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CISSAGLABERRIMINE, AN APORPHINE ALKALOID FROM *CISSAMPELOS GLABERRIMA*

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Key Word Index—*Cissampelos glaberrima*; Menispermaceae; stem; leaves; aporphine; cissaglaberrimine; magnoflorine; oxobuxifoline; NMR.

Abstract—A novel aporphine alkaloid was isolated from dried stems and leaves of *Cissampelos glaberrima*. Spectroscopic analysis established the structure as 1,2-methylenedioxy-3-hydroxyaporphine, to which we have given the name cissaglaberrimine. Unambiguous ^1H and ^{13}C NMR data for magnoflorine and oxobuxifoline are described. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Continuing our phytochemical study of *Cissampelos* species found in Paraiba State (northeast Brazil) [1], and based on the normally large amount of alkaloids of various kinds found in the Menispermaceae [2, 3], we decided to perform an alkaloid extraction procedure [1] using the leaves and stems of *C. glaberrima*. This species is popularly known as 'jarrinha' and is used for the treatment of numerous diseases [4]. After extraction, a mixture of different alkaloids was isolated. Herein, we describe the isolation and structural elucidation of the novel aporphine alkaloid cissaglaberrimine (**1**). We also isolated (+)-magnoflorine and oxobuxifoline and give, for the first time, unambiguous assignments of all the protons and carbon atoms, based on one- and two-dimensional NMR data of magnoflorine (**2**), previously reported from many sources [5], and of oxobuxifoline (**3**), which is encountered only in *Duguetia obovata* (Annonaceae) [6].

RESULTS AND DISCUSSION

From the total tertiary alkaloid (**TTA**) fraction obtained from dried leaves and stems, a mixture of two aporphine alkaloids was obtained. Fractionation of the ethanol extract with petrol (60–80°), $CHCl_3$ and MeOH, led to the isolation of oxobuxifoline (4 mg) from 5 g of the petrol fraction after silica gel column chromatography.

Cissaglaberrimine was a red–brown amorphous powder and its UV spectrum showed absorptions at 225, 242, 278 and 300 (shoulder) nm which are characteristic of 1,2,3-substituted aporphinoids [7, 8]. The IR spectrum showed bands at 3450, 2925, 1636, 1458, 1387 and 1060 cm^{-1}. The electron impact mass spectrum showed a $[M]^+$ which agreed with the molecular formula $C_{17}H_{15}NO_3$; no other significant fragments were observed.

The NMR study (^1H, ^{13}C, HMBC (optimized for $J = 7$ Hz), HC-COBI, ^1H–^1H COSY and NOESY) led to unambiguous assignment of all functional groups. The ^1H NMR (400 MHz, $CDCl_3$) spectrum showed an AB quartet ($J = 1.2$ Hz) with doublets centred at δ 6.11 and 5.95, which is characteristic of a methylenedioxy group at C-1,2 in aporphines [8]. The presence of an ABCD system with signals at δ 7.24 (*dt*, $J = 1.1$, 7.2 Hz), 7.30 (*br d*, $J = 7.2$ Hz), 7.41 (*br t*, $J = 7.2$ Hz) and 8.30 (*d*, $J = 7.2$ Hz) indicated that ring D is unsubstituted. The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum showed a signal at δ 101.5 (*s*, C-1,2 methylenedioxy). In the ^1H–^1H NOESY spectrum one of the methylenedioxy protons (δ 6.11) showed a correlation with H-11 (δ 8.30). H-8 (δ 7.30) correlated with the H-7 methylene protons (δ 2.95–3.10) and the latter proton correlated with H-6a (δ 4.06). Complete assignments of all protons and carbon atoms are given in Table 1.

The full ^1H and ^{13}C NMR (CD_3OD) assignments of magnoflorine (**2**) are presented in Table 2. Previous

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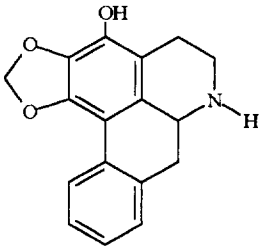

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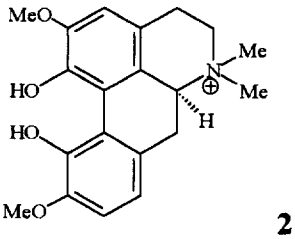

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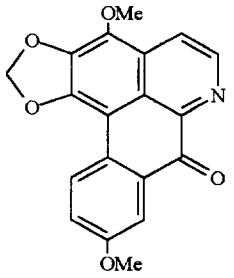

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reports of magnoflorine may have been confused with *N,N*-dimethyllindcarpine [9]; many of the NMR spectra were recorded using mixtures of solvents, such as $CDCl_3$+TFA [10], and some proton or carbon assignments were designated as interchangeable. Full ^1H and ^{13}C NMR (C_5D_5N) data for oxobuxifoline are also presented in Table 2. This compound has previously been isolated from *D. obovata*, but only ^1H NMR data were reported [6].

EXPERIMENTAL

General. CC: alumina (activity II–III, 70–230 mesh ASTM) and silica gel. Prep. TLC (1 mm thick layer) and TLC were carried out on silica gel 60 PF_{254}; spots were detected using UV light at 254 and 360 nm and also spraying with Dragendorff's reagent. Mps are uncorr. UV spectra were obtained in MeOH, IR in KBr. EIMS was obtained using direct insertion probe at 70 eV. NMR data were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to C_5D_5N, unless otherwise stated.

Plant material. Leaves and stems 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 of the Universidade Federal da Paraiba.

Extraction and isolation. Dried ground leaves and stems (3 kg) were extracted with 80% EtOH at room temp. for 4 days. This extract, after being concd under vacuum, was dissolved in 3% HCl, filtered over Celite and extracted several times with $CHCl_3$. The H_2O fr. was alkalinized with NH_4OH to pH 9 and extracted again with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4) and the solvent evapd to afford the TTA. This was subjected to prep. TLC,

Table 1. ^{13}C and ^1H NMR (C_5D_5N) data for cissaglaberrimine (**1**)

C	δ	^1J	^2J	^3J
1	144.5	–	–	–
2	134.5	–	–	–
3	140.1	–	–	–
3a	118.4	–	–	–
4	25.1	2.98–3.15 2H *m*	44.1	130.6
5	44.1	2.98–3.15 1H *m*, 3.47 1H *m*	25.1	54.9, 118.4
6a	54.9	4.06 1H *dd*, J = 5.3, 13.4 Hz	38.7, 130.6	135.6
7	38.1	2.95–3.10 2H *m*	54.9, 135.6	128.9, 130.6, 133.1
7a	135.6	–	–	–
8	128.9	7.30 1H *br d*, J = 7.2 Hz	–	38.7, 127.7, 133.1
9	127.0	7.24 1H *dt*, J = 1.1, 7.2 Hz	–	127.1, 135.6
10	127.7	7.41 1H *br*, J = 7.2 Hz	–	128.9, 133.1
11	127.1	8.30 1H *d*, J = 7.2 Hz	–	127.0, 135.6
11a	133.1	–	–	–
11b	109.1	–	–	–
11c	130.6	–	–	–
O–CH_2–O	101.5	5.95, 6.11 2H A–B *q* J = 1.2 Hz	–	134.5, 144.5

Table 2. ^{13}C and ^{1}H NMR data for magnoflorine (**2**) and oxobuxifoline (**3**)

	Magnoflorine (CD_3OD)		Oxobuxifoline (C_5D_5N)	
C	C	H	C	H
1	150.4	–	150.7	–
2	153.0	–	138.2	–
3	109.7	6.45 1H *s*	136.2	–
3a	116.3	–	131.5	–
4	–	–	119.7	8.21 1H *d*, J = 5.2 Hz
4_{ax}	24.8	3.10 1H *m*	–	–
4_{eq}	24.8	2.56 1H *br d*, J = 15.6 Hz	–	–
5	–	–	145.0	9.06 1H *d*, J = 5.2 Hz
5_{ax}	62.4	3.30 1H *m*	–	–
5_{eq}	62.4	3.40 1H *m*	–	–
6a	71.1	3.80 1H *br t*	146.3	–
7	–	–	182.5	–
7_{ax}	31.8	2.37 1H *br t*, J = 12.8 Hz	–	–
7_{eq}	31.8	2.91 1H *br d*	–	–
7a	126.2	–	125.7	–
8	117.4	6.47 1H *d*, J = 8.0 Hz	111.4	8.30 1H *d*, J = 2.9 Hz
9	110.8	6.66 1H *d*, J = 8.0 Hz	160.2	–
10	151.7	–	122.6	7.48 1H *dd*, J = 2,9, 8.9 Hz
11	149.6	–	129.4	8.63 1H *d*, J = 8.9 Hz
11a	123.5	–	133.6	–
11b	123.4	–	124.4	–
11c	121.3	–	123.3	–
C-2–OMe	56.2	3.75 3H *s*	–	–
C-3–OMe	–	–	60.7	3.87 3H *s*
C-9–OMe	–	–	56.0	4.22 3H *s*
C-10–OMe	56.5	3.82 3H *s*	–	–
N–Me	43.7	2.75 3H *s*	–	–
N–Me	54.1	3.21 3H *s*	–	–
O–CH_2–O	–	–	103.2	6.66 2H *s*

eluting with $CHCl_3$–MeOH (19:1). From this procedure, cissaglaberrimine (**1**, 358 mg) was isolated. The alkaline aq. fr. was acidified with HCl to pH 3 and treated with a soln of picric acid for 24 hr, forming a ppt. This ppt. was dissolved in MeOH, treated with activated charcoal and filtered over Celite. The soln was treated with the ion-exchange resin Amberlite IRA 400 (Cl^-) for 24 hr under agitation. The MeOH soln obtained was the total quaternary alkaloids fr., which, after prep. TLC on silica gel eluting with $CHCl_3$–MeOH (1:1), yielded magnoflorine (140 mg).

Cissaglaberrimine (**1**). Red–brown amorphous powder, mp 203–205° (dec.). UV λ_{max} nm 225, 242, 278, 300 (shoulder). IR ν_{max} cm^{-1} 3450, 2925, 1636, 1458, 1387, 1060. EIMS showed only $[M]^+$ at 281.0. NMR in Table 1.

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