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LABRIFORMIN, A CARDIAC GLUCOSIDE FROM *ASCLEPIAS GLAUCESCENS*[1]

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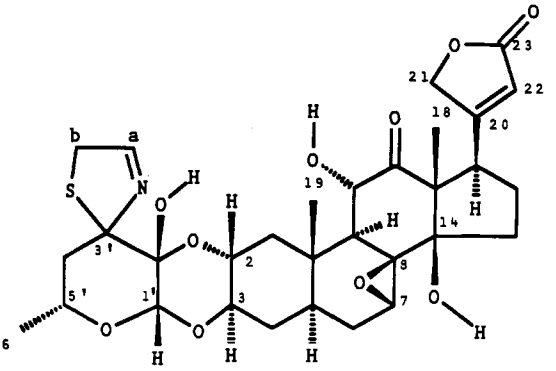
ABSTRACT.—Labriformin [**1**], a known cardenolide, was isolated from the latex of *Asclepias glaucescens*. Its identity was established by comparison of the spectroscopic data with those reported. The ^{13}C-nmr spectrum of **1** is presented. The major constituents of the latex were identified as a mixture of taraxasterol and ψ-taraxasterol acetates.

Various genera of the Asclepiadaceae, particularly *Asclepias* spp. and *Calotropis procera*, produce cardenolides which are important in the defense mechanisms developed by the monarch butterflies (*Danaus plexippus*) (1,2). Some of these plants have been used in the treatment of cancerous states (3,4). Plants of the *Asclepias* genus have been also studied for their high proteolytic enzyme content (5,6). These enzymes were named asclepains.



1

Asclepias glaucescens H.B.K. is widely distributed in Mexico. In a study of the characterization of the multiple forms of asclepain g isolated from the latex of *A. glaucescens* (6,7), we studied the secondary metabolites contained in the solid residue obtained as a by-product. We have also analyzed the secondary metabolites obtained from the aerial parts of the plant.

The CH_2Cl_2-soluble fraction of the solid residue obtained on centrifugation of the latex of *A. glaucescens* was submitted to chromatography using solvents of increasing polarity (see Experimental). The major component (75%) was identified as a mixture of taraxasterol and ψ-taraxasterol acetates by comparison with authentic samples. A small amount of taraxasterol and ψ-taraxasterol (1%) was also isolated.

Elution with 2% MeOH in CH_2Cl_2 yielded labriformin [**1**] (0.75%) which was identified by comparison of its spectral data with those described (8,9). The ^{13}C-nmr chemical shift assignments (Table 1) were made by APT and SFORD experiments and by comparison with those of labriformidin (9), into which **1** is transformed upon acid treatment.

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TABLE 1. ^{13}C-nmr Data for Labriformin [**1**].[a]

Carbon	δ (ppm)	Carbon	δ (ppm)
C-1′	95.17 (d)	C-11	73.66 (d)
C-2′	91.66 (s)	C-12	212.59 (s)
C-3′	99.70 (s)	C-13	63.25 (s)
C-4′	47.21 (t)	C-14	81.17 (s)
C-5′	68.99 (d)	C-15	28.44 (t)
C-6′	20.90 (q)	C-16	26.88 (t)
C-1	44.24 (t)	C-17	42.64 (d)
C-2	68.18 (d)	C-18	18.33 (q)
C-3	71.46 (d)	C-19	13.60 (q)
C-4	31.68 (t)	C-20	170.58 (s)
C-5	40.85 (d)	C-21	73.66 (t)
C-6	36.08 (t)	C-22	118.86 (d)
C-7	54.11 (d)	C-23	173.71 (s)
C-8	62.32 (s)	C=N	160.06 (d)
C-9	48.42 (d)	C-S	42.64 (t)
C-10	37.80 (s)		

[a]Recorded at 20 MHz in $CDCl_3$ with TMS as internal standard. SFORD multiplicity in parentheses.

From the more polar fractions a mixture of cardenolides was isolated which could not be separated into its components even through hplc.

From the aerial parts of *A. glaucescens*, the same products were isolated (see Experimental). Labriformin was obtained in a much smaller proportion than from the latex. This confirms the observation described earlier as a result of the study of the cardenolides contained in the latex and the leaves of several *Asclepias* species and *C. procera*.

Labriformin [**1**], a 3′-thiazoline-4,6-dideoxy-β-D-hexulose cardenolide, is commonly found in *Asclepias* and *Calotropis* spp. (10).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected. ^{1}H nmr and ^{13}C nmr were performed in $CDCl_3$ with TMS as internal reference in a Varian FT80 apparatus. Ir spectra were determined on a Perkin-Elmer 552 spectrometer. Hplc analysis was carried out on a Varian 8500 apparatus.

PLANT MATERIAL.—*A. glaucescens* was collected in Yautepec, Morelos (voucher 11944 FCME, Herbarium, Faculty of Science, UNAM). The latex was collected in the same location and transported at −40°.

ISOLATION OF SECONDARY METABOLITES FROM LATEX.—The latex (200 ml) was centrifuged at 70,000 *g* at 5° for 1 h. The residue (11) obtained was dried under vacuum in a desiccator for 3 days to yield 26 g of crude solid residue which was extracted with MeOH. The solution obtained was evaporated to dryness and dissolved in EtOH-H_2O (1:1), and the solution was extracted with CH_2Cl_2. The organic solution was washed with brine and dried, and the solvent was removed under vacuum.

The residue obtained (12.3 g) was submitted to dry cc on Si gel. Elution was performed with hexane/CH_2Cl_2 mixtures of increasing polarity, CH_2Cl_2, and CH_2Cl_2/MeOH. Elution with hexane-CH_2Cl_2 (9:1) gave a mixture of taraxasterol and ψ-taraxasterol acetates (8.5 g) identified by comparison with authentic samples (ir, ^{1}H nmr). From the fraction eluted with hexane-CH_2Cl_2 (1:1), a mixture of taraxasterol and ψ-taraxasterol was obtained (100 mg) and identified by comparison with an authentic sample.

Elution with CH_2Cl_2/MeOH (2%) yielded a white solid product **1** (75 mg) which showed mp 222–225° (from Me_2O/hexane): ir ν max ($CHCl_3$) Me_2CO cm^{-1} 3504, 1784, 1749, 1704, 1635; ^{1}H nmr δ ppm 7.5 (s, 1H, -C*H*=N-), 6.00 (d, J = 2 Hz, 1H, H-22), 5.10 (s, 1H, H-1), 4.80 (br d, J = 2 Hz, H-21), 3.85 (d, J = 2 Hz, 2H, -S-CH_2-), 4.67 (dd, J = 12 and 4 Hz, H-11, collapsed into a d, J = 12 Hz on D_2O addition), 3.9–4.25 (m, 3H, H-2, -3, -5), 3.42 (br d, J = 5 Hz, H-7α), 2.7 (dd, J = 4, 12 Hz, H-4′), 1.25 (d, J = 7 Hz, 3H, H-6′), 1.20 (s, 3H, H-19), 1.12 (s, 3H, H-18); ^{13}C nmr see Table 1. This product was identified as labriformin [**1**] [lit. (11) mp 212–215° from MeOH].

FROM AERIAL PARTS.—Dried aerial parts (1.5 kg) were macerated with MeOH. The residue obtained (200 g) was submitted to liquid-liquid partition between C_6H_6-hexane (1:1) and MeOH-H_2O (4:1). The nonpolar fraction yielded 51 g of gummy residue from which a mixture of taraxasterol and ψ-taraxasterol acetates was isolated. The polar fraction was extracted with CH_2Cl_2. Si gel chromatography of the gummy residue, using mixtures of *n*-hexane/CH_2Cl_2 and CH_2Cl_2/MeOH of increasing polarity, yielded labriformin [**1**] (10 mg) and a mixture of cardenolides (40 mg) that was not successfully purified by hplc of the original mixture or its acetylated derivatives.

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