

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'*-dimethylurine, 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.

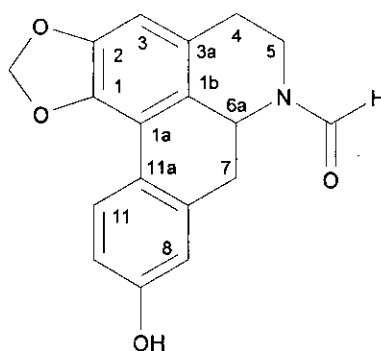
Sciaferine (**1**) was isolated as dark green needles (CH₂Cl₂/MeOH) which decomposed about 293 °C. In the IR spectrum there was a carbonyl absorption at 1626 cm⁻¹. The MS of **1** established a molecular weight of 337 daltons with one exchangeable hydrogen present, and high resolution electrospray MS data (HRESMS) indicated that the molecular formula was C₁₉H₁₅NO₅. 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, oxoaporphine (**1**) possessed two methoxyl groups on the A ring, OCH₃-2 at δ 4.14 and OCH₃-3 at δ 4.03, as evidenced by mutual nOes between the two methoxyl groups and an nOe

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 ^1H 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 $\text{CDCl}_3/\text{CD}_3\text{OD}$ (10:1)

C#	^{13}C	^1H (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 CDCl₃/CD₃OD (10:1) at 25 °C 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 NH₄OH:H₂O 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 CH₂Cl₂ (1.25 L) and 2% H₂SO₄ (3 x 0.5L) to obtain the CH₂Cl₂ soluble neutral fraction. This neutral fraction (19.5 g) was chromatographed over silica gel-60 eluting with various hexane-CH₂Cl₂ mixtures (progressing from 4:1 to 1:9), then pure CH₂Cl₂, then CH₂Cl₂-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 CH₂Cl₂), followed by various sphingolipids (from a fraction in CH₂Cl₂-MeOH 99:1), and finally the two new alkaloids, sciaferine (1) (1 mg) and *N*-formylanobine (2) (2 mg), both from a fraction in CH₂Cl₂-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, $\text{CH}_2\text{Cl}_2/\text{MeOH}$), mp $>293^\circ\text{C}$; 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 $\text{C}_{19}\text{H}_{15}\text{NO}_5$, 337.09499; DCIMS (positive, NH_3) m/z 338 (100, $[\text{M} + \text{H}]^+$), 324 (5); ^1H NMR ($\text{CDCl}_3/\text{CD}_3\text{OD}$ 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 $\text{C}_{18}\text{H}_{15}\text{NO}_4$, 309.10011; DCIMS (positive, NH_3) m/z 327 (100, $[\text{M} + \text{NH}_4]^+$), 310 (67, $[\text{M} + \text{H}]^+$), 299 (5), 282 (6), 280 (5); DCIMS (negative, NH_3) m/z 344 (10, $[\text{M} + \text{Cl}]^-$ from residual CH_2Cl_2), 308 (100, $[\text{M} - \text{H}]^-$), 279 (2); ^1H and ^{13}C NMR in $\text{CDCl}_3/\text{CD}_3\text{OD}$ (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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