Turk J Chem
26 (2002) , 457 – 463.

Terpenoids and Aromatic Compounds from *Euphorbia heteradena*

Sevil ÖKSÜZ, Ayhan ULUBELEN, Aslı BARLA
Faculty of Pharmacy, University of Istanbul, 34452 Istanbul-TURKEY
e-mail: oksuzfatma@hotmail.com
Wolfgang VOELTER
Physiological Institute, University of Tübingen
Hoppe-Seyler Street 4, 7400 Tübingen-GERMANY

Received 30.07.2001

Euphorbia heteradena Jaub. & Spach (Euphorbiaceae), a plant endemic to Turkey, has not been investigated previously. The CH_2Cl_2 extract of the aerial parts of *Euphorbia heteradena* yielded 24-methylenecycloartanol, cycloart-25-en-3β,24-diol, cycloart-22-en-3β,25-diol, vomifoliol, vomifoliol 9-*O* – β-D-glucopyranoside, 3,4,3′-tri-*O*-methoxy ellagic acid, syringic acid and scopoletin. The structures of the isolates were identified by high field spectroscopic methods including 1D and 2D NMR techniques.

Key Words: *Euphorbia heteradena*, Euphorbiaceae, triterpenes, norisoprenoids, coumarins, aromatic acid.

Introduction

The genus *Euphorbia* is the largest genus in the Spurge (Euphorbiaceae) family, comprising more than 1000 species and consists, of about 91 species in Turkey. It contains a toxic, skin-irritant, milky latex. Many biologically active compounds have been isolated from *Euphorbia* plants, which are well known to contain irritant and tumor-promoting constituents[1,2]. *Euphorbia* species have been used in folk medicine in Turkey for the treatment of diarrhea, inflammation and swellings and it is known as a wart remover.[3] In our continuing search for biologically active compounds from the Turkish Euphorbiaceae, we have examined the chemical constituents of *Euphorbia heteradena*, a plant endemic to Turkey, and isolated cyclortanol type triterpenes; 24-methylenecycloartanol (**1**)[4], cycloart-25-en-3β,24-diol (**2**)[5] and cycloart-22-ene-3β,25-diol (**3**)[6], two norisoprenoids; vomifoliol (**4**)[7], vomifoliol 9-*O*-β-D-glucopyranoside (**5**)[8], two coumarins: scopoletin (**6**) and 3,4,3′-tri-*O*-methoxy ellagic acid (**7**)[9], and an aromatic acid: syringic acid (**8**)[10]. We report herein the isolation and structure determination of the above mentioned compounds. A detailed literature survey revealed that no chemical and biological studies have been performed previously with *Euphorbia heteradena*. Therefore, this is the first report on the chemical constituents of *Euphorbia heteradena*.

Experimental

General Procedures

IR spectra were recorded on a Perkin Elmer 983. UV spectra were measured on a Shimadzu UV-1601; optical rotations were measured in an Opt. Act. Ltd. AA-5 model polarimeter. ^{1}H (200 and 250 MHz) and ^{13}C NMR spectra (50.32 and 62.5 MHz) were run on Bruker AC 200 and 250 L. EIMS were recorded on a VG Zabspec instrument (Micromass).

Plant material

Euphorbia heteradena Jaub. & Spach was collected from the south-eastern part of Turkey (Van) in June 1997. The plant was identified by Dr. Şükran Kültür (Faculty of Pharmacy, Department of Botany). A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE:74281).

Extraction and Isolation

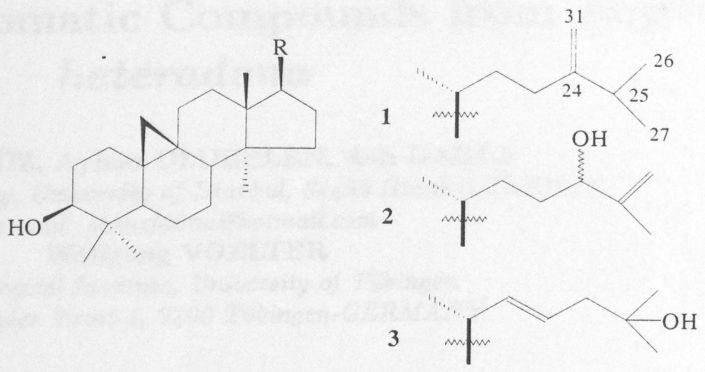
Two kilograms of air-dried and powdered aerial parts of *Euphorbia heteradena* were macerated with EtOH for 3 days and evaporated to dryness under vacuum and kept in a refrigerator at 4°C until use. The residue was dissolved in a small amount of EtOH-H_2O (1:1) and extracted with hexane, in order to remove relatively nonpolar constituents, and then dichloromethane in a separatory funnel. The dichloromethane extract (52.9 g) was subjected to Si gel column (4 x 80 cm) chromatography and eluted with hexane and a gradient of CH_2Cl_2 up to 100% and a gradient of Ac_2CO was added in 10 ml increments into 100 ml CH_2Cl_2 until reaching 100% and followed by EtOH (5%) to yield 48 fractions. Each fraction was monitored on TLC and similar fractions were combined. Further separations were carried out on small Si gel columns when necessary. One-millimeter-thick preparative TLC plates (ready-to-use plates by Merck) were used to purify single compounds.

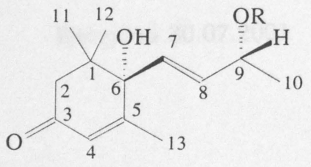
The fractions from which the compounds were isolated and the yields of pure compounds were as follows: **1** [hexane / CH_2Cl_2 (80:20), 19.5 mg], **2** [hexane / CH_2Cl_2 (50:50), 9.7 mg], **3** [hexane / CH_2Cl_2 (50:50), 7.5 mg], **4** [CH_2Cl_2 / Ac_2CO (70:30), 6.3 mg], **5** [CH_2Cl_2 / Ac_2CO (30:70), 4.7 mg], **6** [CH_2Cl_2 / Ac_2CO (40:60), 14.3 mg], **7** [CH_2Cl_2 / Ac_2CO (50:50), 11.3 mg], **8** [CH_2Cl_2 / Ac_2CO (20:80), 5 mg].

24-Methylenecycloartanol (**1**)- UV λ_{max}. (MeOH) nm: 203.5 (ε 2.048); IR ν_{max} ($CHCl_3$) cm^{-1}: 3310, 2917, 2848, 1640, 1462, 1061, 719; EIMS m/z (rel. int %): 440 [M$^+$] $C_{31}H_{52}O$ (11), 409 (100), 393 (81), 355 (15), 313 (6), 301 (25), 269 (12), 173 (29); ^{1}H and ^{13}C NMR see Tables 1 and 2.

Cycloart-25-en-3β,24-diol (**2**) - UV λ_{max}. (MeOH) nm: 203.5 (ε 1.977); IR ν_{max} ($CHCl_3$) cm^{-1}: 3434, 2916, 2848, 1653, 1472, 756; EIMS m/z (rel. int %): 442 [M$^+$] $C_{30}H_{50}O_2$ (8), 424 [M$^+$-H_2O, $C_{30}H_{48}O$] (47), 409 (100), 406 (29), 391 (18), 381 (12), 355 (9), 315 (11), 303 (34), 295 (12), 173 (30); ^{1}H and ^{13}C NMR see Tables 1 and 2.

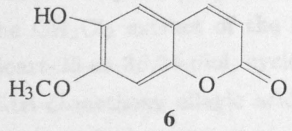
Cycloart-22-en-3β,25-diol (**3**) - UV λ_{max}. (MeOH) nm: 203 (ε 2.003); IR ν_{max} ($CHCl_3$) cm^{-1}: 3423, 3261, 1636, 1457, 1373, 1095, 757; EIMS m/z (rel. int %): 442 [M$^+$] $C_{30}H_{50}O_2$(25), 424 [M$^+$-H_2O, $C_{30}H_{48}O$] (48), 409 ($C_{29}H_{45}O$) (45), 406 (20), 380 (17), 363 (7), 355 (16), 313 (9), 302 (35), 269 (5), 175 (23); ^{1}H and ^{13}C NMR see Tables 1 and 2.



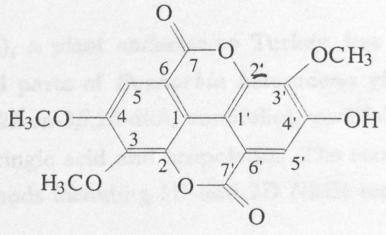


4 R = H

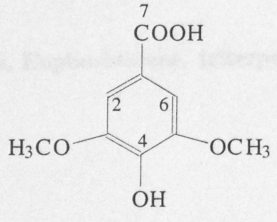
5 R = glucopyranoside



6



7



8

Table 1. ^{1}H NMR Spectral Data of Compounds **1-3** (200 MHz, $CDCl_3$)

H	1	2	3
3	3.28 (dd, J= 5,11 Hz)	3.27 (dd, J= 5, 11 Hz)	3.28 (dd, J=5, 10.5 Hz)
18	0.95 (s)	0.96 (s)	0.96 (s)
19	0.33 (d, J=4 Hz)	0.33 (d, J=4 Hz)	0.33 (d, J=4 Hz)
	0.55 (d, J=4 Hz)	0.54 (d, J=4 Hz)	0.55 (d, J=4 Hz)
21	0.90 (d, J=6 Hz)	0.88 (d, J=6 Hz)	0.86 (d, J=H Hz)
22			5.60 (dd, J=1, 3.5 Hz)
23			5.60 (dd, J=1, 3.5 Hz)
24	4.67 (brs)	4.05 (t, J=7 Hz)	
	4.72 (brs)		
26	1.03 (d, J=7 Hz)	4.82 (brs)	1.31 (s)
		4.93 (brs)	
27	1.03 (d, J=7 Hz)	1.72 (s)	1.31 (s)
28	0.81	0.80	0.81
29	0.90 (s)	0.90 (s)	0.88 (s)
30	0.95 (s)	0.95 (s)	0.96 (s)

Table 2. ^{13}C NMR Spectral Data of Compounds **1-5**, **7-8** (62.89 MHz, $CDCl_3$)

C	1^c	2^c	3^c	C	4^b	$5^{a,b}$	C	7^c	8^b
1	32.0 t	31.9 t	30.5 t	1	41.2 s	31.0 s	1	111.5 s	121.0 s
2	30.4 t	26.5 t	25.5 t	2	49.7 t	50.9 t	2	141.0 s	108.7 d
3	78.9 d	78.9 d	80.8 d	3	207.0 s	197.0 s	3	139.9 s	147.7 s
4	40.5 s	40.45s	39.5 s	4	135.8 d	127.4 d	4	152.5 s	142.0 s
5	47.2 d	47.1 d	47.3 d	5	162.4 s	167.2 s	5	110.2 d	147.7 s
6	21.2 t	21.1 t	20.9 t	6	78.2 s	79.3 s	6	112.3 s	108.7 d
7	28.2 t	28.2 t	28.1 t	7	128.9 d	133.9 d	7	157.7 s	168.9 s
8	48.0 d	48.0 d	47.8 d	8	126.9 d	133.5 d	1′	111.4 s	
9	19.4 s	21.2 s	20.2 s	9	68.0 d	75.0 d	2′	141.5 s	
10	26.5 s	26.5 s	29.6 s	10	27.9 q	22.8 q	3′	140.7 s	
11	26.0 t	26.0 t	25.8 t	11	24.1 q	25.2 q	4′	152.8 s	
12	32.9 t	35.6 t	35.6 t	12	23.7 q	24.0 q	5′	109.8 d	
13	45.4 s	45.4 s	45.4 s	13	18.9 q	20.2 q	6′	112.5 s	
14	48.0 s	48.9 s	48.9 s	1′		1.1.3 d	7′	158.0 s	
15	29.7 t	32.9 t	32.9 t	2′		74.7 d	OCH_3	56.8 q	56.6 q
16	26.6 t	26.6 t	25.5 t	3′		78.3 d	OCH_3	60.0 q	
17	52.3 d	52.2 d	52.1 d	4′		71.6 d	OCH_3	61.4 q	
18	18.1 q	18.1 q	18.0 q	5′		77.9 d			
19	29.7 t	29.8 t	26.6 t	6′		63.0 t			
20	32.9 d	35.9 d	36.5 d	OCH_3					
21	18.4 q	18.4 q	18.3 q						
22	35.1 t	30.4 t	139.3 d						
23	31.4 t	29.5 d	125.7 d						
24	159.8 s	77.1 d	39.1 d						
25	33.9 d	143.5 s	70.7 s						
26	21.9 q	112.5 t	29.1 q						
27	19.4 q	18.4 q	29.8 q						
28	18.1 q	19.4 q	19.3 q						
29	14.0 q	14.0 q	15.2 q						
30	25.5 q	25.5 q	25.5 q						
31	106.1 t								

[a] in CD_3OD, [b] Assignments were done by HETCOR experiments, [c] Assignments were done by comparison with reported data[9,15].

Vomifoliol (Blumenol A) (**4**) - UV λ_{max}. (MeOH) nm: 236 (ε 1.080); IR ν_{max} ($CHCl_3$) cm^{-1}: 3446, 2854, 1667 (enone), 1465, 1374, 1260, 916 (*trans*-disubstituted double bond), 757; EIMS m/z (rel. int %): 223 [M^+-1] $C_{13}H_{20}O_3$ (4), 206 [$C_{13}H_{18}O_2$](10), 168 [$C_9H_{12}O_3$] (27), 151, (14), 150 (24), 135 (21), 125 (34), 124 [$C_7H_8O_2$](100), 123 (26), 111 (28), 107 (20), 83 (76), 79 (21), 69 (20), 57 (12); $[\alpha]_D$ + 231 (*c* 0.97, $CHCl_3$) (lit.[7], $[\alpha]_D$ + 233 (*c* 1, $CHCl_3$);^1H and ^{13}C NMR see Tables 2 and 3.

Vomifoliol 9-*O*-β-D-glucopyranoside (**5**) - UV λ_{max}. (MeOH) nm: 237(ε 1.073); IR ν_{max} ($CHCl_3$) cm^{-1}: 3421, 2924, 1734, 1654 (enone), 1463, 1260, 1077, 970 (*trans*-disubstituted double bond) 757; EIMS m/z (rel. int %): 386 [M^+] $C_{19}H_{30}O_8$ (5), 347 (11), 297 (25), 254 (13), 225 (19), 207 (71), 179 (26), 165 (16), 150 (100), 135 (38), 124 (96), 113 (16), 107 (27), 95 (44), 85 (51), 73 (24), 69 (22), 57 (18); $[\alpha]_D$ + 215 (*c* 0.15, MeOH) (lit.[13], $[\alpha]_D$ + 218 (*c* 0.12, MeOH);^1H and ^{13}C NMR see Tables 2 and 3.

Scopoletin (**6**) - UV λ_{max}. (MeOH) nm: 345.5, 298, 228.5; IR ν_{max} ($CHCl_3$) cm^{-1}: 3333, 1710, 1607, 1566, 1510, 1435, 840, 744; EIMS m/z (rel. int %): 192 [M^+] $C_{10}H_8O_4$ (100), 177 (74), 164 (42), 149

(53), 121 (33), 79 (28), 69 (45);^{1}H and ^{13}C NMR see Tables 2 and 3.

Table 3. ^{1}H NMR Spectral Data of Compounds **4-8** (250 MHz)

H	4*	5**	6*	7*	8*
2a	2.24 (d, J=17 Hz)	2.48 (d, J =17 Hz)			
2b	2.47 (d, J =17 Hz)	2.48 (d, J =17 Hz)			7.37 s
3			6.28 (d, J =9.5 Hz)		
4	5.90 brs	5.90 brs	7.61 (d, J =9.5 Hz)		
5			6.84 s	7.69*** s	
5′				7.78*** s	7.37 s
6					
7	5.85 (d, J =14 Hz)	5.86 (d, J =15 Hz)			
8	5.81 (dd, J =14, 5 Hz)	5.73 (dd, J =7, 15 Hz)	6.92 s		
9	4.38 (dq, J = 5, 6 Hz))	4.45 (dq, J =6.5, 7 Hz)			
10	1.31 (d, J =6 Hz)	1.33 (d, J =6.5 Hz)			
11	1.08 s	1.07 s			
12	1.00 s	1.01 s			
13	1.90 (d, J =1.5 Hz)	1.94 brs			
5′					
1′		4.28 (d, J =7 Hz)			
2′,3′,4′		3.30-3.42 m			
5′		3.23 m			
6′a		3.75 (dd, J =5, 12 Hz)			
6′b		3.87 (dd, J =3, 12 Hz)			
OMe			3.96 s	4.40 s, 4.24 s, 4.05 s	3.95 s
OH					2.64 s

*$CDCl_3$, ** CD_3OD, *** interchangeable

3,4,3′-Tri-O-methoxy ellagic acid (**7**) - UV λ_{max}. (MeOH) nm: 373 (ε 0.375), 358.5 (sh) (ε 0.324), 252 (ε 1.347); IR ν_{max} ($CHCl_3$) cm^{-1}: 3422 (OH), 2923, 2850, 1734 (lactone carbonyl), 1605, 1574, 1458, 1362, 1094, 756; EIMS m/z (rel. int %): 344 [M^{+}] $C_{17}H_{12}O_8$ (100), 329 (35), 301 (16), 296 (27), 258 (9), 230 (8), 172 (10), 103 (6), 64 (28); ^{1}H and ^{13}C NMR see Tables 2 and 3.

Syringic acid (**8**) - UV λ_{max}. (MeOH) nm: 263 (ε 0.423), 210 (ε 1.384); IR ν_{max} ($CHCl_3$) cm^{-1}: 3470 (OH), 2926, 1682 (COOH), 1520, 1463, 1382,1264; EIMS m/z (rel. int %): 198 [M^{+}] $C_9H_{10}O_5$ (100), 183 (39), 167(22), 149 (54), 127 (29), 117 (31), 104 (33), 97 (18), 87 (61), 83 (83), 71 (24), 63 (26);^{1}H and ^{13}C NMR see Tables 2 and 3.

Results and Discussion

From the ^{1}H and ^{13}C NMR assignments, compounds **1-3** were identified as tetracyclic cycloartanol-type triterpenoids, namely, 24-methylenecycloartanol, cycloart-25-en-3β,24-diol, and cycloart-22-ene-3β,25-diol, respectively. The presence of cycloartanol derivatives followed from the typical doublets of the cyclopropane ring bearing two non-equivalent protons (approx. δ_H0.55 and 0.33, d, J= 4.5 Hz) and the chemical shifts of the methyl signals.[11] The differences between **1-3** were on the side chains. Compound **1** showed an exocyclic methylene group at C-24 (δ_H 4.67 brs and 4.72 brs; δ_C 159.8 C-24 and 106.1 C-31); while compound **2** has a side chain terminating in an isopropenyl group (δ 4.82 brs and 4.93 brs, H_2-26 and δ 1.72 s, H-27, δ_C143.5 C-25, 112.5 C-26) and a secondary OH group at C-24 (δ_H 4.05 t, J= 7 Hz), the lowfield shift of the proton under OH group suggested that it was allylic. Compound **3** has an OH group at C-25 (δ_C 70.7 s) and a

double bond between C-22 and C-23 (δ_H 5.60, dd, 2H, J=1, 3.5 Hz, δ_C 139.3 s and 125.7 s) on the side chain. All the spectral data of compounds **1-3** were in good agreement with the data given in references 4, 5 and 6, respectively.

Compounds **4** and **5** were determined to be vomifoliol (or blumenol A) and vomifoliol 9-*O*-β-D-glucopyranoside (or roseoside), respectively, through the comparison of their spectral data with those given in the literature[7,8]. The mass spectra of the two compounds were in full agreement with the proposed structures. The UV absorption of **4** at 236 nm (MeOH) suggested the presence of an enone system, and the IR band at 1667 cm^{-1} supported this finding. The ^1H NMR spectrum showed two tertiary methyls at δ 1.08 (3H, s, H-11) and 1.00 (3H, s, H-12), a secondary methyl at δ 1.31 (3H, d, J= 6 Hz, H-10), a methyl attached to an olefinic carbon at δ 1.90 (3H, d, J= 1.5 Hz, H-13), an isolated methylene group α-to CO at δ 2.24 (2H, d, J = 17 Hz, H-2$_a$) and 2.47 (2H, d, J = 17 Hz, H-2$_b$), and an oxymethine proton at δ 4.38 (dq, J= 6 and 5 H-9). The unsaturation on the side chain was followed by ^1H and ^{13}C NMR resonances at δ 5.81 (dd, J= 5 and 14 Hz, H-8, δ_C126.9) and 5.85 (d, J= 14 Hz, H-7, δ_C128.9). This compound was first isolated from *Rauwolfia vomitoria* and its measured optical rotation was very close to that published by Pouset *et al.*[7]. Therefore the stereochemistry of the asymmetric centers must be the same as 6S and 9R.

The ^1H and ^{13}C NMR spectra of **5** showed the resonances of a sugar moiety and it was found to be identical to vomifoliol 9-*O*-β-D-glucopyranoside by comparison of its spectral data (^1H, ^{13}C, mass, IR and UV spectra) with those in the literature[8,13,14]. Compound **5** has previously been found in *Vinca rosea*[8].

Although **4** and **5** were previously isolated from several plants, this is the first report of the isolation of these compounds from an *Euphorbia* species.

Compound **6** was identified as scopoletin, which is a very well-known compound and widely distributed in higher plants.

Compound **7** gave a dark-blue color under UV light$_{254}$ and a yellow color with alkali, and showed absorptions at 373, 358 (sh) and 252 nm consistent with an ellagic acid derivative. The molecular formula of **7**, $C_{17}H_{12}O_8$, was obtained by EIMS ([M^+] = 344) and the ^1H NMR spectrum displayed only two singlets at δ 7.78 (s, H-5) and 7.69 (s, H-5′) for aromatic protons and three methoxyl singlets at δ 4.40, 4.24 and 4.05. In the ^{13}C NMR spectrum, 17 carbon signals were observed including four (C), two (C=O), six (C-O), two (C-H) and three (CH_3). On the basis of spectral data, compound **7** was identified as 3,4,3′-tri-*O*-methyl ellagic acid, which has previously been reported from *Euphorbia acaulis*[9] and found active against *Bacillus substilis*[12].

Compound **8**, $C_9H_{10}O_5$, was obtained as colorless powder. The ^{13}C NMR spectrum showed the presence of 1,3,4,5-tetra-substituted symmetrical aromatic ring carbons, two equivalent methoxyl carbons and a carboxyl carbon. By means of ^1H and ^{13}C NMR, mass, IR and UV spectral data, **8** was identified as syringic acid.

Acknowledgments

This project was partly supported by a JÜLİCH-TÜBİTAK Research Grant and partly by the Research Fund of the University of Istanbul (Grant No: T-632/190299).

References

1. F. J. Evans and S. E. Taylor, **Prog. Chem. Org. Nat. Prod. 44**, 1-99 (1983).
2. F. J. Evans and C. J. Soper, **Lloydia 41**, 193-233 (1978).
3. T. Baytop, **Therapy with Medicinal Plants in Turkey**, pp 385-386 Istanbul University Press, Istanbul 1984.
4. J. De Pascal Teresa, J. G. Urones, I. S. Marcos, P. Basabe, M. J. Sexmero Cuadroado and F. Moro, **Phytochemistry 26**, 1767-1776 (1987).
5. V. Anjaneyulu, G. Sambasiva and J. D. Connolly, **Phytochemistry 24**, 1610-1612 (1985).
6. S. Öksüz, H-L. Shieh, J. M. Pezzuto, N. Özhatay and G. A. Cordell, **Planta Medica 59**, 472-473 (1993).
7. J. L. Pouset and J. Poisson, **Tetrahedron Lett.**, 1173-1174 (1969).
8. D. S. Bhakuni, P. P. Joshi, H. Uprety and R. S. Kapil, **Phytochemistry 13**, 2541-43 (1974).
9. R. S. Bindra, N. K. Satti and O. P. Suri, **Phytochemistry 27**, 2313-2315 (1988).
10. S. Inoshiri, M. Sasaki, H. Kohda, H. Otsuka and K. Yamasaki, **Phytochemistry 26**, 2811-2814 (1987).
11. V. Anjanejulu, K. H. Prasad, K. Ravi and J. D. Connolly, **Phytochemistry 24**, 2359- 2367 (1985).
12. M. Tada and K. Sakurai, **Phytochemistry 30**, 1119-1120 (1991).
13. H. Achenbach, R. Waibel, B. Raffelsberger and I. A. Mensah, **Phytochemistry 20**, 1591-1595 (1981).
14. R. Andersson and L. N. Lundgren, **Phytochemistry 27**, 559-562 (1988).
15. D. D. Khac, S. T. Van, A. M. Campos, J. Y. Lallemand and M. Fetizon, **Phytochemistry 29**, 251-256 (1990).