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CHEMICAL STUDIES ON MEXICAN PLANTS USED IN TRADITIONAL MEDICINE, V. CUCURBITACIN GLUCOSIDES FROM *CIGARRILLA MEXICANA*[1,2]

RACHEL MATA,* PERLA CASTAÑEDA, MARÍA-DEL-RAYO CAMACHO,

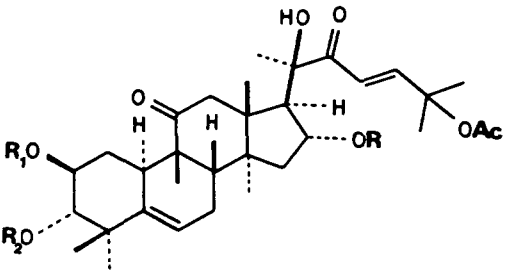
División de Bioquímica y Farmacia, Facultad de Química

and GUILLERMO DELGADO

Instituto de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, Coyoacán, 04510 México, DF

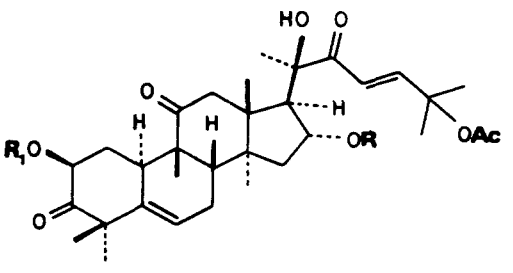
ABSTRACT.—The new cucurbitacin glucoside 2-*O*-β-D-glucopyranosylcucurbitacin F 25-acetate and arvenin I were isolated from the more polar fractions of the MeOH extract of *Cigarrilla mexicana*.

Previously we reported the isolation and structure elucidation of six terpenoids from the aerial parts of *Cigarrilla mexicana* (Zucc. et Martius ex DC.) Aiello (Rubiaceae), commonly used for the treatment of amebiasis in some parts of Mexico (1). In the continuation of our work on the triterpene constituents, we have now characterized the new cucurbitacin glycoside **1** and arvenin I [**2**].



	R	R_1	R_2
1	H	β-D-glucopyranosyl	H
1a	Ac	tetraacetyl β-D-glucopyranosyl	Ac
1b	H	H	H
1c	Ac	Ac	Ac
1d*	H	H	H

*$\Delta^{23,24}$ tetrahydro derivative



	R	R_1
2	H	β-D-glucopyranosyl
2a	Ac	tetraacetyl-β-D-glucopyranosyl
2b	H	H
2c	Ac	Ac

[1]For Part IV see R. Mata *et al.* (1).

[2]Taken in part from the BS research work of P. Castañeda.

The more polar fractions obtained from the MeOH extract of the aerial parts of the plant as previously described (1) were subjected to extensive cc to yield **1** and **2**.

2-*O*-β-D-glucopyranosylcucurbitacin F 25-acetate [**1**], mp 125–127°, $[\alpha]_D$ +5.5 ($c = 2$, MeOH), possesses a molecular formula of $C_{38}H_{58}O_{13}$, which was established by elemental analysis and ^{13}C-nmr spectroscopy. The presence of hydroxyl, α,β-unsaturated ketone, and acetate groups was inferred from the ir spectrum (3483, 1733, and 1689 cm^{-1}, respectively). Treatment with Ac_2O/pyridine gave the hepta-acetate **1a**. The ^{13}C-nmr data of **1** supported assignment of an unsaturated, tetracyclic, triterpene nucleus with two quaternary (δ 79.81, 79.65) and three secondary oxygenated functionalities (δ 83.16, 80.53, 70.84), two ketone groups (δ 213, 204.09), and one acetate (δ 169.76, 22.21), as well as a glucopyranosyloxy moiety (δ 106.20, 78.39, 78.20, 75.79, 71.57, 62.71) (2,3). The ^{1}H-nmr spectrum of the acetyl derivative showed similarities to literature data for cucurbitacins (3,4): signals attributable to seven acetyl groups and eight methyls were present; three 1- proton signals occurred in the olefinic region; the two lower field signals (δ 6.35 and δ 7.12) comprised an AB system with a coupling constant (16 Hz) characteristic of a *trans* double bond and were assigned to the α,β-unsaturated function known to be present.

Initial attempts at acid hydrolysis of **1** led to extensive decomposition, but enzymatic hydrolysis with cellulase or β-D-glucosidase gave β-D-glucose and aglycone **1b**. The mass spectrum of **1b** showed important fragments at *m/z* 405, 387, 369, 113, 111, and 96 (base peak), which strongly supported a cucurbitacin structure that possesses a normal side chain with the C-22 α,β-unsaturated ketone and an acetate group at C-25 (5,6). The ^{1}H-nmr of its triacetate **1c** was typified by the presence of eight quaternary methyl groups, four acetate methyl groups, three olefinic protons, and three methine protons on carbons bearing acetoxy groups at δ 4.66, δ 4.93, and δ 5.15. The signals at δ 4.66 (d, $J = 10$ Hz) and δ 4.93 (dd, $J = 10, 5$ Hz) were mutually coupled and were assigned to H-3β and H-2α, respectively.

Hydrogenation of compound **1b** over 10% Pd/C yielded, after tlc purification, 23,24-dihydrocucurbitacin F 25-acetate [**1d**], which was identical to a standard sample previously isolated from *Hintonia latiflora* (Sesse et Mociño ex DC.) Bullock (7). That the sugar was attached to position two was clearly demonstrated by the chemical conversion of **2** into **1** by $NaBH_4$ reduction and by decoupling experiments.

Irradiation at δ 4.61 (H-3) simplified the multiplet between δ 3.75–3.50 (H-2 and H-5′). Conversely, irradiation at δ 3.61 collapses the H-3 doublet to a singlet and also simplified the -CH_2OH signal located at 4.20.

Compound **2** analyzed for $C_{38}H_{56}O_{13}$. ^{13}C-nmr and ^{1}H-nmr information was in agreement with a cucurbitacin glucoside structure. Enzymatic hydrolysis afforded β-D-glucose and cucurbitacin B [**2b**], which was identified by comparison with an authentic sample. The physicochemical data of **2** and its acetyl derivative **2a** were identical to those previously reported by Yamada *et al.* (3) for arvenin I. However, their assignment of the signal at δ 3.64 to H-10 in the ^{1}H nmr of their acetyl derivative of **2** was not consistent with the decoupling experiments carried out on our acetyl derivative. Upon irradiation of the signal at δ 4.16 (H-6′) the signal they attributed to H-10 (δ 3.64) was simplified. Conversely, irradiation of the resonance at δ 3.64 produced changes in the signal at δ 4.16. Therefore, this signal should be assigned to H-5′.

This is to our knowledge the first report of cucurbitacin glycosides in a member of the Rubiaceae family. Also, as we suggested previously, the presence of cucurbitacins seems to be the common chemical character in those *Hintonia*-related genera (e.g., *Cigarrilla*) which possess extremely bitter barks (1,7,8). The potential antiamoebic activity of both cucurbitacins **1** and **2** is being evaluated by the International Organization for Chemical Sciences in Development (IOCD).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected; ir spectra were recorded on a Nicolet FT-IR instrument; uv spectra were recorded on a Hitachi 2205 double beam spectrophotometer with MeOH as the solvent; ^{1}H-nmr spectra were recorded on a Varian FT spectrometer in $CDCl_3$, pyridine-d_5, or DMSO-d_6, with TMS as internal standard; ^{13}C-nmr spectra were taken in a 50-MHz instrument; mass spectra were determined on a Hewlett-Packard 5985-B spectrometer. Si gel 60 (70–230 mesh) was used for cc; tlc was done on Si gel 60 GF 254 plates (Merck), and the compounds were visualized with uv radiation, 0.4 N H_2SO_4, or anisaldehyde reagent.

PLANT MATERIAL.—The plant *C. mexicana* was collected in la Barranca de Tolantongo, Estado de Hidalgo, Mexico, and was identified by Dr. David Lorence, Instituto de Biologia, UNAM. A voucher specimen (DL 5040) is deposited at the National Herbarium.

EXTRACTION AND PRELIMINARY FRACTIONATION.—Extraction with MeOH of the aerial parts of *C. mexicana* and preliminary fractionation of the MeOH extract were previously described (1).

ISOLATION OF ARVENIN I [**2**].—Fractions 759–787 (6 g) eluted with EtOAc-MeOH (9:1) from the column previously described (1) were rechromatographed on Si gel (395 g). Elution was accomplished with $CHCl_3$ with increasing amounts of MeOH. Fractions 555–561 eluted with $CHCl_3$-MeOH (95:5) yielded 1.743 g (0.1120% of the dry wt) of **2**, mp 140–144° [lit. (3) mp 141–146°]; $[\alpha]_D$ +25 (c = 2, MeOH); ir ν max (KBr) 3425, 2977, 2925, 1720, 1691, 1630, 1464, 1372, 1263, 1127, 1079, 989 cm^{-1}; ^{13}C nmr (pyridine-d_5) 212.70 (s, C-11), 211.20 (s, C-3), 204.20 (s, C-22), 170.0 (s, -*O*COMe), 150.10 (d, C-24), 140.8 (s, C-5), 122.52 (d, C-23), 120.50 (d, C-6), 104.08 (t, C-1′), 79.80 (s, C-20), 79.70 (s, C-25), 78.50 (d, C-2), 78.30 (d, C-3′), 75.70 (d, C-2′), 71.40 (d, C-4′), 70.70 (d, C-16), 62.60 (t, C-6′), 59.70 (d, C-17), 51.50 (s, C-14), 50.90 (s, C-13), 49.1 (t, C-12), 48.80 (s, C-9), 48.50 (s, C-4), 46.32 (t, C-15), 42.90 (d, C-10), 35.17 (d, C-8), 34.30 (d, C-1), 28.68 (q, C-29), 26.60 (q, C-27), 26.75 (q, C-26), 25.47 (q, C-28), 24.20 (t, C-7), 21.70 (q, C-18), 20.4 (t, C-19), 20.28 (s, O-CO*Me*), 19.90 (q, C-21), 18.86 (q, C-30). *Anal.* calcd for $C_{38}H_{56}O_{13}$: C 63.33, H 7.77; found C 63.03, H 7.70.

ISOLATION OF 2-*O*-β-D-GLUCOPYRANOSYLCUCURBITACIN F 25-ACETATE [**1**].—Fractions 613–638 (0.950 g) of the above column eluted with $CHCl_3$-MeOH (93:7) were in turn chromatographed on Si gel (42 g) using as eluent EtOAc-MeOH-H_2O (80:1:3). Fractions 29–49 afforded 0.429 g (0.0316% dry wt) of **1** as a white powder: mp 122–127°, $[\alpha]_D$ +5.5 (c = 2, MeOH); ir ν max (KBr) 3438, 2974, 2929, 2879, 1733, 1689, 1630, 1462, 1372, 1264, 1127, 1080, 1058, 1024, 990; ^{13}C nmr (pyridine-d_5) 213.0 (s, C-11), 204.09 (s, C-22), 169.76 (s, -*O*COMe), 149.9 (d, C-24), 141.74 (s, C-5), 122.49 (d, C-23), 119.0 (d, C-6), 106.20 (d, C-1′), 83.16 (d, C-2), 80.53 (d, C-3), 79.81 (s, C-20), 79.65 (s, C-25), 78.39 (d, C-3′), 78.20 (d, C-5′), 75.79 (d, C-2′), 71.57 (d, C-4′), 70.84 (d, C-16), 62.71 (t, C-6′), 59.50 (d, C-17), 51.03 (s, C-14), 49.11 (s, C-13), 48.87 (t, C-12), 48.54 (s, C-9), 46.27 (t, C-15), 43.24 (d, C-10), 34.31 (t, C-1), 33.22 (d, C-8), 29.93 (q, C-29), 26.64 (q, C-26), 26.18 (q, C-27), 25.31 (q, C-21), 24.20 (t, C-7), 22.21 (q, -O-CO*Me*), 21.74 (q, C-28), 20.35 (q, C-30), 20.28 (q, C-18), 19.04 (q, C-19), 42.51 (s, C-4). *Anal.* calcd for $C_{38}H_{58}O_{13}$: C 63.15, H 8.03; found C 63.10, H 8.06.

ACETYLATION OF **1**, **1b**, **2**, AND **2b**.—To separate solutions of **1** (60 mg), **1b** (10 mg), **2** (50 mg), and **2b** (10 mg) in 1 ml of pyridine was added 1 ml of Ac_2O. The mixtures were kept at room temperature for 24 h, and after usual workup, the acetyl derivatives, **1a**, **1c**, **2a**, and **2c**, respectively, were obtained.

Compound **1a**.—Yield 47 mg, mp 107–110°; ir ν max (KBr) 3452, 2976, 2939, 1742, 1695, 1630, 1433, 1371, 1241, 1176, 1129, 1040, 983; ^{1}H nmr ($CDCl_3$) 0.97 (s, 6H, H-18, H-30), 1.09 (s, 3H, H-29), 1.20 (s, 3H, H-28), 1.25 (s, 3H, H-21), 1.39 (s, 3H, H-19), 1.55 (s, 6H, H-26, H-27), 1.84 (s, 3H), 1.95 (s, 3H), 2.00 (s, 6H), 2.03 (s, 3H), 2.10 (s, 3H), 2.11 (s, 3H), 2.65 (d, J = 14 Hz, 1H, H-12), 3.50–4.05 (m), 4.20 (m, 2H, H-6′), 4.60 (d, J = 7 Hz, 1H, H-1′), 4.61 (d, J = 10 Hz, 1H, H-3), 5.67 (m, 1H, H-6), 6.35 (d, J = 16 Hz, 1H, H-23), 7.12 (d, J = 16 Hz, 1H, H-24).

Compound **1c**.—Yield 10 mg, mp 102°; ^{1}H nmr ($CDCl_3$) 0.99 (s, 3H, H-18), 1.04 (s, 3H, H-30), 1.08 (s, 3H, H-29), 1.21 (s, 3H, H-28), 1.24 (s, 3H, H-21), 1.40 (s, 3H, H-19), 1.56 (s, 6H, H-26, H-27), 1.84 (s, 3H), 1.97 (s, 3H), 2.02 (s, 3H), 2.04 (s, 3H), 2.65 (d, J = 7 Hz, 1H, H-17), 3.20 (d, J = 14 Hz, 1H, H-12′), 4.24 (s, 1H, OH-20), 4.66 (d, J = 10 Hz, 1H, H-3), 4.93 (dd, J = 10, 5 Hz, 1H, H-2), 5.15 (t, J = 7 Hz, 1H, H-16), 3.73 (d, J = 5 Hz, 1H, H-6), 6.43 (d, J = 16 Hz, H-23), 7.14 (d, J = 16 Hz, 1H, H-24); eims (rel. int.) $[M-60]^+$ 584 (0.4), 566 (0.4), 531 (2), 524 (0.4), 471 (0.4), 455 (10), 411 (1), 369 (2), 351 (4), 309 (0.2), 219 (1), 113 (24.7), 111 (25.3), 96 (100), 43 (92.7).

Compound **2a**.—Yield 48 mg, mp 104–106° [lit. (3) mp = 108°]. Its spectral properties were identical to those previously described (3).

Compound **2c**.—Yield 9 mg, mp 112–116° [lit. (9) mp 114–117°]. The spectroscopic parameters were identical to those previously reported (9).

ENZYMATIC HYDROLYSIS OF **1** AND **2** WITH CELLULASE.—Compounds **1** and **2** (100 mg each) were suspended in H_2O (10 ml), and cellulase (Type I, Sigma, 200 mg) was added. The mixtures were incubated for 72 h at 36°. The reaction mixture was then extracted with $CHCl_3$. The $CHCl_3$ extracts were evaporated and chromatographed on Si gel plates [$CHCl_3$-MeOH (8:2)] to yield 35 mg of **1b** and 30 mg of **2b**.

Compound **1b**.—Mp 205–210°; ir ν max (KBr) 3400, 2970, 1739, 1693, 1630, 1240, 985; eims (rel. int.) $[M-60]^+$ 500 (1.2), 482 (0.8), 457.0 (0.7), 405 (1.3), 387 (12), 369 (12), 351 (5), 113 (18), 112 (13), 111 (19), 96 (100).

Compound **2b**.—Mp 165° [lit. (8) mp 160–162°]; the spectroscopic properties were identical to those in the literature (8). Glucose was separated from the reaction mixtures as previously described (3).

ENZYMATIC HYDROLYSIS OF **1** AND **2** WITH β-GLUCOSIDASE.—Compounds **1** and **2** (5 mg each) suspended in 1 ml of H_2O were mixed with 5 mg of β-glucosidase (Sigma). The mixture was incubated 72 h at 36°. Glucose and aglycones **1b** and **2b** were readily identified by tlc in the hydrolysates of **1** and **2**, respectively.

CATALYTIC HYDROGENATION OF **1b**.—To a solution of 15 mg of **1b** in 5 ml of EtOH was added 5 mg of 10% Pd. The mixture was stirred under hydrogen for 45 min, and the resulting product was filtered. Evaporation of the solvent yielded a solid which after purification by preparative tlc afforded 5 mg of 23,24-dihydrocucurbitacin F 25-acetate, mp 230–235°, identical to a standard sample previously isolated from *Hintonia latiflora* (7).

$NaBH_4$ REDUCTION OF **2**.—To a solution of 60 mg of $NaBH_4$ in absolute EtOH was added 100 mg of **2**. The mixture was stirred for 40 min at room temperature. Workup in the normal way gave a solid product (19 mg). The major component **1** was separated by tlc. The yield of **1** was 9 mg, and it was identical to the natural product.

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