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MILONINE, AN 8,14-DIHYDROMORPHINANDIENONE ALKALOID FROM LEAVES OF *CISSAMPELOS SYMPODIALIS*

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Key Word Index—*Cissampelos sympodialis*; Menispermaceae; leaves; 8,14-dihydromorphinandienone; milonine.

Abstract—A novel 8,14-dihydromorphinandienone alkaloid was isolated from the dried leaves of *Cissampelos sympodialis*. Spectroscopic analysis established its structure as (+)-(9β,13β,14α)-5,6-didehydro-4-hydroxy-3,6-dimethoxy-17-methylmorphinan-7-one, to which we have given the name, milonine.

INTRODUCTION

The family Menispermaceae is well known for the production of alkaloids of various types [1, 2], including morphinandienones [3]. In our work with the dried leaves of *Cissampelos sympodialis*, a plant used in the treatment of numerous diseases in north-eastern Brazil, where it is popularly known as milona, several alkaloids were detected. Herein, we report the isolation and structure elucidation of the new alkaloid, milonine (**1**).

RESULTS AND DISCUSSION

From the total tertiary alkaloid (TTA) fraction, obtained from the leaves of *C. sympodialis*, a mixture of several alkaloids was obtained. The structural elucidation of milonine only is described here.

Milonine was a reddish amorphous powder and its UV spectrum showed absorptions at 224 nm attributable to a α,β-unsaturated carbonyl chromophore, 262 nm due to an aromatic ring and a shoulder at 300 nm, which is characteristic of 8.14-dihydromorphinandienone alkaloids [4]. The IR spectrum showed bands at 3515 cm^{-1} (hydroxyl group), 2950 (C–H olefinic), 1685 cm^{-1} (α,β-unsaturated carbonyl), 1615 (C=C olefinic) and 1500 cm^{-1} (C=C aromatic). The HR EI mass spectrum indicated the molecular formula to be $C_{19}H_{23}NO_4$. The following fragments were observed, 314 $[M - CH_3]^+$ (100), 286 $[314 - CO]^+$ (17), 192 $[M - C_8H_9O_2]^+$ (24) and 42 $[H_2C{=}C{=}O]^+$ (17), being characteristic of morphinandienone alkaloids [5].

The NMR study—^1H, ^{13}C, HMBC (optimized for J = 7 Hz), HC-COBI, COSY and NOESY (mixing times, 0.6, 0.8 and 1.2 sec)—led to the unambiguous assignment of all functional groups. The ^1H NMR (400 MHz, pyridine-d_5) showed an AB quartet (J = 8.4 Hz) with doublets centred at δ6.95 and 6.79. Three singlets which integrated for three protons each were observed, one at δ2.28 (N–Me), the other two at δ3.65 and 3.78 for two methoxyls. The ^{13}C NMR (100 MHz, pyridine-d_5) showed a signal at δ194.5 (*s*, C-7) for the α,β-unsaturated carbonyl, δ43.3 (*s*, *N*–Me), δ55.1 (*s*, C-6-OMe) and δ56.7 (*s*, C-3-OMe). Complete assignments of all the protons and carbons are given in Table 1. The strong NOE interaction between H-8ax and one of the protons at C-15 in the NOESY experiment proved that the junction between the rings B and C must be *trans* and not *cis*. In the latter case, one would expect a NOE interaction between H-14 and one of the protons in C-15, but this was not observed (Fig. 1). The absence of a fragment at *m/z* 59 in the mass spectrum also supported the contention that the junction between the rings B and C is *trans*, since the *cis*-compounds all give a high intensity peak for this fragment [3, 6]. The positive optical rotation indicated that the absolute configuration for milonine may be depicted as shown in **1**, the isomer of (−)-8,14-dihydrosalutaridine, previously isolated from a number of *Croton* species [6].

EXPERIMENTAL

CC was carried out on alumina (activity II–III, 70–230 mesh ASTM). Prep. TLC (1.0 mm layer) and TLC were

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Table 1. ^{13}C and ^{1}H NMR data for milonine

	C	^{1}J	^{2}J	^{3}J
1	119.9	6.79 *br d* (J = 8.4 Hz)		28.6, 128.3, 147.2
2	111.0	6.93 *d* (J = 8.4 Hz)	119.9	132.3, 145.7
3	147.2			
4	145.7			
5	125.5	8.34 *s*	152.0	42.2, 128.3, 194.5
6	152.0			
7	194.5			
8	40.6	ax 3.65 *dd* (J = 14.0, 17.6 Hz) eq. 2.61 *dd* (J = 17.6, 4.4 Hz)	42.2, 194.5	38.9
9	57.5	2.81 *br d* (J = 5.8 Hz)	28.6, 42.2	38.9, 42.2, 47.6, 132.3
10	28.6	ax 3.11 *d* (J = 17.2 Hz) eq 2.76 *dd* (J = 17.2, 5.8 Hz)	57.5, 132.3	42.2, 119.9, 128.3
11	132.3			
12	128.3			
13	38.9			
14	42.2	2.41 *m*		
15	33.2	2.22 to 2.12 *m* (2H)	47.6	128.3
16	47.6	2.42 *m* (1H)/2.30 *m* (1H)	33.2	38.9, 57.5
MeO-3	56.7	3.78 *s* (3H)		147.2
MeO-6	55.1	3.65 *s* (3H)		152.0
Me–N	43.3	2.28 *s* (3H)		47.6, 57.5
HO		10.77 *br s* (1H)		

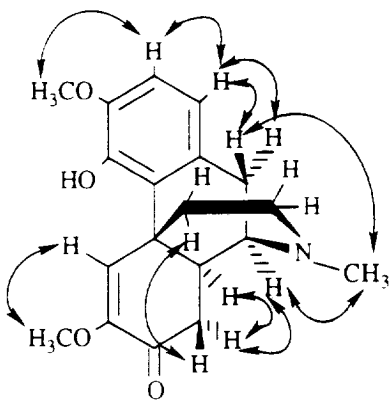
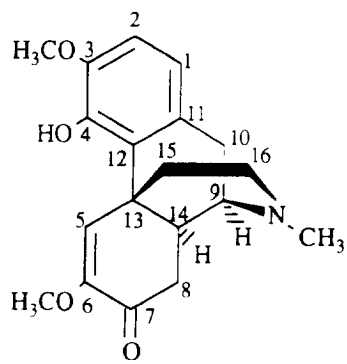


Fig. 1. NOE interactions between the protons of milonine.



1 - Milonine

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. Mp is uncorr. UV spectra were obtained in MeOH, IR in $CHCl_3$. HREIMS were obtained using a direct insertion probe at 70 eV. NMR data were obtained at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Chemical shifts are reported in ppm relative to the solvent (pyridine-d_5).

Plant material. Leaves of *C. sympodialis* Eichl. were collected during July and August of 1992 in the Botanical Garden of the Laboratorio de Technologia Farmaceutica of the Universidade Federal da Paraiba, Brazil. A voucher specimen (Agra 1456) has been deposited at the herbarium of this University.

Extraction and isolation. Dried ground leaves (542 g), were extracted with 80% EtOH at room temp. for 12 days. This extract (130 g), was dissolved in 3% HCl and extracted several times with $CHCl_3$. The aq. fr. was basified with NH_4OH to pH 9 and again extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried ($MgSO_4$) and the solvent evapd to afford the TTA (1.5 g). The TTA was subjected to CC over alumina, eluting with hexane containing increasing amounts of $CHCl_3$, $CHCl_3$ with increasing amounts of MeOH and finally with MeOH. The fr. eluted with $CHCl_3$–MeOH (49:1), after further purification by prep. TLC, yielded milonine (0.076 g, 0.014%).

Milonine (**1**). Reddish amorphous powder, mp 74–76°. $[\alpha]_D^{25}$ + 50° (MeOH; *ca* 0.6). UV λ_{max} nm: 224, 262, 300. IR ν_{max} cm^{-1} 3515, 2950, 1685, 1615, 1500. Found $[M]^+$ 329.1537; $C_{19}H_{23}O_4N$ requires 329.1546. EIMS *m/z* (rel. int.): 329 $[M]^+$ (58), 314 (100), 286 (17), 192 (24), 84 (12), 42 (17).

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