619	11357
PT,YF	19621	11358
PT	19622	
VEE	19623	11359
PT	19624	
PT	19626	
YF,VEE	19631	11360
VEE	19634	11361
PT,VEE	19635	11362
VEE	19636	11363
PT,VEE	19640	11364
PT,VEE	19641	
PT	19642	
VEE	19643	11365
VEE	19647	11366
PT	19649	11367
?	19653	11368
VEE	19655	11369
PT	19657	11370
PT,YF	19664	11371
PT,VEE	19670	11372
VEE	19671	11373
VEE	19672	11374
PT,YF	19674	11375
PT,YF	19675	11376
PT,YF	19676	11377
PT	19679	11378
?	19680	11379
PT	19681	11380
PT	19685	11381
PT,VEE	19686	11382
VEE	19689	
PT,VEE	19691	11383
PT	19692	
PT	19695	
YF	19696	11384
PT	19698	
PT,VEE	19699	
PT,VEE	19700	11385
PT	19701	11386
PT,YF,VEE	21188	11161
PT,YF	21189	11162
YF	21190	11163

PT,YF	21191	11164
PT	21194	11165
PT	21195	11166
PT,VEE	21197	11167
VEE	21199	11168
YF	21201	11169
VEE	21205	11170
PT,YF,VEE	21207	11171
VEE	21214	11172
PT,VEE	21220	11268
PT	21221	11269
PT	21222	11270
PT	21223	11271
PT,VEE	21224	11272
PT,YF	21225	11273
PT,YF,VEE	21226	11274
VEE	21228	11275
PT	21229	11276
PT,YF,VEE	21232	11277
PT,VEE	21233	11278
PT,YF,VEE	21234	11279
YF	21235	
PT,YF,VEE	21236	11280
PT,YF	21238	11281
VEE	21242	11282
PT,YF	21247	11283
YF,VEE	21248	11284
YF	21251	11285
PT,VEE	21253	11286
YF,VEE	21255	11287
YF	21261	11288
PT	21263	11289
PT,YF,VEE	21264	11290
PT	21266	11291
PT,VEE	21267	11292
PT	21268	11293
PT,YF,VEE	21272	11294
YF	21275	11295
PT,YF	21278	11296
YF	21281	11431
YF	21282	11297
PT	21285	11298
PT	21286	11299
PT	21287	11300
YF	21288	11301
PT	21289	11302
PT,YF	21291	11303
PT,YF	21292	11304
?	21293	11305
VEE	21294	11306

PT	21297	11307
PT,YF	21301	11308
YF,VEE	21303	11309
PT,YF	21306	11310
PT	21307	11311
PT,YF	21314	11312
PT,YF	21315	11313
PT	21317	11314
PT	21320	11315
PT,YF	21321	11316
YF	21323	11317
PT,YF,VEE	21325	11318
VEE	21326	11319
PT	21328	11320
PT	21329	11321
PT	21330	11322
YF	21668	11409
PT	21669	
YF	21671	11410
PT,YF,VEE	21680	11411
PT	21681	
PT	21682	
PT	21683	
YF	21687	11412
YF	21688	
PT,VEE	21689	11413
PT	21690	11414
VEE	21691	11415
PT	21695	
YF	21696	
VEE	21693	11416
PT	21700	
YF	21703	11417
VEE	21704	11418
PT,YF,VEE	21705	11419
PT,VEE	21706	11420
PT,VEE	21708	11421
PT,VEE	23174	11422
PT,VEE	23175	11423
PT	23176	
VEE	23184	11424
PT,VEE	23185	11425
PT,VEE	23186	11426
PT	23187	

PT	23188	11427
PT	23189	11428
PT,YF,VEE	23190	11429
PT	23191	11430
YF	23192	11432
YF	23193	11433
YF	23194	11434
YF,VEE	23195	11435
YF	23198	11436
YF	23199	11437
PT,YF	23200	11438
YF	23202	11439
PT,YF,VEE	23203	11440
PT,YF	23204	11441
PT,VEE	23206	11442
?	23207	11443
PT,YF,VEE	23210	11444
VEE	23211	11445
PT,YF	23213	11446
YF	23214	11447
VEE	23216	11448
VEE	23218	11449
VEE	23221	11450
PT,YF	23222	11451
PT,VEE	23225	11452
PT	23226	11453
PT	23227	11454
VEE	23229	11455
VEE	23230	11456
PT,VEE	23233	11457
VEE	23234	11458
YF,VEE	23235	11459
VEE	23236	11460
VEE	23237	11461
PT,VEE	23238	11462
PT,VEE	23240	11463
PT	23242	11464
PT,YF,VEE	23243	11465
VEE	23244	11466
PT,YF,VEE	23245	11467
PT,VEE	23247	11468
YF,VEE	23248	11469
PT,VEE	23249	11470
VEE	23250	11471
PT,VEE	23252	11472
YF,VEE	23253	11473
PT,VEE	23254	11474
PT,YF,VEE	23255	11475
YF	23256	11476
?	23257	11477

YF,VEE	23258	11478
YF	23260	11479
YF,VEE	23261	11480
YF,VEE	23262	11481
YF	23263	11482
YF	23264	11483
PT,VEE	23265	11484
VEE	23267	11485
VEE	23269	11486
PT,YF,VEE	23270	11487
VEE	23271	11488
VEE	23272	11489
VEE	23273	
PT	23274	11490
VEE	23275	11491
YF	23276	11492
VEE	23278	11493
PT,VEE	23279	11494
YF	23280	11495
PT,VEE	23282	11496
?	23283	11497
PT,YF,VEE	23284	11498
VEE	23285	11499
PT	23286	11500
PT,YF	23287	11501
VEE	23288	11502
YF	23290	11503
VEE	23291	11504
VEE	23292	11505
PT,YF	23293	11506
VEE	23306	11507
PT	23307	
PT,VEE	23308	11508
PT	23311	11509
VEE	23316	11510
VEE	23317	11511
VEE	23318	11512
PT	23319	11513
VEE	23322	11514
VEE	23323	11515
VEE	23325	11516
YF	23326	11517
?	23327	11518
?	23328	11519
VEE	23329	11520
PT	23330	11521
PT,VEE	23331	11522
PT	23333	11523
VEE	23334	11524
PT,VEE	23335	11525

PT	23337	11526
PT,VEE	23338	11527
PT,YF	23339	11528
VEE	23342	11529
PT,VEE	23343	11530
PT,VEE	23344	11531
PT,YF	23346	11532
?	23347	11533
YF	23348	11534
YF	23349	11535
YF	23350	11536
?	23353	11537
PT,VEE	23354	11538
PT	23355	11539
VEE	23356	11540
VEE	23357	11541
YF	23359	11542
VEE	23361	11543
PT	23363	11544
YF	23364	11545
VEE	23365	11546
VEE	23366	11547
VEE	23369	11548
PT,VEE	23370	11549
VEE	23371	11550
PT,YF	23372	11551
VEE	23374	11552
VEE	23375	11553
VEE	23376	11554
PT,VEE	23377	11555
PT	23378	11556
VEE	23379	11557
VEE	23380	11558
VEE	23382	11559
VEE	23383	11560
VEE	23386	11561
VEE	23387	11562
VEE	23388	11563
VEE	23389	11564
VEE	23391	11565
VEE	23392	11566
VEE	23394	11567
VEE	23396	11568
VEE	23397	11569
VEE	23398	11570
PT	23399	
PT,VEE	23400	11571
?	23401	11572
VEE	23402	11573
VEE	23404	11574

PT	23405	11575
VEE	23407	11576
PT,YF,VEE	23408	11577
VEE	23410	11578
PT,YF,VEE	23412	11579
PT	23417	
VEE	23418	11580
?	23426	11581
PT	23427	
PT,VEE	23428	11582
PT,VEE	23429	11583
VEE	23430	11584
?	23433	11585
VEE	23434	11586
PT	23437	11587
PT	23439	11588

Table IV

Full Screen Submissions for Grant Period

ASU No.	Number of Fractions	Virus	AVS No.	Priority
B611679	5		6975-6979	High
	8	SFS	9439-9446	
	3		11181-11183	
B619208	4		6980-6983	
B619315	5		6984-6988	High
	6	SFS,VEE,VV	9447-9452	
B619467	7		6989-6995	
	1		6994	
B624784	1		6000	
B630654	5		6996-7000	
B636725	7		7001-7007	
B662371	6		7008-7013	
	1		7009	
B677577	5		7014-7018	
	1		7017	
B680433	1		3966	
B705008	2	HIV	8472-8473	
	6	HIV	8480-8485	
B705028	5	HIV	8433-8437	High
B706399	6	HIV	702, 8467-8471	
B708007	2	HIV	8460-8461	
B708116	6	HIV	8454-8459	
B708122	12	HIV	8419-8430	
B708143	12	HIV	5426-5437	
B710052	16	HIV	8438-8453	
B711932	2	HIV	8465-8466	
B712294	2	HIV	8431-8432	

ASU No.	Number of Fractions	Virus	AVS No.	Priority
B712550	1	HIV	703	
	8	VEE, YF	8343-8350	
B715449	13	HIV	8486-8498	
B716543	3	HIV	8462-8464	
B721116	5	Dengue,JE,YF	6579-6583	
	3	HIV		
B721160	5	VEE	6733-6737	
B721557	4	HIV	8474-8477	
B721562	2	HIV	8478-8479	
B723123	5	YF	9453-9457	High
B723344	14	JE	6738-6751	
B724441	2	SFS	8351-8352	
B724957	3	RNA		
B805951	5		7019-7023	
B818539	5		7024-7028	
B827298	5		7029-7033	High
	5		11184-11188	
	1		7031	
B832245	5		7034-7038	
B832248	5		7039-7043	
B833909	9		7044-7052	
	1		7048	
B835741	6		7053-7058	
B836710	6		7059-7064	
B836749	7		7065-7071	High
	3	YF	9458-9460	
	1			
B841474	4		7072-7075	
B842649	7		7076-7082	

ASU No.	Number of Fractions	Virus	AVS No.	Priority
B848528	5		7083-7087	High
	2		7085, 7087	
	5		11260-11265	
	2		7083, 7087	
B848990	2		11190-11191	High
B853791	11		7088-7098	High
	1		11265	
GRP-23148	3 (a plant)			
GRP-23465	1 (a plant)			

Table V

Special Sample Submissions for Grant Period

Sample	Weight	AVS No.
Lycorine	2 gm.	2563
Pancratistatin	114.9 mg. 41.8 mg. 200 mg.	361
Crude extract with Pancratistatin	1.56 gm.	
TRK-BS-1-10		5787-5796
TRK-BS-5		5791
TRK-BS-8		5794
TRK-BS-13		5797
Trichosanthin (GLQ 223)		5999
Narciclasine	4.09 gm.	2812
Isonarciclasine	200 mg. 1.58 gm.	4223
cis-Dihydronarciclasine	200 mg. 2.0 gm.	4590
trans-Dihydronarciclasine	50 mg. 20 mg. 200 mg.	4591
Balanitin 4-7 (B816351)		6001-6004
cis-Dihydro-7-deoxynarciclasine	40.1 mg. 200 mg.	4592
Streptimidone	2.068 gm.	4796
trans-Dihydro-7-deoxynarciclasine	18.6 mg. 10.1 mg. 100 mg.	4609
iso-7-Deoxynarciclasine	43.7 mg.	4527
Justicidin B	2.0 gm. 2.0 gm.	346
GRP-B3-5B		4742
GRP-B5-5F		4747

Sample		Weight	AVS No.
GRP-B45-1C			6789
GRP-B45-3A			6790
GRP-18072	DiMeVal-Val-MeVal-Pro (a synthetic)		
GRP-18073	Dolapyrrolidone (a synthetic)		
GRP-18074	(a synthetic)		
GRP-18056	(a synthetic)		6793
GRP-18075	(a synthetic)		
GRP-18076	(a synthetic)		
GRP-18077	(a synthetic)		
GRP-18078	(a synthetic)		
GRP-18079	(a synthetic)		
GRP-18080	(a synthetic)		
GRP-22906	(a microorganism)		6791
GRP-23148	(a plant)		6792
SB-D-45E			4102
B631963 - KO84 (NSC 374923)			2793
Dolastatin 15 (1 segment and 3 units)			
Dolastatin 15			
Bryostatin 1		10 x 100 μg	2712
Dolastatin 10		10 x 100 μg	2715
Stylotellin 1	(B722095)		

A P P E N D I X   B

Experimental Summaries
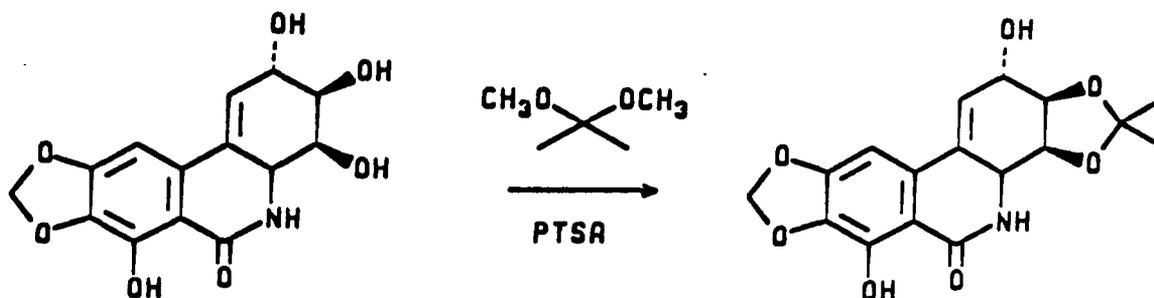
Narciclasine-3,4-acetonide

To a solution of narciclasine (1.0 g, 3.25 mmol) in dimethylformamide (5 mL) and dimethoxypropane (5 mL) was added p-toluene sulfonic acid (100 mg). The solution was stirred at room temperature overnight. Acetonide precipitated out of solution. Pyridine (1 mL) and water (50 mL) was added and the mixture was stirred at room temperature for 30 minutes. The precipitate was collected by filtration, washed with water and dried at 64°C over P_2O_5 under high vacuum to give as an amorphous powder, narciclasine-3,4-acetonide (1.05 g, 92.9%), mp. 275-7°, IR (NaCl) ν_{max} 3500, 3150, 1637, 1625, 1596, 1464, 1437, 1337, 1201, 1079, 1038, 1019 cm^{-1}, ^{1}HNMR δ (CDCl$_3$) 1.39 (s, 3H, CH$_3$), 1.53 (s, 3H, CH$_3$), 2.47 (d, J = 4.1 Hz, 1H, OH), 4.10-4.13 (m, 3H, H-3,4,4a), 4.39 (dd, J = 6.7, 4.1 Hz, 1H, H-2), 6.05 (ABq, J = 1.2 Hz, 2H, -OCH$_2$O-), 6.21 (brs, 1H, NH), 6.32 (dd, J = 3, 1.2 Hz, 1H, H-1), 6.70 (s, 1H, H-10), 9.2 (s, 1H, OH).
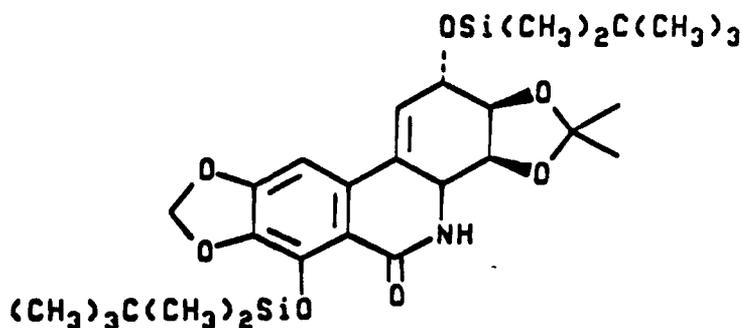
2,7-Di-[(tert-butyldimethyl)-silyloxy]-narciclasine-3,4-acetonide

Diisopropylethyl amine (1.8 ml, 10.35 mmol) was added (under argon) to a heated (60°C) solution of narciclasine 3,4-acetonide (800 mg, 2.3 mmol) in dimethylformamide (8 ml) followed by tert-butyldimethylsilyl chloride (1.04 g, 6.9 mmol). The resulting reddish solution was stirred at room temperature overnight and monitored by TLC (hexane: acetone, 4:1). After completion, water (50 ml) was added and the viscous mixture was poured into ether (450 mL). The ethereal solution was washed with 10% aqueous citric acid (50 mL), water (2 x 100 mL), dried and evaporated under reduced pressure to give a gum which was

crystallized from ethanol to afford colorless flakes of disilyloxy derivative (1.2 g, 90.5%), mp. 207-9°C, $[\alpha]_D^{30}$ +61.2°, (c, 2.5, CHCl$_3$), IR (NaCl) ν_{max} 3250, 2952, 2930, 2857, 1676, 1480, 1381, 1362, 112, 1057, 837 cm^{-1}, ^{1}HNMR δ (CDCl$_3$) 0.148, 0.152 (s, 6H, 2xCH$_3$), 0.219, 0.225 (s, 6H, 2xCH$_3$), 0.945 (s, 9H, C(CH$_3$)$_3$), 1.335 (s, 3H, CH$_3$), 1.467 (s, 3H, CH$_3$), 3.969-4.019 (m, 2H, 2xCH), 4.065 (dd, J = 7.1, 5.2 Hz, 1H, CH), 4.305 (quint, J = 2.5 Hz, 1H, CH), 5.902 (brs, 1H, NH), 5.967 (d, J = 1.2 Hz, 1H, 1/2OCH$_2$O), 5.984 (d, J = 1.2 Hz, 1H, 1/2OCH$_2$O), 6.153 (brt, J = 2.3 Hz, 1H, H-1), 6.799 (s, 1H, H-10).
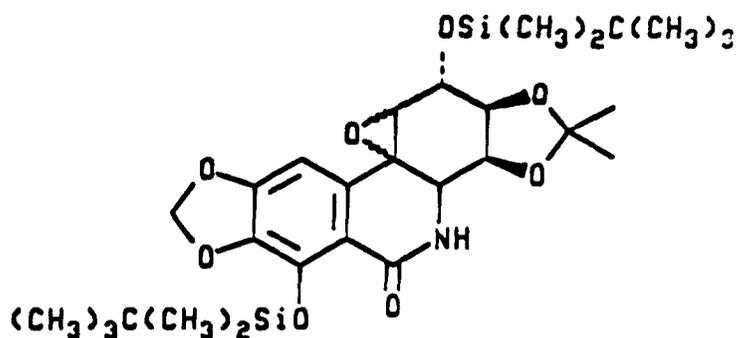
1,10b-(α)-and-(β)-Epoxy-2,7-di-[(tert-butyldimethyl)silyloxy]-narciclasine-3,4-acetonide

To a stirred solution of 2,7-di-[(tert-butyldimethyl)-silyloxy]-narciclasine-3,4-acetonide (240 mg, 0.42 mmol) in CH$_2$Cl$_2$ (10 mL) was added 0.2M phosphate (pH 8.0) buffer (10 ml, prepared from Na$_2$HPO$_4$ and NaH$_2$PO$_4$). The biphasic mixture was cooled to 0°C and m-chloroperbenzoic acid (215 mg, 1.2 mmol) was added and the mixture was stirred 20 min. at 0°C and then 4 hrs. at room temperature. The reaction was carefully monitored (TLC, hexane:acetone, 17:3) and upon completion CH$_2$Cl$_2$ (100 mL) was added. The organic phase was separated and washed with 5% aqueous sodium thiosulfate (2 x 25 mL), water (25 mL), 5% aqueous sodium carbonate (3 x 25 mL), water (25 mL), dried (Na$_2$SO$_4$), and evaporated under reduced pressure to produce a colorless powder. Chromatography (VLC) on neutral SiO$_2$ and elution with hexane-acetone (95:5) gave an inseparable mixture (α:β, ^{1}HNMR of hydrogenolyzed product) of epoxides, (205 mg, 83.3% combined yield), mp. 196-9°C, $[\alpha]_D^{30}$ +97.1° (c, 1.05, CHCl$_3$), IR (NaCl) ν_{max} 3350, 2953, 2930, 1680, 1473, 1361, 1344, 1252, 1106, 1063, 1034, 839, 777 cm^{-1}, ^{1}HNMR δ (CDCl$_3$) 0.155 (s, 6H, SiC(CH$_3$)$_2$), 0.215, 0.242 (s, 3H each, SiCH$_3$), 0.950 (s, 9H, C(CH$_3$)$_3$), 1.007 (s, 9H, C(CH$_3$)$_3$), 1.293 (s, 3H, 1/2C(CH$_3$)$_2$), 1.427 (s, 3H, 1/2C(CH$_3$)$_2$), 3.811 (s, 1H), 3.869 (d, J = 8.0Hz, 1H), 4.158-4.266 (m, 3H), 5.763 (brs, 1H, NH), 5.972 (d, 1H, J = 1.6 Hz, 1H, 1/2OCH$_2$O), 6.014 (d, J = 1.6 Hz, 1H, 1/2OCH$_2$O), 7.294 (s, 1H, H-10), EIMS (m/z) 591 (2%), 576 (10), 534 (100), 518 (4), 476 (10), 430 (10), 344 (4), 316 (6).

OSi(CH₃)₂C(CH₃)₃

(CH₃)₃C(CH₃)₂SiO
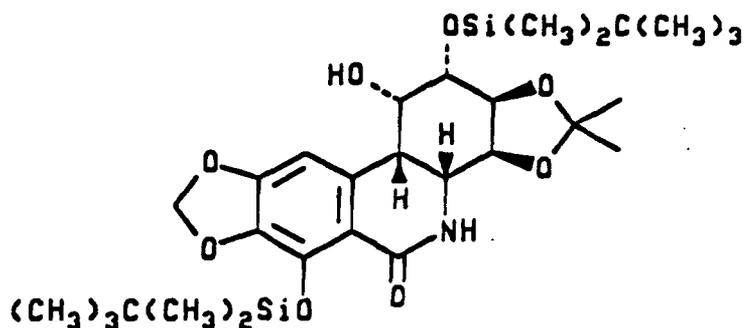
(major product)

1α and 1β-Hydroxy-2,7-di-[(tert-butyldimethyl)silyloxy]-10b, 4a-<u>cis</u> and <u>trans</u>-dihydro-narciclasine-3,4-acetonide

To a solution of epoxide mixture (see above) (100 mg, 0.17 mmol) in methanol:ethylacetate (1:3, 20 mL) was added 10% palladium supported on carbon (100 mg). The reaction mixture was evacuated and flushed with hydrogen (5x) and then hydrogenated at ambient temperature and pressure for 1 hr using a hydrogen filled balloon. The catalyst was removed by filtration and the filtrate concentrated to dryness to give a powder (97 mg), purified on PLC (SiO₂, hexane:ethylacetate, 4:1) to give a mixture (1:17, ¹HNMR analysis) of 1β, 10bα and 1α, 10bβ alcohols (80 mg, 80%, found to lose the phenolic silyl group in solution), as an amorphous powder from acetone-hexane, mp 116-9°, [α]$_D^{30}$ +7.5° (c, 0.55, CHCl₃), IR (NaCl) ν$_{max}$ 3250, 2952, 2929, 1675, 1473, 1382, 1360, 1250, 1220, 1111, 1069, 1042, 839 cm⁻¹, ¹HNMR of major product (1α-hydroxy isomer), δ (CDCl₃) 0.096, 0.158 (s, 3H each, Si(CH₃)₂), 0.231 (s, 6H, C(CH₃)₃), 1.403 (s, 3H, CH₃), 1.531 (s, 3H, CH₃), 2.310 (brs, 1H, OH), 2.926 (brs, 1H, H-10b), 3.745 (dd, J = 7.5, 3.3 Hz, 1H, H-2), 3.828 (brs, 1H, H-4a), 4.109 (dd, J = 5.4, 1.5 Hz, 1H, H-4), 4.196 (d, J = 4.0 Hz, 1H, H-1), 4.217 (dd, J = 7.3, 5.3 Hz, 1H, H-3), 5.115 (brs, 1H, NH), 5.930 (d, J = 1.3 Hz, 1H, 1/20CH₂O), 5.993 (d, J = 1.3 Hz, 1H, 1/20CH₂O), 6.415 (s, 1H, H-10).

OCOCH₃

CH₃CO₂

OCOCH₃

OCOCH₃

CH₃CO₂
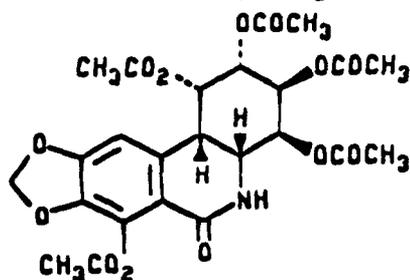
(major product)

1α,2α,3β,4β,7-pentacetyl-10b,4a-<u>cis</u>-dihydro-narciclasine (1α,10bβ-isopancratistatin pentaacetate) and Pancratistatin pentaacetate

To a cooled (0°C) solution of the mixture of silylether (see above, 15 mg, 0.025 mmol) in methanol (2 mL) and water (0.5 ml) was added acetic acid (0.5 ml) and trifluroacetic acid (0.5 ml). The solution was stirred at 0°C for

2 hrs and then stored in a refrigerator overnight. Solvents were removed
under reduced pressure and the resulting product was dried under high vacuum
over phosphorous pentoxide for 4 hrs. The product was then acetylated using
pyridine (0.5 mL) and acetic anhydride (0.5 mL) at room temperature overnight
followed by heating at 60°C for 1 hr. The reaction mixture was quenched with
methanol and the volatile materials were evaporated through azeotropic
distillation with methanol and cyclohexane. Product was found to be a (1:9)
mixture of pancratistatin pentaacetate (detected only by NMR spectrum of the
mixture) and 1α, 10bβ-isopancratistatin pentaacetate. The products were
separated on a column of silica gel by elution with $CH_2Cl_2:CH_3OH$, 99:1 to give
9.0 mg of an amorphous powder from CH_2Cl_2-hexane of 1α,2α,3β,4β,7-pentaacetyl-
10b,4a-cis-dihydro-narciclasine, mp.165-9°, $[\alpha]_D^{30}$ +135 (c, 0.2, $CHCl_3$), IR
(NaCl) ν_{max} 3341, 1778, 1751, 1677, 1481, 1371, 1248, 1226, 1192, 1084, 1035
cm^{-1}, ^1HNMR δ ($CDCl_3$) assignment based on ^1H, ^1H-COSY spectra, 1.893, 1.979,
2.027, 2.166, 2.351 (each s, 3H each, 5 x $OCOCH_3$), 3.305 (t, J - 3.8 Hz, 1H,
H-10b), 3.933 (t, J - 2.5 Hz, 1H, H-4a), 5.405 (dd, J - 10.7, 3.2 Hz, 1H, H-
2), 5.460 (brs, 1H, NH), 5.470 (dd, J - 10.7, 2.3 Hz, 1H, H-3), 5.488 (brs,
1H, H-4), 5.532 (t, J - 3.4 Hz, 1H, H-1), 6.068 (d, J - 1.2 Hz, 1H, 1/2OCH_2O),
6.085 (d, J - 1.2 Hz, 1H, 1/2OCH_2O), 6.626 (s, 1H, H-10), the chemical shift
for NH shifted downfield at δ 5.590 in dilute solutions (ca. 1.5 mg/0.5 mL).
Cis relationship of the protons at H-4a, H-10b and H-1 established by NOE
measurement. Thus strong NOE's were observed between H-10b, H-1, H-2, H-10, H-
4a, and H-4a also gave NOE enhancement to NH). The NOE's also establishes
proof for the chair conformation of ring C.
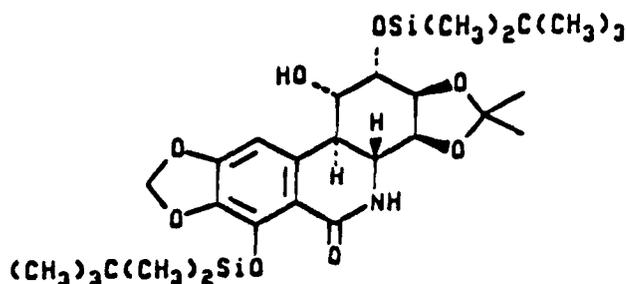
(major product)

1α-Isopancratistatin

Palladium/carbon (10%, 80 mg) was added to the epoxide mixture described
above (80 mg, 0.14 mmol) in anhydrous THF (45 mL) and the hydrogenolysis was
performed as described in the previous experiment for 8 hrs. The filtrate
obtained after removal of the catalyst was concentrated to dryness to give a
2:1 mixture of trans:cis dihydro product, by ^1HNMR analysis. The products
were separated on PLC (hexane-acetone (17:3) to give trans dihydro product (42
mg, 52.3%) and cis dihydro product (20 mg, 24.8%), identical (^1HNMR and TLC)
with the cis product obtained in the previous hydrogenolysis reaction.
Slightly impure trans dihydro product was obtained as an amorphous powder from
acetone-hexane; ^1HNMR δ ($CDCl_3$) of major product: 0.104, 0.138, 0.190, 0.199
(each s, 3H each, 2 x $Si(CH_3)_2$), 0.902, 0.971 (each s, 9H each, 2 x $C(CH_3)_3$),
1.312, 1.433 (each s, 3H each, $C(CH_3)_2$), 2.814 (dd, J - 14.3, 7.7 Hz, 1H, H-
10b), 3.085 (d, J - 3.7 Hz, 1H, OH), 3.457 (dd, J - 14.1, 8.0 Hz, 1H, H-4a),
3.866 (dd, J - 7.2, 5.0 Hz, 1H, H-2), 4.055 (m, 1H, H-1), 4.207 (t, J - 8.5

Hz, 2H, H-3,4), 5.696 (s, 1H, NH), 5.910 (d, J - 1.2 Hz, 1H, 1/2OCH$_2$O), 5.950 (d, J - 1.2 Hz, 1H, 1/2OCH$_2$O), 6.998 (s, 1H, H-10).
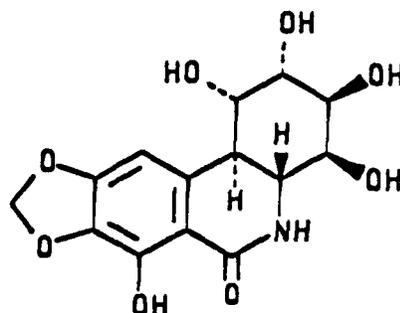

To a cooled (0°C) solution of _trans_ product (25 mg 0.042 mmol) in THF:CH$_3$OH:H$_2$O (1.5:2:1, 4.5 mL) was added acetic acid (0.5 mL) and trifluoroacetic acid (1.0 mL) and stirred at the same temperature for 1 hr. After storing overnight in the refrigerator, the reaction was not complete and required heating to 40°C for 8 hrs. Solvents were removed under reduced pressure and the product was purified by flash chromatography on silica gel. The product, 1α-isopancratistatin (11.1 mg, 81%), eluted with a 9:1 mixture of CH$_2$Cl$_2$:CH$_3$OH and was obtained as an amorphous powder, mp. 325-7, IR (KBr) ν_{max} 3500-3300, 1679, 1470, 1337, 1285, 1210, 1141, 1089, 1064, 802, 725 cm^{-1}, ^{1}HNMR δ (DMSO-d$_6$+D$_2$O) 2.80 (dd, 10.9, 10.9 Hz, 1H, H-10b), 3.31 (dd, J - 13.2, 10.5 Hz, 1H, H-4a), 3.76 (m, 2H), 3.81 (t, J - 3.6 Hz, 1H), 3.84 (brs, 1H), 5.97, 6.00 (only two AB lines visible, 2H, OCH$_2$O), 7.27 (s, 1H, H-10).
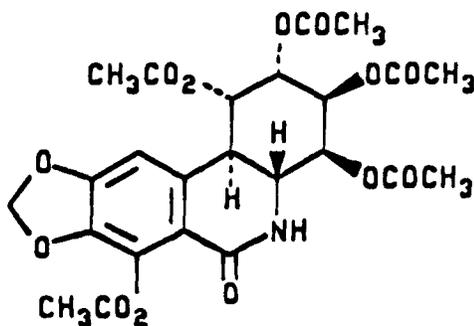
1α,2α,3β,4β,7-Pentaacetyl isopancratistatin

1α-Isopancratistatin (2.7 mg) was treated with acetic anhydride (0.2 mL) in pyridine (0.2 ml) at 50°C for 2 hrs. The mixture, quenched with methanol, was reduced to dryness under a nitrogen stream. Product was chromatographed on a column of silica gel and eluted with CH$_2$Cl$_2$:CH$_3$OH (49:1) to give an amorphous powder of 1α-isopancratistatin pentaacetate (3.4 mg, 76.5%), mp. 146-8, IR (NaCl) ν_{max} 3350, 2930, 2850, 1755, 1676, 1482, 1370, 1248, 1226, 1176, 1084, 1059, 1033 cm^{-1}, ^{1}HNMR δ (CDCl$_3$) 2.06 (s, 3H, COCH$_3$), 2.09 (s, 3H, COCH$_3$), 2.12 (s, 3H, COCH$_3$), 2.18 (s, 3H, COCH$_3$), 2.36 (s, 3H, COCH$_3$), 3.44 (t, J - 11.9 Hz, 1H, H-10b), 3.84 (t, J - 11.0 Hz, 1H, H-4a), 5.25 (dd, J - 10.8,

3.0 Hz, 1H, H-4), 5.42 (dd, J - 11.4, 3.3 Hz, 1H, H-1), 5.44 (t, J - 3.3 Hz, 1H, H-1), 5.53 (t, J - 3.8 Hz, 1H, H-2), 6.06 (d, J - 1.2 Hz, 1H, 1/2OCH₂O), 6.07 (d, J - 1.2 Hz, 1H, 1/2OCH₂O), 6.54 (s, 1H, H-10), spectrum was assigned on the basis of 2D-COSY, and the stereochemistry by NOEDS data.
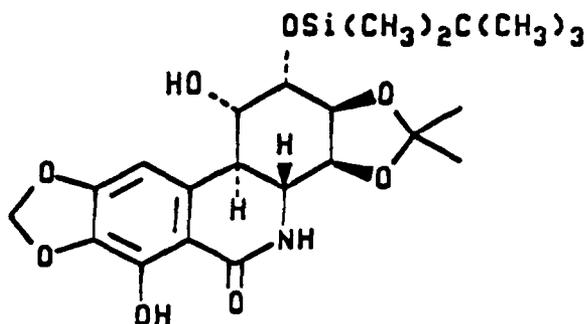
1α-Hydroxy-2-[(tert-butyldimethyl)silyloxy]-10b, 4a-cis and trans-isopancratistatin-3,4-acetonide and 1α-hydroxy-2-[(tert-butyldimethyl)silyloxy]-Δ(10b,4a)-isopancratistatin-3,4-acetonide

The silyloxy epoxide mixture (150 mg) was dissolved in THF (10 mL) and hydrogenolyzed using hydrogen-10% Pd/C (50 mg) as described. Chromatography on a silica gel column and elution with hexane-acetone (7:3) gave 7-desilylated products (interestingly desilation was occurring during the hydrogenolysis reaction as crystallized epoxide was free of benzoic acid by NMR) trans:cis:Δ(10b,4a) in the ratio of (5:3:5). The 10b,4a trans product (50 mg, 38%), crystallized from methanol as shining flakes, mp. 274-5; IR (NaCl) ν_{max} 3530, 3360, 2952, 2939, 1678, 1466, 1373, 1361, 1345, 1260, 1230, 1085, 1071 cm⁻¹; ¹H NMR δ (CDCl₃) 0.15 (s, 3H, SiCH₃), 0.18 (s, 3H, SiCH₃), 0.94 (s, 9H, SiC(CH₃), 1.37 (s, 3H, CH₃), 1.48 (s, 3H, CH₃), 2.90 (dd, J - 14.3, 6.9 Hz, 1H, H-10b), 3.13 (d, J - 2.7 Hz, 1H, OH), 3.57 (dd, J - 14.5, 7.9 Hz, 1H, H-4a), 3.92 (dd, J - 6.8, 5.2 Hz, 1H, H-2), 4.13 (ddd, J - 7.4, 4.6, 2.2 Hz, 1H, H-1), 4.25 (t, J - 6.7 Hz, 1H, H-4), 4.29 (t, J - 6.7 Hz, 1H, H-3), 6.03 (ABq, J - 3.0 Hz, 2H, OCH₂O), 6.04 (brs, 1H, NH), 6.93 (s, 1H, H-10), 12.48 (s, 1H, ArOH), (assignment was made on the basis of a 2D-COSY analysis and stereochemical assignment was accomplished by NOEDS measurement).
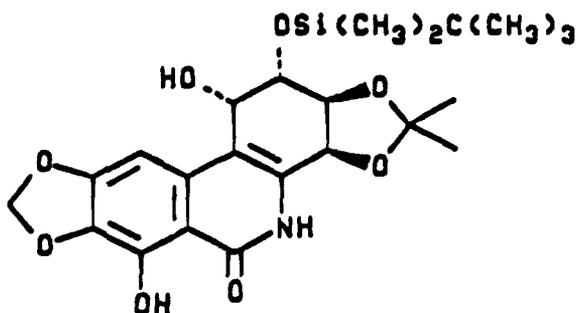
Continued elution of the column with the same solvent gave a mixture of cis and 10b,4a (Δ) product. The cis product could not be separated

but crystallization of the mixture from methanol yielded pure 10b, 4a (Δ) olefinic product (55 mg, 41.4%), recrystallized from methanol as flakes, mp. 266-8; IR (NaCl) ν_{max} 3535 (brs), 2989, 2959, 2931, 2897, 2857, 1677, 1625, 1485, 1422, 1373, 1253, 1215, 1117, 1086, 1036 cm^{-1}; ^{1}H NMR δ (CDCl$_3$) 0.17 (s, 3H, SiCH$_3$), 0.21 (s, 3H, SiCH$_3$), 0.97 (s, 9H, SiC(CH$_3$), 1.49 (s, 3H, CH$_3$), 1.50 (s, 3H, CH$_3$), 2.90 (s, 1H, OH), 3.86 (dd, J = 7.8, 3.5 Hz, 1H, H-2), 4.50 (t, J = 7.5 Hz, 1H, H-3), 4.76 (d, J = 3.4 Hz, 1H, H-1), 5.11 (d, J = 7.0 Hz, 1H, H-4), 6.10 (d, J = 1.6 Hz, 1H, 1/2OCH$_2$O), 6.11 (d, J = 1.6 Hz, 1H, 1/2OCH$_2$O), 6.83 (s, 1H, H-10), 9.85 (s, 1H, NH), 12.65 (s, 1H, OH). Assignment is based on 2D-COSY spectrum and stereochemistry was determined by NOEDS measurement.

Hydrogenolysis of the silyloxy epoxide mixture on a scale better than the one reported here in different solvents (ethyl acetate, mixture of ethyl acetate and methanol) produced similar products. Hydrogenolysis in methanol mostly produced the Δ (10b, 4a) product.
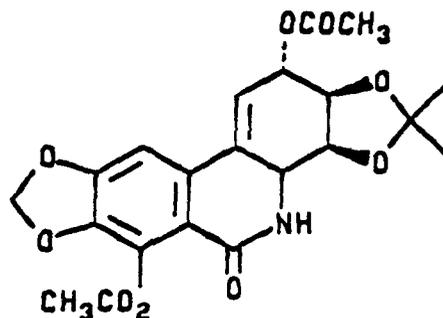

2,7-Diacetoxy-narciclasine-3,4-acetonide

Acetonide was prepared from narciclasine (1 g) as described before and all the solvents were evaporated under reduced pressure to give a crude product which was acetylated with acetic anhydride (3 mL) - pyridine (3 mL) at 60°C for 6 hrs. Solvents were evaporated under reduced pressure after addition of methanol and then chromatographed on a silica gel column and eluted with hexane-ethyl acetate-methylene chloride (3:1:2) to give pure diacetate (1.2 g, 85.4%) as an amorphous powder from acetone-hexane, mp. 130-33 °C; IR (NaCl) ν_{max} 3350, 1775, 1745, 1671, 1482, 1373, 1233, 1210, 1177, 1081, 1031 cm^{-1}; ^{1}H NMR δ (CDCl$_3$) 1.39 (s, 3H, CH$_3$), 1.52 (s, 3H, CH$_3$), 2.21 (s, 3H, COCH$_3$), 2.39 (s, 3H, COCH$_3$), 4.12 (dd, J = 7.8, 7.8 Hz, 1H, H-3), 4.16 (brs, 1H, H-4a), 4.31 (dd, J = 7.5, 5.8 Hz, 1H, H-4), 5.39 (dd, J = 4.9, 2.3 Hz, 1H, H-2), 6.02 (brs, 1H, NH), 6.09 (brs, 2H, OCH$_2$O), 6.12 (t, J = 3.2 Hz, 1H, H-1), 6.98 (s, 1H, H-10).
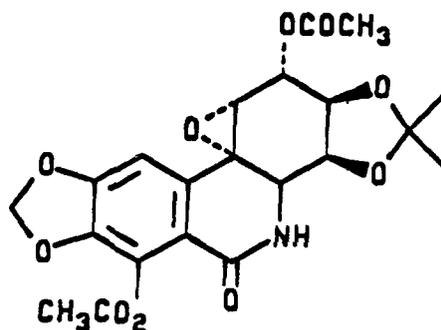
1,10b-(α)-Epoxy-2,7-diacetoxy-narciclasine-3,4-acetonide

To a solution of narciclasine acetonide diacetate (1.0 g, 2.32 mmol) in CH_2Cl_2 (60 mL) was added 0.2 M soadium phosphate buffer (pH 8, 60 mL) followed by m-chloroperbenzoic acid (1.4 g, 3.5 molar equivalent). The reaction mixture was stirred at room temperature overnight and then CH_2Cl_2 (500 mL) was added and the organic layer was separated, washed with 5% solution of sodium thiosulfate (3 x 200 mL), 5% solution of sodium carbonate (3 x 200 mL), water (2 x 100 mL) , dried (Na_2SO_4) and evaporated to give almost pure epoxide as a powder, crystallized from acetone-hexane (700 mg, 67.5%) as amorphous granules, mp. 231-232 °C; IR (NaCl) ν_{max} 3370, 1792, 1749, 1682, 1500, 1365, 1345, 1209, 1175, 1083, 1032 cm^{-1}; ^1H NMR δ (CDCl$_3$) 1.31 (s, 3H, CH$_3$), 1.43 (s, 3H, CH$_3$), 2.20 (s, 3H, COCH$_3$), 2.36 (s, 3H, COCH$_3$), 4.00 (d, J = 6 Hz, 1H, H-4a), 4.03 (s, 1H, H-1), 4.27 (apparent t, J = 8.1 Hz, H-1, H-3), 4.38 (dd, J = 7.9, 6.3 Hz, 1H, H-4), 5.33 (d, J = 6.1 Hz, 1H, H-2), 5.82 (brs, 1H, NH), 6.07 (ABq, J = 1.2 Hz, OCH$_2$O), 6.43 (s, 1H, H-10).

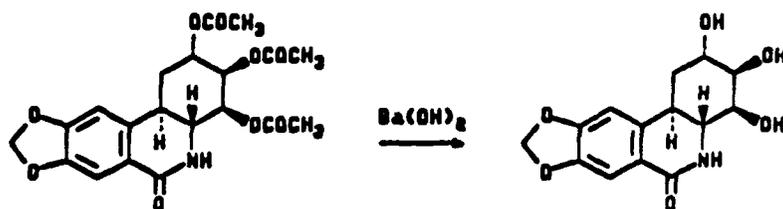SYNTHETIC TRANSFORMATION OF 7-DEOXYNARCICLASINE

7-deoxynarciclasine   iso-7-deoxynarciclasine   trans-dihydro-7-
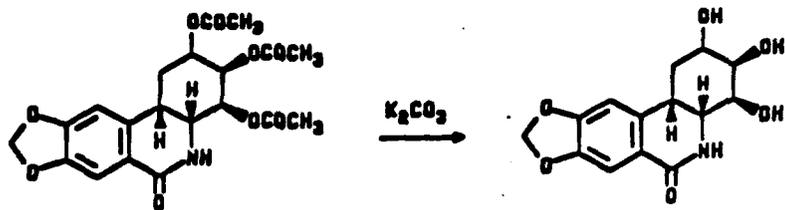-deoxynarciclasine

cis-dihydro-7-deoxynarciclasine
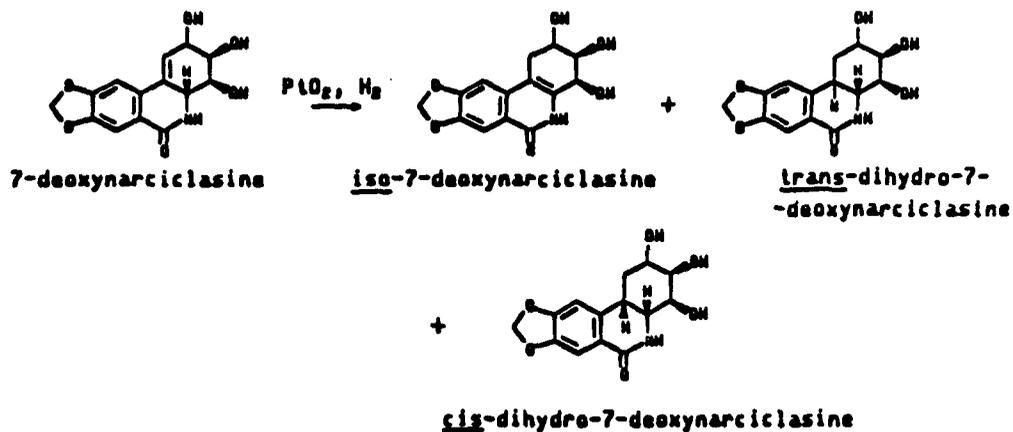
Hydrogenation of 7-deoxynarciclasine. A solution of 7-deoxynarciclasine (1.02
g, 3.4 mmol) in methanol-ethanol (400 ml, 1:1) was degassed with nitrogen,
platinum oxide (57 mg) was carefully added and the resulting mixture was
hydrogenated at ambient temperature and pressure for 24 hrs. The reaction
mixture was filtered through Celite and concentrated in vacuo to afford crude
iso-7-deoxynarciclasine (150 mg, dark brown solid) which crystallized from
pyridine-hexane as a powder (100 mg, 9.8% yield). Identity with earlier
sample of the compound was confirmed by mp and nmr. The mother liquor
contained cis and trans-dihydro-7-deoxynarciclasine.

cis- and trans-isomers   cis- and trans-isomers
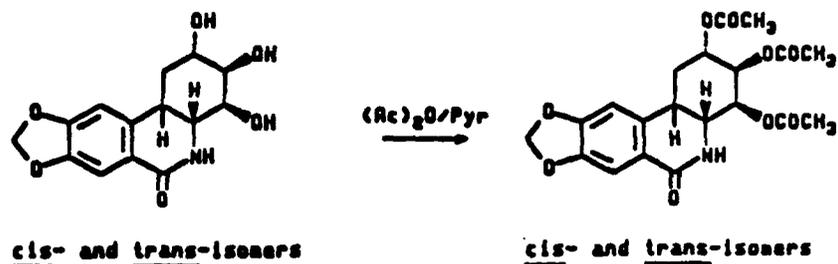
<u>cis and trans-dihydronarciclasine triacetate</u>. The crystallization residue
from <u>iso</u>-7-deoxynarciclasine (see above) was concentrated to dryness and
treated with acetic anhydride (7 ml) and pyridine (10 ml) at 60° for 6 hrs.
Methanol was added and the resulting solution was concentrated to dryness.
The mixture was flash chromatographed over silica gel using CH_2Cl_2:MeOH (99.4-
0.6) twice to furnish <u>trans</u>-dihydro-7-deoxynarciclasine (112 mg, 7.6% yield)
and <u>cis</u>-dihydro-7-deoxynarciclasine (752 mg, 51.2% yield). Identity with
earlier sample of the compound was confirmed by mp and nmr.

<u>Trans-dihydro-7-deoxynarciclasine</u>. To a solution of the triacetate (112 mg)
in methanol (20 ml) was added a saturated solution of barium hydroxide (6 ml).
After heating for 15 min. at 100°C, the mixture was cooled, saturated with
solid CO_2, stirred at room temperature overnight and filtered. The filtrate
was evaporated to dryness and the product was crystallized from methanol to
give <u>trans</u>-dihydro-7-deoxynarciclasine (51 mg, 65% yield). Identity with
earlier sample of the compound was confirmed by mp and nmr.

OCOCH₃ / OCOCH₃ / OCOCH₃ ... K₂CO₃ → OH / OH / OH

cis-dihydro-7-deoxynarciclasine. To a solution of the triacetate (750 mg) in methanol (80 ml) was added potassium carbonate (300 mg). The mixture was stirred at room temperature for 2 hrs, then filtered through a column of Sephadex LH-20. Elution with CH_2Cl_2:MeOH (3:2) removed the product from the column. Crystallization from acetone-MeOH afforded cis-dihydro-7-deoxynarciclasine as crystals (419 mg, 80% yield). Identity with earlier sample of the compound was confirmed by mp and nmr.