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SECONDARY METABOLITES FROM THE STEM BARK OF *CELAENODENDRON MEXICANUM*[1]

PERLA CASTAÑEDA, ALMA BAHENA, ERIC GARCÍA, DANIEL CHÁVEZ, RACHEL MATA,*

Laboratorio de Fitoquímica, Departamento de Farmacia, Facultad de Química, Universidad Nacional Autónoma de México, Apartado Postal 70-265, México D.F., 04511, México

and ATILANO GUTIÉRREZ

Laboratorio de Resonancia Magnética Nuclear, Instituto de Química, Universidad Nacional Autónoma de México, Coyoacán 04510, México D.F., México

ABSTRACT.—Two new naturally occurring compounds, celaenodendrolide I [**1**], a picrotoxane type of terpenoid, and ellagic acid-4-*O*-β-D-xylopyranoside-3,3′-dimethyl ether [**3**] were isolated from the stem bark of *Celaenodendron mexicanum* (Euphorbiaceae). Friedelin, friedelan-3β-ol, dihydroisohyenanchin, and (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid were also obtained. The structures were elucidated by chemical and spectroscopic evidence.

Celaenodendron mexicanum Standl. (Euphorbiaceae), indigenous to Mexico, is used in folk medicine as an antiseptic (1). Previous phytochemical investigation conducted on the leaves of this species afforded three biflavones and three friedelane triterpenoids (1). In this paper, we report details of the isolation and structure elucidation of the major constituents of the stem bark of *C. mexicanum*.

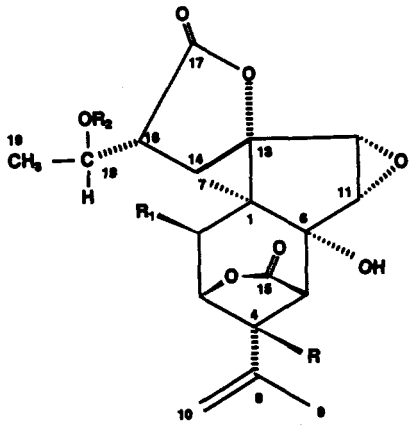
RESULTS AND DISCUSSION

Cc on Si gel of the $CHCl_3$ extract of the stem bark of *C. mexicanum* resulted in the isolation of friedelin, friedelan-3β-ol, dihydroisohyenanchin, (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid, celaenodendrolide I [**1**], and ellagic acid-4-*O*-β-D-xylopyranoside-3,3′-dimethyl ether [**3**]. Compounds **1** and **3** appear to be new naturally occurring substances.

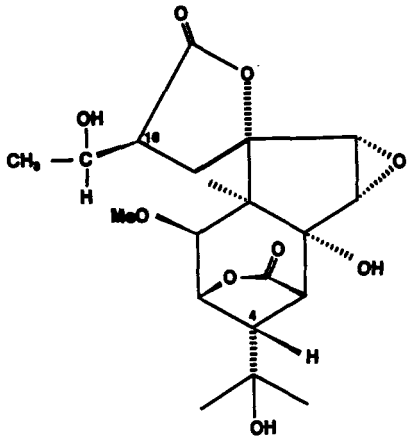
Celaenodendrolide I [**1**] was obtained as a white crystalline solid, and the molecular formula $C_{20}H_{26}O_9$ was indicated by eims and ^{13}C nmr. The ir spectrum of **1** showed absorption maxima for hydroxyl groups (3500 cm^{-1}), γ-lactone (1772 and 1760 cm^{-1}), and a methylenic double bond (1630 and 900 cm^{-1}). The nmr data (Table 1) strongly support a picrotoxane type of compound (2–5). Comparison of the nmr spectrum of **1** (Table 1) with that recently described for picrodendrin E [**2**] indicated that the spectral patterns were very similar (2), except that the signals for the hydroxyisopropyl at C-4 and the C-4 methine of **2** were missing. In their place, signals for an isoprenyl moiety (δ_C 145.6, 112.7, and 20.8; δ_H 2.40 and 5.22) and for one quaternary carbon bearing a hydroxyl group (δ_C 80.6) were observed. Moreover, the proton resonances attributable to H-14 (δ 3.05 and 4.40) and H-16 (δ 3.30) were paramagnetically shifted in comparison with the corresponding signals in compound **2**. However, they were similar to those of pretoxine [**4**] which, like picrodendrin E, has an identical but α-oriented side chain at C-16 (5).

Double resonance experiments further supported the assignments of H-3, H-5, and H-2, since irradiation of the signal at δ 5.41 (H-3) changed the broad singlet at δ 3.65 (H-5) to a sharp singlet. Conversely, irradiation of H-3 caused sharpening, not only of

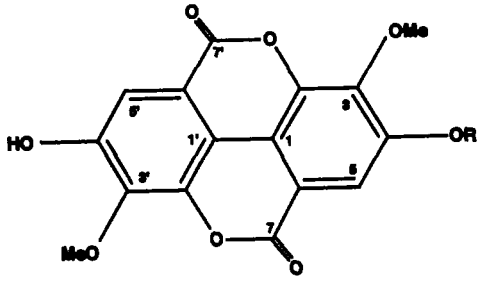
[1]Chemical Studies on Mexican Plants Used in Traditional Medicine, XXIX. Taken in part from the BS and MS theses of A. Bahena, E. García, and D. Chávez.



1 R=OH, R_1=OMe, R_2=H
4 R=H, R_1=OH, R_2=H
5 R=OAc, R_1=OMe, R_2=Ac



2



3 R=β-D-xylopyranosyl
6 R=H

H-5, but also of H-2 (δ 4.30). The W-type long range coupling between H-3 and H-5 is a common feature of the picrotoxinin type of picrotoxans (2,3,5).

Acetylation of **1** with Ac_2O and pyridine afforded the diacetyl derivative **5** as the major product. The downfield shifts observed for the signals of H-18 (Δδ 0.99) and H-3 (Δδ 0.97) in the ^{1}H nmr of **5** were in agreement with the placement of the secondary and one of the tertiary carbinolic groups at C-18 and C-4 (6), respectively. The chemical shift observed for C-7 (δ 25.3) was consistent with the disposition of the remaining tertiary hydroxyl group at C-6 (2–4). Finally, the relative stereochemistry of **1** was determined by analysis of the ^{1}H-^{1}H NOESY experiment (Table 2). From the NOESY spectrum, H-16 had a cross peak with H-14β, indicating that they were on the same face and that they had a β orientation. Similarly, H-14α had correlations not only with H-14β but also with H-18; this required H-14α and H-18 to be on the same face of the molecule and also supports the α orientation of the side chain at C-16. The other interactions indicated in Table 2 demonstrated that the remaining chiral centers are compatible with those of picrodendrine E [**2**]. Thus, structure **1** was proposed for celaenodendrolide I.

Compound **3** was isolated as a white amorphous powder. The molecular formula $C_{21}H_{18}O_{12}$ was established by elemental analysis, and it showed hydroxyl (3400–3290 cm^{-1}), carbonyl (1745 cm^{-1}), and aromatic (1605 and 1590 cm^{-1}) absorptions in the ir spectrum. Acid or enzymatic hydrolysis of compound **3** gave the aglycone **6**, which was

TABLE 1. ^{1}H- and ^{13}C-nmr Data of Celaenodendrolide I [**1**].[a]

Position	δ ^{13}C (pyridine-d_5)	δ ^{13}C (DMSO-d_6)	δ ^{1}H (pyridine-d_5)	δ ^{1}H (DMSO-d_6)
1	52.4	50.82	—	—
2	87.7	86.02	4.30 s	3.88 s
3	83.3	81.3	5.41 d (1)	4.82 d (3.2)
4	80.6	79	—	—
5	58.2	58.4	3.65 d (1)	2.98 d (3.2)
6	77.8	75.8	—	—
7	25.3	23.8	1.87 s	1.32 s
8	145.6	143.2	—	—
9	20.8	19.5	2.40 brs	1.95 s
10	112.7	112.08	5.22 m	5.03 m
11	62.9	61.1	4.07 d (2.7)	3.48 d (3)
12	61.6	60.3	4.22 d (2.7)	3.7 d (3)
13	91.5	90.17	—	—
14	30.2	28.7	α 4.40 dd (13.2, 12) β 3.05 dd (13.2, 9.0)	α 3.50 dd (13.6, 12.0) β 2.25 dd (13.6, 9.2)
15	175.9	173.8	—	—
16	48.4	46.9	3.30 ddd (12, 9, 3.6)	2.82 ddd (11.7, 9.3, 4)
17	176.6	176.4	—	—
18	65.4	64.6	4.67 m	4.13 m
19	22.2	20.1	1.45 d (6.4)	1.25 d (6.4)
-OMe	58.9	60.3	3.33 s	3.43 s
-OH	—	—	5.18 bs	4.22
-OH	—	—	8.01 s	5.18
-OH	—	—	8.15	5.18

[a]Chemical shifts are in ppm with TMS as the internal standard. The assignments were verified by COSY, HETCOR, and DEPT experiments. Coupling constants in Hz are given in parentheses.

identified by spectral data as ellagic acid 3,3′-dimethylether (6), and xylose. The sugar was identified by tlc comparison with an authentic sample. The nmr properties of compound **3** (see Experimental) were very similar to those previously described for ellagic acid-4-*O*-β-D-glucopyranoside-3,3′-dimethylether (7), differing only in the signals for the sugar portion. The ^{13}C-nmr data supported the assignment of β-D-xylopyranose as the glycone moiety (δ_c 103.7, 78.1, 74.5, 70.7, 67.3) (8). The β-D-glycosidic linkage was confirmed from the coupling constant observed for the anomeric proton at δ 5.82 (d, J=6.6 Hz). The position of the glycosidic linkage was confirmed on the basis of the NOESY experiment. The NOESY spectrum of **3** showed that the anomeric proton of xylose (δ 5.82) correlated with H-5 (δ 8.42); this interaction was in agreement with the placement of the sugar at C-4. It is important to point out that none of the signals due to the methoxyl groups correlated with those of the aromatic protons in the NOESY spectrum; this observation was consistent with the disposition of the methoxyl groups at C-3 and C-3′. Thus, the structure of **3** was elucidated as ellagic acid-4-*O*-β-D-xylopyranoside-3,3′-dimethylether. The known compounds dihydroisohyenanchin (4,9), friedelin (1), friedelan-3-β-ol (10) and (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid (11) were also isolated.

The only metabolite found both in aerial parts and stem bark of *C. mexicanum* was friedelin. To our knowledge, this is the second report of picrotoxan terpenoids in a member of the Euphorbiaceae family. Dihydroisohyenanchin was first isolated from *Hyenanche globosa* Lamb. (9), which belongs to the same tribe (Hyaenancheae) as *C. mexicanum*.

TABLE 2. NOESY interactions of Celaenodendrolide I [1].

Proton (δ)	nOe
H-2 (4.30)	H-3, H-7
H-3 (5.42)	H-2, H-5
H-5 (3.65)	H-3, H-11
H-7 (1.87)	H-2, H-10, H-19
H-9 (5.23)	H-10
H-10 (2.40)	H-7, H-9
H-11 (4.24)	H-5, H-12
H-12 (4.08)	H-11, H-14β
H-14α (4.4)	H-14β, H-18
H-14β (3.0)	H-12, H-14α, H-16
H-16 (3.28)	H-14β
H-18 (4.68)	H-14α, H-19
H-19 (1.45)	H-7, H-18

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were obtained in KBr on a Perkin Elmer 599 B spectrophotometer; uv spectra were recorded on a Beckman DU-7 spectrophotometer; ^{1}H- and ^{13}C-nmr spectra were recorded at 300 MHz and 75 MHz, respectively, on a Varian VXR-300S apparatus; optical rotations were measured with a JASCO DIP 360 Digital polarimeter. Mass spectra were taken on a Hewlett-Packard 599 B spectrometer. Melting points were determined in a Fisher Johns apparatus and are uncorrected.

PLANT MATERIAL.—Plant material (stem bark) was collected in Chamela, Jalisco, Mexico in January 1990. A voucher specimen (Martijena 90-II) has been deposited in the National Herbarium (MEXU), Instituto de Biologia, Universidad Nacional Autonoma de Mexico.

EXTRACTION AND ISOLATION.—The air-dried plant material (850 g) was ground into powder and extracted exhaustively by maceration at room temperature with $CHCl_3$. After filtration, the extract was concentrated in vacuo to give a brownish residue (59.3 g). The crude $CHCl_3$ extract was chromatographed on a Si gel column (593 g) using a hexane-$CHCl_3$ (1:1→0:1) and a $CHCl_3$-MeOH (1:0→0:1) stepwise gradient. A total of 369 fractions (150 ml each) were collected. Combined fractions 41–57, eluted with hexane-$CHCl_3$ (4:6), yielded 25 mg of friedelan-3β-ol and 27 mg of friedelin. Fractions 142–147 eluted with $CHCl_3$-MeOH (95:5) afforded **1** (73 mg) and dihydroisohyenanchin (87 mg). From fractions 194–252 eluted with $CHCl_3$-MeOH (95:5) crystallized 250 mg of **3**. Finally, fractions 325–341 yielded 1.5 g of (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid. Friedelin was identified by comparison with an authentic sample (1). Friedelan-3β-ol (10), dihydroisohyenanchin (4,9), and (−)-(2*S*,4*R*)-1-methyl-4-hydroxypyrrolidine-2-carboxylic acid (11) were identified by comparison of ir, mp, and ^{1}H- and ^{13}C-nmr data with those from the literature.

Celaenodendrolide I [**1**].—Colorless needles: mp 255°; $[\alpha]^{20}$ +18 (*c*=1, MeOH); ir ν max (KBr) 3500, 1772, 1760, 1630, 1230, 1080, 1030, 980, 900, 820, cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 410 (0.7), $[M-H_2O]^+$ 392 (0.7), $[M-15]^+$ 395 (1.4) $[M-15-H_2O]^+$ 377 (1), 366 (15), 330 (18), 126 (100), 111 (50), 99 (83), 69 (76), 45 (88).

Ellagic acid-4-O-β-*D-xylopyranoside-3,3′-dimethyl ether* [**3**].—White amorphous powder: mp 240–242°, $[\alpha]^{20}$ −22.7 (*c*=2, pyridine); ir ν max (KBr) 3400–3290, 1745, 1605, 1590, 1480, 1355, 1095, 1065, 990, 910 cm^{-1}; uv λ max (MeOH) 262, 293 (sh), 356; elemental analysis C 54.42, H 3.83 (calcd for $C_{21}H_{18}O_{12}$, C 54.54, H 3.89); ^{1}H nmr (pyridine-d_5) δ 3.93 (1H, m, H-4″), 4.20 (3H, s, -OMe), 4.28 (3H, s, -OMe), 4.21–4.55 (1H, m, H-3″), 5.82 (1H, d, H-1′), 8.04 (1H, s, H-5′), 8.42 (1H, s, H-5); ^{1}H nmr (DMSO-d_6) δ 3.81 (1H, m, H-4″), 4.05 (1H, s, -OMe), 4.09 (1H, s, OMe), 5.16 (m, H-3″), 7.52 (1H, s, H-5′), 7.74 (1H, s, H-5); ^{13}C nmr (pyridine-d_5) δ 61.3 (3′-OCH_3), 61.9 (3-OCH_3), 67.3 (C-5″), 70.7 (C-4″), 74.5 (C-3″), 78.1 (C-2″), 103.7 (C-1″), 111.7 (C-1′), 112.7 (C-6′), 113.0 (C-5′), 113.2 (C-5), 113.9 (C-6), 115.01 (C-1), 141.3* (C-3′), 141.8* (C-2′), 142.3* (C-2), 143.0* (C-3), 152.3 (C-4), 154.3 (C-4′), 159.0 (C-7, C-7′). Values with an asterisk are interchangeable.

ACETYLATION OF CELAENODENDROLIDE I.—Compound **1** (10 mg) was treated 48 h with Ac_2O and pyridine at room temperature, and the reaction mixture was worked up at usual. The major product was

purified by tlc [$CHCl_3$-MeOH (95:5)] to yield 6 mg of **5** as a glassy white solid: ir ν max (KBr) 3454, 2926, 1774, 1760, 1744, 1606, 1370, 1220, 1024, 902, 812, 756 cm^{-1}; ^1H nmr (pyridine-d_5) δ 1.37 (3H, d, J=6.4 Hz, H-19), 1.81 (3H, s, H-7), 1.97 (3H, s, Ac), 2.04 (3H, s, Ac), 2.18 (3H, brs, H-9), 2.91 (1H, dd, J=13.2, 9 Hz, H-14β), 3.49 (3H, s, -OMe), 3.54 (1H, m, H-17), 3.80 (1H, brs, H-5), 4.06 (1H, dd, J=13.2, 9 Hz, H-14α), 4.12 (1H, d, J=2.7 Hz, H-12), 4.25 (1H, d, J=2.7 Hz, H-11), 4.28 (1H, bs, H-2), 5.51 (2H, m, H-10), 5.68 (1H, m, H-18), 6.38 (1H, bs, H-3), 8.55 (1H, s, OH).

ACID HYDROLYSIS OF **3**.—Compound **3** (5 mg) was refluxed for 30 min with 1 ml of 1 N HCl. The acid solution was extracted with EtOAc (3×20 ml) and washed with H_2O (3×20 ml). The organic phase was dried over Na_2SO_4 and, after removal of solvent, 3 mg of **6**, mp 330° [lit. (6) mp 333°], was obtained. Ir, uv, eims, and ^1H- and ^{13}C-nmr data were identical to those previously described (6).

ENZYMATIC HYDROLYSIS OF **3** WITH CELLULASE.—To 2 mg of **3** were added 1 ml of H_2O and 2 mg of cellulase (Sigma Type 1). The mixture was incubated at 36° for 72 h. After the usual workup, the aglycone **6** (1 mg) and xylose (tlc) were obtained.

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