mutually coupled, high-field multiplets were also present (δ = 1.49–2.05), accounting for a sequence of two methylenes. Selective decoupling and ^{1}H-^{1}H COSY experiments showed the coupling of these methylenes with the previous oxymethine, therefore assigning a gluc-O-CH(CH_3)-CH_2-CH_2-sequence.

The presence in the ^{13}C-NMR spectrum of the resonances corresponding to the saturated aliphatic carbonyl and the carboxyl functions, at 201.1 and 171.1 ppm, respectively, together with the signal of a quaternary carbon atom at 43.9 ppm, allowed us to assign to **1** the structure of 9-*O*-β-D-glucopyranoside of the 9-hydroxy-2,2,4-trimethyl-deca-3-en-6-on-1-oic acid. Finally, a *Z*-configuration was assigned to the three-substituted double bond on the basis of the chemical shift value of the methyl group at C-4, which did not show the shielding effect expected for an *E*-configuration (7, 8). In the NOESY experiment, evident correlations between the two methyl singlets and the protons of the methylene at δ = 2.14 and 2.65 were also in accordance with these assignments.

Concerning the biosynthetic origin of the aglucone, it could be considered as derived from the junction of three isoprenoid units and the subsequent loss of two terminal carbons.

Materials and Methods

Plant material

Mentzelia incisa Urban et Gilg. was collected in the Monterrey district (N. L. Mexico) and identified by Prof. H. Sanchez at ITESM in Monterrey (voucher specimen SH-346).

Isolation

The aerial parts (350 g) were exhaustively extracted with MeOH at room temperature and the extract evaporated to an aqueous suspension. After a further addition of water (0.5 l), the resulting solution was extracted with EtOAc and *n*-BuOH (0.5 l, each) in the sequence. The CC separation of the *n*-BuOH extract was performed on SiO_2 (ca. 100 g) in CH_2Cl_2 : MeOH, 85 : 15, 1 ml/min; elution vol. of **1** 700–750 ml.

^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AM-500 spectrometer. The assignments were confirmed by COSY and NOESY experiments.

Compound **1**

Amorphous powder; $[\alpha]_D^{20}$: −26.5 (*c* = 1.0, MeOH). IR (KBr) ν_{max} cm^{-1}: 3400, 1710, 1670, 1210. ^{1}H-NMR (CD_3OD, TMS): δ = 1.01 (3H, s, H_3-11), 1.08 (3H, s, H_3-12), 1.23 (3H, d, *J* = 5.0 Hz, H_3-10), 1.49 (1H, m, H-8b), 1.74–1.87 (2H, m, H-8a and H-7b), 2.03 (3H, d, *J* = 1.3 Hz, H_3-13), 2.05 (1H, m, H-7a), 2.14 (1H, dd, *J* = 18.0 and 1.3 Hz, H-5b), 2.65 (1H, d, *J* = 18.0 Hz, H-5a), 3.13 (1H, dd, *J* = 8.0 and 8.5 Hz, H-2′), 3.26–3.33 (3H, m, H-4′, H-5′ and H-3′), 3.67 (1H, dd, *J* = 12.0 and 5.8 Hz, H-6′b), 3.80 (1H, m, H-9), 3.84 (1H, dd, *J* = 12.0 and 3.0 Hz, H-6′a), 4.31 (1H, d, *J* = 8.0 Hz, H-1′), 5.82 (1H, m, H-3). ^{13}C-NMR (CD_3OD, TMS): δ = 22.2 (C-10 or C-13), 22.3 (C-13 or C-10), 24.3 (C-11 or C-12), 24.7 (C-12 or C-11), 33.2 (C-8), 34.9 (C-7), 43.1 (C-2), 51.2 (C-5), 62.8 (C-6′), 71.8 (C-4′), 75.2 (C-2′), 77.8 (C-5′ or C-3′), 78.0 (C-9), 78.3 (C-3′ or C-5′), 104.2 (C-1′), 127.1 (C-3), 133.7 (C-4), 171.7 (C-1), 201.0 (C-6).

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Alkaloids from Leaves and Stems of *Vallesia glabra*

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Abstract

Eleven known indole alkaloids: vallesine, aspidospermine, 11-methoxydichotine, apparicine, tubotaïwine, vincadifformine, condylocarpine, (−)-rhazinilam, aspidospermatine, haplocidine, and 18-oxo-haplocidine, have been isolated from leaves and stems of *Vallesia glabra* (Cav.) Link. (Apocynaceae) from Bolivia. Analysis of 2 D NMR spectra complete previous ^{1}H and ^{13}C data for vallesine, aspidospermine, and 11-methoxydichotine.

The genus *Vallesia* includes 12 species, 8 of which are American, spreaded out from Florida to Argentina. To the best of our knowledge, only 3 species were chemically studied: *Vallesia dichotoma* Ruiz. and Pav. (from Peru) (1, 2). *Vallesia antillana* (from Cuba) (3, 4), and *Vallesia glabra* (Cav.) Link. (5) from which only two alkaloids, vallesine and aspidospermine, were isolated.

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According to some botanical authorities *V. glabra* and *V. dichotoma* are synonyms but they may represent different varieties of the same species (6).

We report here on the alkaloidal contents of leaves and stems of *Vallesia glabra* from Bolivia. *Vallesia glabra* is a small shrub common in the arid valleys of Bolivia and Paraguay. Plant materials were collected in the Bolivian Province of Misque, in September 1991 during an ethnobotanical field work (ORSTOM – U.M.S.S. project: Medicinal plants of Bolivian tropical region). Voucher specimens (Moretti N° 1510) are deposited in the National Herbarium of Bolivia, La Paz. Botanical identification was confirmed by L. Allorge (Museum National d'Histoire Naturelle de Paris).

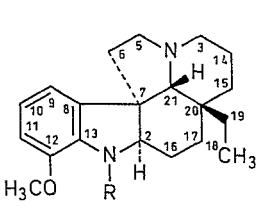
The extraction of the alkaloid mixtures (A. M.) was carried out according to the usual acid-base process (7), and the following yields were obtained: 2.4 % (stems), 2.9 % (leaves). The purification of A.M. was performed by column chromatography (silica gel, CH_2Cl_2-MeOH with increasing amounts of MeOH), and the isolation of pure alkaloids was realized by preparative TLC, CC (with the same solvents), or by crystallization. Eleven known alkaloids were obtained (Table 1) and their structures were elucidated by comparing their spectra with literature data, or with those of reference samples available in our laboratory.

Table 1 Alkaloids from *Vallesia glabra*.

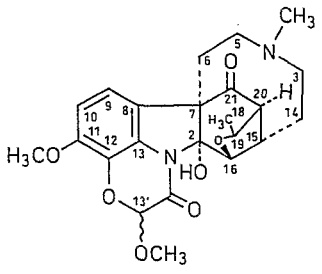
Alkaloids	Leaves %*	Stems %*	Isolation	Spectral analysis	Ref.
Vallesine (1)	31.91		TLC	a + b + c	(5)
Aspidospermine (2)	5.52	7.42	TLC	a + b + c	(5)
11-methoxydichotine (3)	0.13		crystallization	a + b + c	(9)
(–)-apparicine	1.02	0.13	TLC	a + b	(9)
Tubotaïwine**	0.22	0.16	TLC	a	(12)
Vincadifformine**		0.39	TLC	a	(9)
Condylocarpine		0.32	TLC	a	(9)
(–)-rhazinilam	0.33		TLC	a + b + c	(14)
Aspidospermatine	0.28	0.64	CC	a + b	(13)
Haplocidine		1.91	TLC	a + b	(11)
18-oxohaplocidine		0.53	CC	a + b	(11)

* Yields obtained from alkaloid mixture.
** Alkaloids compared with reference samples.
a = UV, IR, MS, and ^{1}H-NMR; b = ^{13}C-NMR and COSY ^{1}H-^{1}H; c = HMBC, HMQC.

The novel isolation of vallesine (1), aspidospermine (2), and 11-methoxydichotine (3) has provided the opportunity to complete previous ^{1}H- and ^{13}C-NMR data, by means of 2D NMR experiments (COSY H-H, HMQC, HMBC). These techniques were specially helpful to solve the problem of assignments of the eight methylene groups of 1 and 2. Furthermore, HMBC spectra of 1 and 2 permitted us to detect C-7 (superimposed with C-5) by its long-range correlations (^3J) with H-9 and H-16. In 2, the presence of this quaternary carbon was also confirmed by the signal at δ = 52.4 in the ^{13}C-NMR spectrum using the Spin Echo Broad Band Off-Resonance Decoupling (SEBBORD) technique (8).



1 R = CHO, vallesine
2 R = $COCH_3$, aspidospermine



3 11-methoxydichotine

Vallesine (1)

$[\alpha]_D$: −91° (*c* 1, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS, δ values in ppm): 9.3 (s, CHO), 7.0 (t, J = 7.5 Hz, H-10), 6.85 (d, J = 7.5 Hz, H-9), 6.8 (d, J = 7.5 Hz, H-11), 4.53 (dd, J = 11, 6.1 Hz, H-2), 3.87 (s, OCH_3), 3.1 (td, J = 9, 3.3 Hz, H-5), 3 (br. d, J = 11 Hz, H-3), 2.25 (s, N-CO-CH_3 and H-21), 2.23 (m, H-5′), 2.15 (m, H-16), 2.08 (m, H-17), 2.03 (dd, J = 12, 3.3 Hz, H-6), 1.93 (dd, J = 11, 2.8 Hz, H-3′), 1.75 (qt, J = 13, 4 Hz, H-14), 1.63 (br. d, J = 13 Hz, H-15), 1.5 (dd, J = 12, 3 Hz, H-6′), 1.45 (dd, J = 13, 3 Hz, H-14′), 1.4 (dq, J = 14, 7.3 Hz, H-19), 1.27 (m, H-16′), 1.1 (dd, J = 13.4, 4.4 Hz, H-17′), 1.05 (dd, J = 13, 4.3 Hz, H-15′), 0.85 (dq, J = 14, 7.3 Hz, H-19′), 0.63 (t, J = 7.3 Hz, CH_3-18). ^{13}C-NMR δ: 161.2 (CHO), 148.1 (C-12), 140.8 (C-13), 127 (C-8), 124.4 (C-10), 115.6 (C-9), 110.1 (C-11), 70.7 (C-21), 63.9 (C-2), 55.2 (OCH_3), 53.5 (C-3), 52.3 (C-5 and C-7), 39.6 (C-6), 35.3 (C-20), 34.1 (C-15), 29.8 (C-19), 24.2 (C-16), 22.5 (C-17), 21.4 (C-14), 6.6 (C-18).

Aspidospermine (2)

$[\alpha]_D$: −99° (*c* 0.5, $CHCl_3$). ^{1}H-NMR (300 MHz, $CDCl_3$, TMS, δ values in ppm): 7.07 (t, J = 8.7 Hz, H-10), 6.83 (d, J = 8.7 Hz, H-9), 6.8 (d, J = 8.7 Hz, H-11), 3.87 (s, OCH_3), 3.11 (td, J = 9, 3.2 Hz, H-5), 3 (br. d, J = 10.7 Hz, H-3), 2.25 (m, H-5′), 2.23 (s, H-21), 2.2 (s, N-CO-CH_3), 2.05 (m, H-6), 2 (m, H-17), 1.95 (m, H-16), 1.9 (m, H-3′), 1.73 (qt, J = 13, 4 Hz, H-14), 1.6 (m, H-15), 1.55 (m, H-6′), 1.5 (br. d, J = 13 Hz, H-14′), 1.35 (m, H-16′), 1.2 (dq, J = 14, 7.1 Hz, H-19), 1.1 (m, H-17′), 1.05 (m, H-15′), 0.8 (dq, J = 14, 7.1 Hz, H-19′), 0.6 (t, J = 7.1 Hz, CH_3-18). ^{13}C-NMR δ: 160 (N-CO-CH_3), 148 (C-12), 141 (C-13), 128 (C-8), 125.9 (C-10), 115.4 (C-9), 110 (C-11), 71 (C-21), 64 (C-2), 55.3 (OCH_3), 53.5 (C-3), 52.4 (C-5 and C-7), 38 (C-6), 35.4 (C-20), 34.1 (C-15), 29.9 (C-19), 24.7 (C-16), 23 (C-17), 22.9 ($COCH_3$), 21.5 (C-14), 6.7 (C-18). The proton H-2 did not appear on the spectra, but its presence was ascertained by the signal of a methine carbon at δ = 64 in the ^{13}C undecoupled C-H spectrum.

11-Methoxydichotine (3)

Crystallized from acetone; m.p. 121 °C, $[\alpha]_D$: −11° (*c* 1, $CHCl_3$). ^{1}H-NMR as in (2). ^{13}C-NMR ($CDCl_3$, δ values in ppm): 158.7 (C-13), 147.3 (C-11), 126 (C-12), 125 (C-8), 120.3 (C-10), 114 (C-9), 99.8 (C-2), 98.3 (C-13′), 77.7 (C-19), 76.5 (C-16), 60 (C-7), 56.6 (OCH_3-13′), 56.3 (OCH_3-11), 53.5 (C-3), 50 (C-20), 49 (C-5), 42 (C-6), 41.3 (N-CH_3), 39.5 (C-15), 18.6 (C-14), 17.2 (C-18).

This study shows that *V. glabra* possesses a high alkaloid content and a chemical composition similar to that of *V. dichotoma* (9). The most significant feature of this work is the novel isolation of the cytotoxic (–)-rhazinilam, an original mitotic poison which inhibits the polymerization of tubuline to microtubules (10).

Detailed information on the work-up procedure and copies of the original spectra can be obtained from the author of correspondence.

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Methyl Angolensate: The Antiulcer Agent of the Stem Bark of *Entandrophragma angolense*

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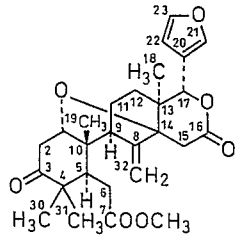
Abstract

Methyl angolensate (**1**), the major compound isolated from the methanol extract of the stem bark of *Entandrophragma angolense* produced a dose-related inhibition of gastric ulceration, 40 mg/kg body weight (B. W.) being more effective than 40 mg/kg B. W. of propranolol. The highest dose used (80 mg/kg B.W.) completely inhibited gastric ulceration and significantly reduced gastric acidity ($P < 0.05$). Furthermore, **1** (40 mg/kg B. W.) significantly reduced gastric acid secretion induced by histamine (1.0 mg/kg B. W.) and carbachol (1.0 mg/kg B. W.). These results suggest that **1** produces its antiulcer activity by inhibition of gastric acid secretion.

We have recently shown (1) that the methanol extract of the stem bark of *Entandrophragma angolense* (Welw.) C.DC. (Meliaceae) is highly potent in inhibiting indomethacin-induced gastric ulceration in rats. We also showed that the crude extract was non-toxic to the rats at a wide range of doses (20–200 g/kg B. W.) tested. These observations prompted us to undertake a phytochemical study of the plant aimed at isolating and identifying the antiulcer agent(s). This paper describes the isolation and identification of methyl angolensate (**1**) from the methanol extract of the stem bark of *E. angolense* and provides evidence that **1** is the antiulcer agent of the crude extract of the plant.



1

Fractionation of the methanol extract of the stem bark of *E. angolense* led to the isolation of **1** as the major compound. The identity of **1** was established by spectroscopy (UV, IR, OR, ^{1}H-NMR, EI-mass) and by comparison of our data with those reported in the literature (2–4). Consequently, the efficacy of **1** to protect against experimental indomethacin-induced gastric mucosal damage and to influence gastric acid secretion was investigated as indicated in the Materials and Methods section. The results presented in Table **1** clearly show that **1** produces a dose-related gastroprotective action in indomethacin-induced ulceration in rats. The cytoprotection produced by propranolol (40 mg/kg) was lower than that caused by 40 mg/kg of **1**. Thus, **1** is more potent than propranolol in protecting against gastric ulceration in rats. These results are identical to those that we have previously reported (2) for the methanol extract of *E. angolense* and they confirm that **1** is the antiulcer agent of the stem bark of *E. angolense*.

The significant reduction in total intragastric acidity observed in this study (see Table **1**) strongly suggests that **1** may act by inhibiting gastric acid secretion by the parietal cells. To investigate the mechanism of ulcer inhibition, the effects of **1** (40 mg/kg B. W.) on basal, histamine- (1 mg/kg, B. W.), and carbachol- (1 mg/kg, B. W.) induced gastric acid secretion in male albino rats were studied. The results (mean of 5 experiments ± SEM) are as

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