ALKALOIDS FROM THE NEUTRAL FRACTION OF *TELITOXICUM KRUKOVII*

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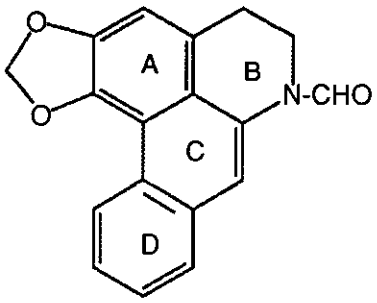
Abstract – Two new aporphinoids *N*-formyldehydroanonaine (**1**), telikovinone (**2**), and the known alkaloids *N*-demethyl-*N*-formyldehydronuciferine, *N*-formylnuciferine, 7-chloro-6-demethylcepharadione B, and norcepharadione B have been isolated and identified from the neutral fraction of *Telitoxicum krukovii*.

South American Menispermaceae have a distinguished place in medicine because eleven species of the four genera, *Chondodendron*, *Abuta*, *Telitoxicum*, and *Sciadotenia* used by native Indians to make pot and/or tube curare[1] were discovered to contain compounds of paralyzing activity.[2] *Telitoxicum* is comprised of eight species.[1,3,4] So far we have reported the presence of oxoaporphines and other aporphinoid compounds from base fractions of two *Telitoxicum* species, *T. glaziovii* and *T. peruvianum* and azafluoranthenes from *T. peruvianum*.[5,6] In continuation of our work on *Telitoxicum* species, we now report the isolation and structural determination of the alkaloids from the neutral fraction of *T. krukovii*. The new alkaloids *N*-formyldehydroanonaine (**1**), telikovinone (**2**), and the known bases *N*-demethyl-*N*-formyldehydronuciferine,[7] *N*-formylnuciferine,[8] two highly fluorescent 4,5-dioxoaporphines, 7-chloro-6-demethylcepharadione B (7-chloronorcepharadione B),[9] and norcepharadione B[10,11] were isolated and identified from spectral data.

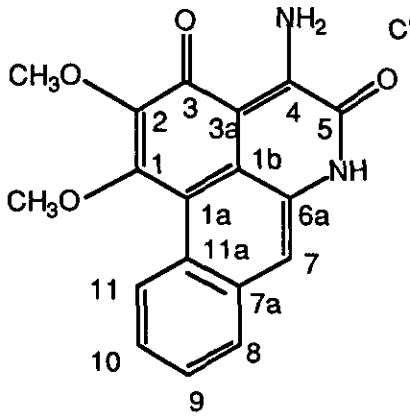
N-Formyldehydroanonaine (**1**) was crystallized from hexane/ethyl acetate to give colorless crystals, mp 185-186°C. The high resolution mass spectrum gave the molecular formula $C_{18}H_{13}NO_3$. The presence of a

carbonyl group at 1674 cm^{-1} and the 6a-7 double bond at 1633 cm^{-1} in the ir along with uv absorption maxima at 246, 268 sh, 288, 330, 356, and 376 nm suggested that this compound might be an *N*-formyldehydroaporphine. The ^1H nmr showed the methylenedioxy group at δ 6.28, H-3 at δ 7.07 and H-7 at δ 7.25 as singlets. The signal at δ 8.92 (1H) indicated the presence of an *N*-formyl group. The remainder of the spectrum consisted of four aromatic multiplets from ring D and two aliphatic triplets from ring B of the aporphine structure. Several aporphines having an *N*-formyl group have been isolated from Menispermaceae; one of them, *N*-demethyl-*N*-formyldehydronuciferine was found to be a mutagen to *Salmonella typhimurium* TA 98 and TA 100.[7,12-14] This is the first time that *N*-formyldehydroaporphines and *N*-formylaporphine were found in the genus *Telitoxicum*.



1

Telikovinone (**2**), $C_{18}H_{14}N_2O_4$, crystallized from MeOH-$CHCl_3$ as blood red needles, mp 275 -277°C (decomp.). The uv spectrum exhibited maxima at 242, 262, 284, 308, 324, 388, 406, 426, and 470 nm. The ir spectrum showed the extended conjugation for the carbonyl groups at 1680 and 1610 cm^{-1}. The ^1H nmr (400 MHz, $CDCl_3$/ CD_3OD) of telikovinone showed two 3H singlets at δ 4.12 and δ 4.00, corresponding to two methoxy groups. In the downfield portion of the spectrum there appeared two aromatic multiplets at δ 7.55 and δ 7.48 (H-10 and H-9), two doublet of doublets at δ 9.34 (H-11) and δ 7.86 (H-8) and one aromatic singlet at δ 7.75 (H-7). These proton chemical shift assignments were based on the homodecoupling data and on nOe data. Based on the spectral data, we can propose five different structures (**2, 3, 4, 5** and **6**) for telikovinone.



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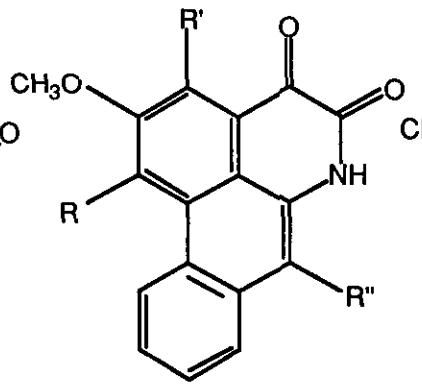
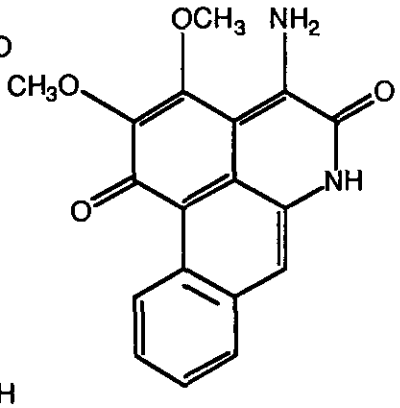


3. R = OCH_3; R' = NH_2; R" = H

4. R = OCH_3; R' = H; R" = NH_2

5. R = NH_2; R' = OCH_3; R" = H



6

A 4,5-dioxo-3-aminoaporphine structure (**3**), a 4,5-dioxo-7-aminoaporphine (**4**), and a 4,5-dioxo-1-aminoaporphine (**5**) were ruled out based on the absence of a M^+ - 28 peak in the mass spectrum, which is due to the loss of C= O from the molecular ion. These 4,5-dioxo and 1,5-dioxo-4-aminoaporphine (**6**) were not possible by nOe data described below.

The nOe saturation of methoxyl at δ 4.12 enhanced the H-11 doublet of doublets at δ 9.34, indicating that it must be attached at C-1. Likewise irradiation of the H-8 doublet of doublets at δ 7.86 enhanced the aromatic singlet at δ 7.75 indicating that it occupies the C-7 position. The nOe and coupling network together described the arrangement of protons in **2**, and these assignments are further confirmed by ^{13}C experiments. In the downfield portion of the ^{13}C nmr spectrum there are eleven quaternary resonances between δ 179.3 and δ 106.7 along with five protonated signals between δ 128.6 and δ 115.9. In the upfield portion of the spectrum there are two methoxyl resonances at δ 61.2 and δ 60.7. The carbon assignments are based on the proton assignments and correlation results.

The HMQC identified the protonated carbons. The HMBC spectrum provided long-range correlations supporting the assignments. Of particular interest are the long range correlations between H-7 at δ 7.75 and C-8 at δ 128.6, C-11a at δ 128.5 and C-1b at δ 116.6. Additionally H-11 at δ 9.34 correlates to C-7a at δ 131.8, C-9 at δ 125.8, and C-1a at δ 117.0. The assignments of C-1 and C-2 at δ 161.2 and δ 145.7 are based on their corresponding long-range heterocorrelations to their respective methoxy protons at δ 4.12

and δ 4.00. The assignments for C-3 through C-5 are made by analogy to another 3,5-dioxo-4-aminoaporphine.[15] Since CD_3OD is necessary to dissolve **2**, the HMBC correlations from the amino group are not available for assignment by correlation. The similarity of chemical shifts, however, ruled out other structural possibilities. For **2** C-3, 3a, 4 and 5 resonate at δ179.3, δ106.7, δ157.3, and δ149.8 respectively as compared to δ179.4, δ106.7, δ156.8, and δ148.6 for another 3,5-dioxo-4-aminoaporphine.[15]

The neutral fraction of *T. krukovii* is found to contain some alkaloids that are not basic enough to be extracted into the alkaloid fraction. 4,5-Dioxoaporphines have been identified from the *Telitoxicum* genus for the first time. In order to address the authenticity of telikovinone as a natural product, extraction of 10g of the plant material left behind was carried out in methanol without ammonium hydroxide. The thin layer chromatography of the crude extract did not show the presence of telikovinone. At this time, we have to conclude that telikovinone is an artifact of the extraction procedure. 7-Chloro-6-demethylcepharadione B was also considered to be an artifact.[16]

EXPERIMENTAL

General Experimental Procedures.- Mps are uncorrected. ^1H- and ^{13}C-nmr spectra were collected using a Bruker AMX 400 system at normal probe temperature in mixed $CDCl_3$/ CD_3OD for telikovinone and at 200 MHz on a WP 200 Bruker spectrometer for the other compounds; chemical shifts are listed in ppm. Ir spectra were recorded on a Mattson Galaxy series Ftir 3000 spectrophotometer and uv spectra on a Hewlett Packard 5842 Diode Array uv/visible spectrophotometer. Mass spectra were recorded on a Kratos-MS 9/50 mass spectrometer at 70 ev.

Plant Material.- The plant material was collected from Peru in 1970 by Schunke. A voucher specimen identified by Dr. B. A. Krukoff was placed in the New York Botanical Garden herbarium.

Extraction and Isolation. - Woody stems were air dried and powdered. Ground plant material (1.6 kg) was moistened with sufficient 1 : 1 NH_4OH : H_2O for five minutes at room temperature and extracted exhaustively with EtOAc-EtOH (9:1) (7 x 5 l). Extract was concentrated and then partitioned between CH_2Cl_2 and 1% H_3PO_4, followed by 2% H_2SO_4 to obtain the CH_2Cl_2 soluble neutral fraction (6.95 g). Acidic extracts were made alkaline with adequate amount of 6M NH_4OH and extracted with CH_2Cl_2. The bases from both phosphoric and sulfuric acid extracts gave identical tlc's and were mixed to give the total

base fraction (0.35 g). The complexity of the thin layer chromatogram and the small amount of the base fraction available prevented the investigation of the alkaloids from it. The neutral fraction was chromatographed over a column of silica gel-60 (420 g) by elution with hexane, hexane-ethyl acetate mixtures, ethyl acetate, ethyl acetate-methanol. Fractions eluted with hexane-ethyl acetate mixtures gave *N*-formyldehydroanonaine, *N*-demethyl-*N*-formyldehydronuciferine, *N*-formylnuciferine while ethyl acetate fractions on repeated column chromatography yielded 7-chloro-6-demethylcepharadione B, norcepharadione B, and telikovinone.

N-Formyldehydroanonaine (**1**).– colorless crystals (1 mg); mp 185-186°C; ir (KBr) ν max 1674, 1633 cm^{-1}; eims (70 ev) m/z (%) $[M]^+$ 291 (100), 263 (33), 262 (44), 235 (11), 232 (11), 204 (24), 176 (18); Hreims m/z calcd for $C_{18}H_{13}NO_3$: 291.0892, found 291.0909; uv λ max (EtOH) 246 (log ε 4.27), 268sh (4.22), 288 (3.79), 330 (3.80), 356 (3.36), and 376 nm (3.31); ^1H nmr {200 MHz, $CDCl_3$} δ 9.03 (1H, m, H-11), 8.92 (1H, s, NCHO), 7.80 (1H, m, H-8), 7.59 (2H, m, H-9 and H-10), 7.25 (1H, s, H-7), 7.07 (1H, s, H-3) 6.28 (2H, s, OCH_2O), 4.16 (2H, t, J = 5.9 Hz, H-5) and 3.22 (2H, t, J = 5.9 Hz, H-4).

Telikovinone (**2**).– blood red crystals (2.2 mg); mp 275-277°C[$CHCl_3$ / MeOH]; ir (KBr) ν max 3420, 3380, 1680, 1610 cm^{-1}; eims (70 ev) m/z (%) $[M]^+$ 322 (100), 307 (76), 291 (26), 279 (63), 264 (22), 236 (58), 208 (32), 179 (20), 153 (31); Hreims m/z calcd for $C_{18}H_{14}N_2O_4$: 322.0950, found 322.0944; uv λ max (EtOH) 242 (log ε 4.01), 262 (4.31), 284 (3.91), 308 (3.56), 324 (3.61), 388 (3.59), 406 (3.83), 426 (3.88), and 470 nm (3.35); ^1H nmr (400 MHz, $CDCl_3$/ CD_3OD) δ 9.34 (1H, dd, J = 2.1 and 8.2 Hz, H-11), 7.86 (1H, dd, J = 2.1 and 8.2 Hz, H-8), 7.75 (1H, s, H-7), 7.55 (1H, m, H-10), 7.48 (1H, m, H-9), 4.12 (3H, s, OCH_3-1) and 4.00 (3H, s, OCH_3-2). ^{13}C Nmr ($CDCl_3$ / CD_3OD) δ 179.3 (C-3), 161.2 (C-1), 157.3 (C-4), 149.8 (C-5), 145.7 (C-2), 131.8 (C-7a), 128.6 (C-8), 128.5 (C-11a), 128.3 (C-6a), 127.2 (C-10), 126.5 (C-11), 125.8 (C-9), 117.0 (C-1a), 116.6 (C-1b), 115.9 (C-7), 106.7 (C-3a), 61.2 (OCH_3-1), 60.7 (OCH_3-2).

Identification of Known Compounds.– *N*-Demethyl-*N*-formyldehydronuciferine (1.7 mg), *N*-formylnuciferine (10.7 mg), 7-chloro-6-demethylcepharadione B (0.5 mg), and norcepharadione B (9.7 mg) were confirmed by ^1H nmr, ir, uv, and ms. Co-tlc was done for norcepharadione B.

ACKNOWLEDGMENTS

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