Eletefine, a Stephaoxocane Alkaloid from *Cissampelos glaberrima*

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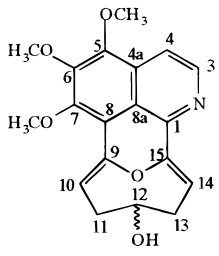
Received January 23, 1998

A novel isoquinoline alkaloid bearing an oxocane ring (stephaoxocane type (Kashiwaba, N.; Morooka, S.; Kimura, M.; Ono, M.; Toda, J.; Suzuki, H.; Sano, T. *J. Nat. Prod.* **1996**, *59*, 803)), has been isolated from the roots of *Cissampelos glaberrima*. This compound was given the trivial name eletefine (**1**) and its structure assigned on the basis of spectroscopic data and conversion to the corresponding ketone (**2**).

Continuing our investigations on the alkaloids of Menispermaceae found in Paraíba State (northeastern Brazil),[2,3] an alkaloid extraction procedure[2] was performed on the roots of *C. glaberrima*. This species is popularly known as "jarrinha" and is used for the treatment of the symptoms of asthma and urinary infections.[4] TLC chromatograms of the extract revealed two yellow fluorescent spots at 360 nm that were easily isolated by preparative TLC. It was interesting though, that after isolation (within about 48 h) the separated bands reverted to the initial mixture. This mixture was eletefine. Herein, we describe the isolation and structure elucidation of the novel alkaloid **1**.



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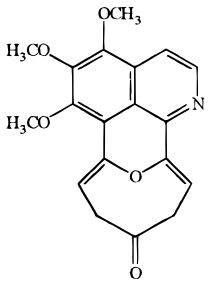

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As outlined above, **1** appeared to consist of a mixture of isomers that may exist in two forms in equilibrium: with, or without, intramolecular H-bonding from the OH to the oxygen of the oxocane ether bridge. It is, however, possible to perform NMR analysis of the isolated isomers before they revert to the equilibrium state. The mixture (**1**) is a reddish-brown wax, and its IR spectrum showed bands at 3403, 2928, 1457, 1400, and 1033 cm^{-1}. The electron impact mass spectrum showed a $[M]^+$ of 341, which is consistent with the proposed molecular formula $C_{19}H_{19}NO_5$.

The NMR study of **1** (^1H, ^{13}C, HMBC (optimized for $J = 7$ Hz), HC-COBI, $^1H-^1H$-COSY, and NOESY) led to unambiguous assignments of all functional groups. The ^1H NMR (400 MHz, $CDCl_3$) spectrum showed one s at δ 3.93 (3H) and one s at δ 4.03 (6H), assigned to three methoxy groups; an AB ($J = 5.8$ Hz) quartet with doublets centered at δ 8.32 and 7.70, integrating for 1 H each assignable to the protons H-3 and H-4, respectively; a dd ($J = 5.7$, 8.4 Hz) at δ 5.86 and another very alike signal at δ 6.02, integrating for 1 H each and indicative of protons in a double bond are assigned to the protons H-10 and H-14, respectively; four sets of m at δ 2.46, 2.50, 3.30, and 3.37 each integrating for 1 H are designated to the methylene protons at C-11 and C-13; one *t* ($J = 7.0$ Hz) at δ 3.59 indicating a proton in a carbinol carbon point to the presence of a hydroxyl at position C-12. The JMOD ^{13}C NMR spectrum confirmed the presence of the three methoxy groups proposed above and also indicated by their chemical shifts at δ 61.4, 61.5, and 61.8 that they are all sterically hindered. The presence of a secondary carbinol is also confirmed by a negative signal at δ 71.8; two olefinic carbons bearing oxygen are also observed by positive signals δ 153.9 and 158.3 and assigned to the carbons C-9 and C-15. Analysis of the HMBC correlations gives strong indications of the actual position for the connections between the oxocane ring and the isoquinoline nucleus; this can be demonstrated by correlations from H-10 (δ 5.86) to C-8 (δ 119.0) and from H-14 (δ 6.02) to C-1 (δ 150.7). The fact that H-10 and H-14 also correlate with C-12 shows clearly the symmetry of the oxocane ring. HMBC also shows to which carbons each methoxy is bonded. Analysis of the NOESY spectrum demonstrated that 7-COMe correlates strongly with H-10,

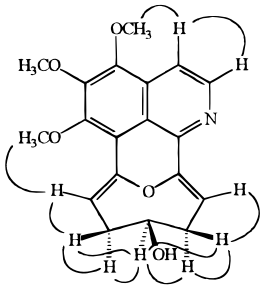

Figure 1. Main NOE correlations for compound **1**.

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S0163-3864(98)00018-4 CCC: $15.00
Published on Web 07/31/1998

Table 1. ^{1}H, ^{13}C, and HMBC NMR Data of Eletefine (**1**) Recorded in $CDCl_3$

			HMBC	
C	H	^1J	^2J	^3J
1		150.7		
3	8.32, 1H, d (J = 5.8 Hz)	141.9	114.5	129.5, 150.7
4	7.70, 1H, d (J = 5.8 Hz)	114.5	141.9	120.7, 146.9
4a		129.5		
5		146.9		
6		147.8		
7		148.8		
8		119.0		
8a		120.7		
9		153.9		
10	5.86, 1H, dd (J = 5.7, 8.4 Hz)	114.8	38.2, 153.9	119.0, 71.8
11	2.46, 1H, m	38.2	71.8, 114.8	38.0, 153.9
	3.37, 1H, m			
12	3.59, 1H, t (J = 7.0 Hz)	71.8		
13	2.50, 1H, m	38.0	71.8, 113.8	38.2, 158.3
	3.30, 1H, m			
14	6.02, 1H, dd (J = 5.7, 8.4 Hz)	113.8	38.0, 158.3	71.8, 150.7
15		158.3		
5-OMe	4.03, 3H, s	61.8		146.9
6-OMe	4.03, 3H, s	61.5		147.8
7-OMe	3.98, 3H, s	61.4		148.8

being one more confirmation for that side of the connection between the isoquinoline nucleus and the oxocane ring. The 5-COMe correlates with H-4, which correlates with H-3. Other important correlations point to the stereochemistry in the oxocane ring, where it is possible to see a strong correlation between the olefinic protons at C-10 and C14 positions with the semiequatorial protons at C-11 and C-13 positions, respectively. The same semiequatorial protons at C-11 and C-13 also present strong correlations with the proton at C-12. Weak correlations are observed between the semiaxial protons at C-11 and C-13 and the proton at C-12. The main NOE correlations of **1** are presented in Figure 1.

Oxidation of the hydroxy group in C-12 to a ketone, using a reaction involving pyridinum dichromate in an aprotic media,[5] led to oxoeletefine (**2**), a single compound. This compound does not convert to a mixture; this feature confirmed that the property of reverting always to the equilibrium state mixture could be somehow related to the hydroxy group at C-12.

C. glaberrima has been shown[3] to produce other benzylisoquinoline-derived alkaloids such as magnoflorine, oxobuxifoline, and cissaglaberrimine.

To date, only four alkaloids of this type have been described in the literature, and all were isolated from plants belonging to the family Menispermaceae. They are as follows: excentricine[6] and 2-*N*-methylexcentricine[7] isolated from *Stephania excentrica* and stephaoxocanine[1] and stephaoxocanidine[8] isolated from *Stephania cepharantha*.

Experimental Section

General Experimental Procedures. VLC was carried out on silica gel H (TLC mesh). Preparative TLC (0.5 mm thick layer) was carried out on silica gel 60 PF_{254} ($CHCl_3$/MeOH 48:1); spots were detected using UV at 254 and 360 nm and Dragendorf's reagent. The IR spectrum was obtained in dry film. EIMS was obtained using direct-insertion probe at 70 eV (HP 5988A). NMR data were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C (BRUKER AMX 400). Chemical shifts are reported in ppm relative to the solvent $CDCl_3$.

Plant Material. Roots of *C. glaberrima* St. Hill (*C. pareira* Vell) were collected in January 1995 at the city of Santa Rita, PB, Brazil. A voucher specimen (Agra &Gois 3326-JPB) is deposited at the Herbarium Lauro Pires Xavier of the Universidade Federal da Paraíba.

Extraction and Isolation. Dried ground root (1 kg) was extracted with 80% EtOH at room temperature for 4 days. This extract, after concentration under reduced pressure, was dissolved in 3% HCl, filtered over Celite, and extracted with $CHCl_3$. The $CHCl_3$ extract was subject to VLC and successive preparative TLC to afford **1** (97 mg).

Eletefine (1): reddish-brown wax; fluorescent under UV (360 nm); IR (dry film) ν_{max} 3403, 2928, 1457, 1400, 1033 cm^{-1}; EIMS *m/z* (rel int) 341 $[M]^+$ (80), 326 $[M - CH_3]^+$, (100), 298 $[326 - CO]^+$ (51), 297 $[M - CH_2{=}CHOH]^+$ (15) and 282 $[297 - CH_3]$ (20); complete NMR assignments are given in Table 1.

Oxoeletefine (2): yellowish-red wax; IR (dry film) ν_{max} 2939, 1699, 1457, 1402 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.37 (1H, d, J = 5.9 Hz, H-3), 7.74 (1H, d, J = 5.9 Hz, H-4), 5.89 (1H, dd, J = 4.6, 7.8 Hz, H-10), 4.02 (1H, dd, J = 4.6, 12.8 Hz, H-11), 3.14 (1H, ddd, J = 1.4, 7.8, 12.8 Hz, H-11), 3.98 (1H, dd, J = 4.4, 13.0 Hz, H-13), 3.17 (1H, ddd, J = 1.4, 7.8, 13.0 Hz, H-13), 6.05 (1H, dd, J = 4.4, 7.8 Hz, H-14), 4.06 (3H, s, 5-OMe), 4.04 (3H, s, 6-OMe), 4.01 (3H, s, 7-OMe); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 149.8 (C-1), 142.3 (C-3), 114.9 (C-4), 129.6 (C-4a), 147.4 (C-5), 147.8 (C-6), 149.3 (C-7), 117.9 (C-8), 120.2 (C-8a), 150.7 (C-9), 112.0 (C-10), 44.3 (C-11), 204.3 (C-12), 44.2 (C-13), 111.1 (C-14), 154.9 (C-15), 61.9 (5-OMe), 61.6 (6-OMe), 61.5 (7-OMe).

Acknowledgment. E.V.L. da-C. and M.L.C. thank CNPq−Brazil for studentships. The group acknowledges M. de Fátima Agra and Gilvani Gois (Laboratório de Tecnologia Farmacêutica, Universidade Federal da Paraíba) for the collection and identification of plant material. NMR spectra were obtained at the NMR laboratory of the University of Strathclyde.

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NP980018B