SCIAFERINE AND *N*-FORMYLANOLOBINE, TWO NEW APORPHINE ALKALOIDS FROM THE NEUTRAL FRACTION OF *SCIADOTENIA TOXIFERA*

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Abstract- The structures of sciaferine (**1**), a new oxoaporphine alkaloid, and *N*-formylanolobine (**2**), both isolated from the neutral fraction of *Sciadotenia toxifera*, were determined by spectral methods.

Sciadotenia toxifera (Krukoff and A. C. Smith), a Peruvian Menispermaceae plant, has been used in folk medicine for the treatment of sterility and malaria as well as an ingredient of curare.[1-3] Seven bisbenzylisoquinolines, (+)-sciadenine, (+)-sciadoline, (+)-sciadoferine, (+) and (-)-isochondodendrines, (+)-*O,O'*-dimethylcurine, and epi-norcycleanine, are the only compounds isolated and identified from the total bases of this species.[3-6] Even though the neutral fraction was not found to contain any antitumor activity, we decided to investigate this fraction in search of new alkaloids. Apart from the typical sterols, trans-stigmasterol, and a variety of sphingolipids, two new minor alkaloids, sciaferine (**1**) and *N*-formylanolobine (**2**), were isolated after repeated column chromatography and preparative TLC followed by crystallization.

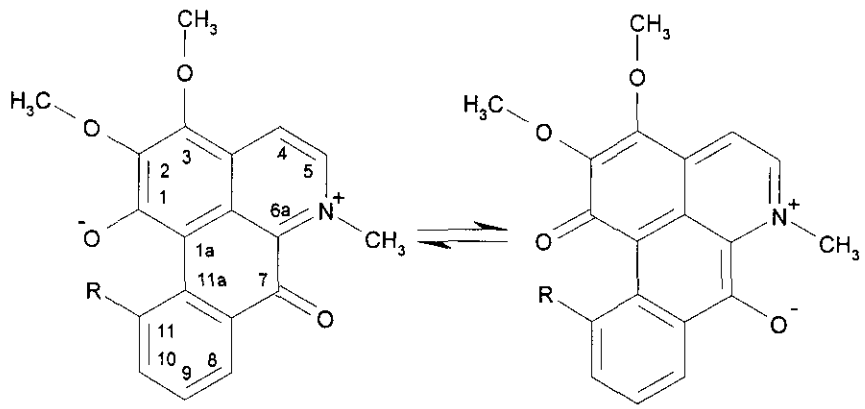
Sciaferine (**1**) was isolated as dark green needles (CH_2Cl_2/MeOH) which decomposed about 293 °C. In the IR spectrum there was a carbonyl absorption at 1626 cm^{-1}. The MS of **1** established a molecular weight of 337 daltons with one exchangeable hydrogen present, and high resolution electrospray MS data (HRESMS) indicated that the molecular formula was $C_{19}H_{15}NO_5$. Compound (**1**) produced a striking dark green color when dissolved in chloroform solution, reminiscent of a recently isolated oxoaporphine alkaloid, teliglazine.[7] Teliglazine, isolated from the basic fraction of *Telitoxicum glaziovii* Moldenke, also produced a dark green color in neutral or basic solution and a pink color in acidic solution due to its ability to tautomerize, as did **1**.

The number of proton signals observed for **1**, along with their chemical shifts, multiplicities, and coupling constants, confirmed that this compound was indeed an oxoaporphine closely related to teliglazine. As was the case with teliglazine, oxoaporhine (**1**) possessed two methoxyl groups on the A ring, OCH_3–2 at δ 4.14 and OCH_3–3 at δ 4.03, as evidenced by mutual nOes between the two methoxyl groups and an nOe

enhancement of the H–4 doublet (J = 6.3 Hz) at δ 8.25 upon saturation of OCH_3–3. Similarly, saturation of the *N*-methyl singlet at δ 4.66 enhanced the H–5 doublet at δ 8.08. Lack of any enhancement at position C-7 was consistent with the proposed 7-oxo position.

The three remaining contiguous aromatic hydrogens in ring D were either at positions 8 – 10 or positions 9 – 11 with a phenolic group at the remaining site. However, since none of the three hydrogens had chemical shifts reflective of teliglazine's downfield H-11 doublet of doublets at approximately δ 10, the hydrogens were positioned at C-8 – 10 with a C-11 hydroxyl group. The chemical shifts of H-8, H-9 and H-10 in ring D were in good agreement with their counterparts in other 7-oxoaporphines. Therefore, **1** is the 11-hydroxy analog of teliglazine. There was an insufficient material available to pursue ^{13}C NMR data.

The tautomers of **1** are analogous to the tautomers previously described for teliglazine.



Tautomers of Sciaferine (**1**, R = OH) and Teliglazine (R = H)

N-Formylanolobine (**2**) was isolated after repeated column chromatography and preparative TLC followed by crystallization. In the IR spectrum there were absorptions at 1650 and 1615 cm^{-1}. The MS of **2** established a molecular weight of 309 daltons with one exchangeable hydrogen present (determined by using ND_3 reagent gas), and HRESMS indicated that the molecular formula was $C_{18}H_{15}NO_4$. A characteristic IR band at 1650 cm^{-1} and the loss of formaldehyde from the parent ion in the CI negative ion MS (ammonia) suggested the presence of a formyl group in **2**.

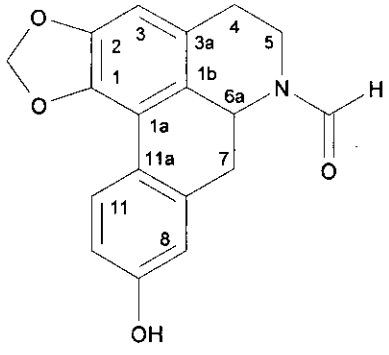
The proton NMR signals of **2** appeared as two discrete sets of resonances (present in a 3:1 ratio) suggesting the possibility of rotamers. The very existence of rotamers and the presence of a sharp one-hydrogen singlet at δ 8.05 (with its minor counterpart at δ 8.18) confirmed the presence of an *N*-formyl group in **2**. There were also four aromatic, two methylenedioxy, and seven aliphatic hydrogens observed for each rotamer. The aromatic signals were separated into two spin systems, an isolated singlet and an ABX spin system, via COSY. In similar fashion the aliphatic signals were also divided into two separate spin systems. The chemical shifts of the major rotamer in $CDCl_3/CD_3OD$ (10:1) are presented in Table 1.

The ^{13}C GASPE NMR spectrum of **2** revealed the anticipated *N*-formyl signal at δ 162.6 along with four aromatic methine, eight aromatic quaternary, one methylenedioxy, one aliphatic methine, and three

aliphatic methylene carbon resonances. By means of HMQC and HMBC correlation data, a traditional aporphine skeleton was mapped out for alkaloid (**2**).

The methylenedioxy group was positioned between C-1 and C-2 of the A ring of **2** based on reciprocal nOe enhancements between the H-3 aromatic singlet at δ 6.37 and the H-4 methylene proton at δ 2.58 in ring B. Confirming HMBC correlations between the methylenedioxy hydrogens at δ 5.91 and 5.80 and C-1 at δ 142.0 and C-2 at δ 147.1 were observed. The phenol group was situated at position C-9 in ring D based on reciprocal nOe enhancements between the H-8 aromatic doublet (J = 1.5 Hz) at δ 6.57 and the H-7 doublet of doublets at δ 2.91 and 2.62 in ring C. Again, HMBC correlations, such as the one between H-11 at δ 7.79 and C-9 at δ 156.5, confirmed this proposal.

The formyl hydrogen singlet at δ 8.05 shared a reciprocal nOe enhancement with H-5 at δ 3.68 and HMBC correlations with C-5 at δ 42.3 and C-6a at δ 49.6 in ring B of the aporphine, verifying that it was an *N*-formyl group. The ^{1}H and ^{13}C assignments, correlations, and nOes for **2** are summarized in Table 1. Based on the spectral data, the structure of **2** was shown to be that of the *N*-formyl analog of the known anolobine.[8]



N-Formylanolobine (**2**)

Table 1. *N*-Formylanolobine (**2**) NMR (400 MHz) Assignments in $CDCl_3/CD_3OD$ (10:1)

C#	^{13}C	^{1}H (ppm)	HMBC to C#	nOe
1	142.0			
1a	117.2			
1b	123.2			
2	147.1			
3	106.2	6.37 (s)	1,1b,2,4	4
3a	126.3			
4	30.6	2.71 (m)	3a,5	3
		2.58 (m)	3,3a,5	3
5	42.3	3.68 (ddd, 2.0, 4.5, 13.0)	3a,4,6a,formyl	formyl

		3.25 (ddd, 3.1, 12.8, 13.0)	formyl	4
6a	49.6	4.80 (dd, 4.7, 13.8)	1b,7	7
7	33.6	2.91 (dd, 4.7, 14.0)	1b,6a,7a,8,11a	6a, 8
		2.62 (dd, 13.8,14.0)	1b,6a,7a	6a, 8
7a	136.6			
8	115.1	6.57 (d, 1.5)	7,9,10,11a	7
9	156.5			
10	113.9	6.62 (dd, 1.5, 8.5)	8,9,11a	11
11	128.5	7.79 (d, 8.5)	1a,7a,9	10
11a	122.0			
O-CH_2-	100.7	5.91 (d, 1.4)	1,2	
		5.80 (d, 1.4)	1,2	
***N*-formyl**	162.6	8.05 (s)	5,6a	5

EXPERIMENTAL

General Experimental Procedures. Melting points were measured on a Fisher Johns melting point apparatus and are uncorrected. UV spectra were collected on a Hewlett Packard 5842 diode array UV/VIS spectrophotometer. IR spectra were collected *via* FT-IR transmission microscopy using a US-Magna IR-760/NICPLAN instrument with no sample preparation. Low resolution DCI MS was performed on a Finnigan Model 4610 quadrupole mass spectrometer using methane, ammonia, and perdeuterioammonia as CI reagent gases in both the positive and negative ion mode. High resolution ESMS were acquired on a Finnigan T70 Newstar FT/MS. The ^1H NMR data were collected in $CDCl_3/CD_3OD$ (10:1) at 25 ^oC using a Bruker AMX400 NMR equipped with an inverse probe. Analytical and preparative TLC were carried out on precoated Si gel G (Kiesel gel G254) plates, and reagent grade chemicals (Fisher) were used throughout.

Plant Material. The plant material (collected from two bush-ropes) was collected in San Martin, Peru in 1977 by Schunke. A voucher specimen identified by Dr. B. A. Krukoff was placed in the New York Botanical Garden herbarium.

Extraction and Isolation. The ground plant material (4.2 Kg) was moistened with 1:1 NH_4OH:H_2O and extracted exhaustively with 9:1 EtOAc-EtOH (4 x 16 L) for two weeks at room temperature. The extract was concentrated and then partitioned between CH_2Cl_2 (1.25 L) and 2% H_2SO_4 (3 x 0.5L) to obtain the CH_2Cl_2 soluble neutral fraction. This neutral fraction (19.5 g) was chromatographed over silica gel-60 eluting with various hexane-CH_2Cl_2 mixtures (progressing from 4:1 to 1:9), then pure CH_2Cl_2, then CH_2Cl_2-MeOH mixtures (progressing from 99.5:0.5 to 70:30) and finally pure MeOH. The earlier fractions yielded stigmasterol (95 mg, from a fraction in pure CH_2Cl_2), followed by various sphingolipids (from a fraction in CH_2Cl_2-MeOH 99:1), and finally the two new alkaloids, sciaferine (**1**) (1 mg) and *N*-formylanolobine (**2**) (2 mg), both from a fraction in CH_2Cl_2-MeOH 98:2. All of the alkaloids from the

neutral fraction were obtained in pure form only after a second, and sometimes third, chromatographic separation and recrystallization.

Sciaferine (1): dark green needles (1.0 mg, CH_2Cl_2/MeOH), mp >293 °C; UV (EtOH) λ_{max} (log ε) 240 (sh, 2.96), 318 (2.84), 422 (2.09), 632 (1.94) nm; UV (EtOH + 5% HCl) λ_{max} (log ε) 256 (sh, 3.35), 316 (3.35), 410 (3.14), 486 (3.10), 582 (3.10) nm; IR ν_{max} 2660, 2587, 2493, 1626, 1586, 1336, 1273 cm^{-1}; HRESMS *m/z* 337.09502; calcd for $C_{19}H_{15}NO_5$, 337.09499; DCIMS (positive, NH_3) *m/z* 338 (100, $[M + H]^+$), 324 (5); ^1H NMR ($CDCl_3/CD_3OD$ 10:1, 400 MHz) δ 8.25 (1H, d, *J* = 6.3 Hz, H-4), 8.08 (1H, d, *J* = 6.3 Hz, H-5), 7.82 (1H, dd, *J* = 1.6, 7.7 Hz, H-8), 7.28 (1H, dd, *J* = 7.7, 7.8 Hz, H-9), 7.21 (1H, dd, *J* = 1.6, 7.8 Hz, H-10), 4.66 (3H, s, NCH_3-6), 4.14 (3H, s, OCH_3-2), 4.03 (3H, s, OCH_3-3).

***N*-Formylanolobine (2):** purple/grey needles (2.0 mg, CH_2Cl_2), mp 284 °C (decomp); UV (MeOH) λ_{max} (log ε) 246 (2.80), 282 (2.80), 294 (2.81), 310 (2.79) nm; UV (MeOH + 0.1M NaOH) λ_{max} (log ε) 244 (2.80), 256 (sh, 2.76), 282 (2.80), 294 (2.81), 316 (2.82), 332 (2.83) nm; IR ν_{max} 3291, 2777, 1650, 1615, 1567, 1390, 1227, 1043, 936 cm^{-1}; HRESMS *m/z* 309.10012; calcd for $C_{18}H_{15}NO_4$, 309.10011; DCIMS (positive, NH_3) *m/z* 327 (100, $[M + NH_4]^+$), 310 (67, $[M + H]^+$), 299 (5), 282 (6), 280 (5); DCIMS (negative, NH_3) *m/z* 344 (10, $[M + Cl]^-$ from residual CH_2Cl_2), 308 (100, $[M - H]^-$), 279 (2); ^1H and ^{13}C NMR in $CDCl_3/CD_3OD$ (10:1) see Table 1.

Identification of known compounds: Trans-stigmasterol (95.0 mg) and the mixed sphingolipids (3.4 mg) were identified by spectral methods.

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