9. Wagner, H., Seitz, R. and Lotter, H. (1978) *J. Org. Chem.* **43**, 3339.
10. San Martín, A., Givovich, A. and Castillo, M. (1986) *Phytochemistry* **25**, 264.
11. Arriaga-Ginger, F. J., Wollenweber, E., Schober, I., Dostal, P. and Braun, S. (1986) *Phytochemistry* **25**, 719.
12. Jolad, S. D., Hoffmann, J. J., Schram, K. H., Cole, J. R., Tempesta, M. S. and Bates, R. B. (1982) *J. Org. Chem.* **47**, 1356.
13. Payne, T. G. and Jefferies, P. R. (1973) *Tetrahedron* **29**, 2575.
14. Voirin, B. (1983) *Phytochemistry* **22**, 2107.
15. Harborne, J. B., Mabry, T. J. and Mabry, H. (1975) *The Flavonoids*. Part 1, pp. 46. Academic Press, New York.

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TRITERPENES FROM *CIGARRILLA MEXICANA**

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Key Word Index—*Cigarrilla mexicana*; Rubiaceae; cucurbitacin E; isocucurbitacin B; *epi*-isocucurbitacin B; 3β-23-dihxdroxy-urs-12-en-28-oic-acid; cucurbitacins.

Abstract—From the aerial parts of *Cigarrilla mexicana* 3β, 23-dihydroxy-urs-12-en-28-oic acid, a new natural product, has been isolated together with the already known cucurbitacin E, isocucurbitacin B, *epi*-isocucurbitacin B, ursolic and oleanoic acids. The structure of the new substance was established by chemical and spectroscopic means.

INTRODUCTION

In continuation of our work on Mexican plants used in Traditional Medicine, we have now investigated aerial parts of *Cigarrilla mexicana* (Zucc *et* Martius *ex* DC) Aiello (Rubiaceae), known in Mexico as cigarro, cigarrilla or cacaloxochilt. Cigarrilla is a monotypic species endemic to Hidalgo, Querétaro and San Luis Potosí, Mexico. The aerial parts, intensely bitter, are used locally for the treatment of amebiasis and as an emetic [1; Lorence, D., unpublished results]. No previous chemical work on the plant has been described.

RESULTS AND DISCUSSION

After repeated column chromatography on silica gel the concentrated methanolic extract of the defatted aerial parts of *C. mexicana* afforded the known compounds cucurbitacin E, isocucurbitacin B, *epi*-isocucurbitacin B as well as oleanoic and ursolic acids. In addition, a new natural ursene **1**, was isolated in 0.006% yield.

Compound **1**, $C_{30}H_{48}O_4$, mp 266–268°, was obtained as colourless needles. Treatment of **1** with pyridine–acetic anhydride afforded diacetate **1b** and methylation with diazomethane yielded methylester **1a**. Finally, treatment with acetone–H_2SO_4 gave the stable acetonide **1c**, thus indicating the presence of a 1–3 or 1–2 glycol moiety in the molecule.

The electron impact mass spectrum showed ions at *m/z* 248 (base), 223, 205 [223 − H_2O] and 203 [248 − COOH], the typical retro-Diels–Alder fragments of a triterpene acid of the Δ^{12} oleanene or ursane type [2, 3]. Furthermore the peaks at *m/z* 223 and 205 indicated that **1** had two hydroxyl groups on the ring A and/or ring B [4].

The ^{1}H NMR spectrum of **1** (Table 1) exhibited signals for two secondary methyl groups, four methyl singlets, one proton doublet ($J = 11$ Hz) at δ 2.17 (H-18) and one proton multiplet at δ 5.20 (H-12), as expected for an urs-12-ene skeleton [5–7]. Also, it showed an AB system (δ 3.29, 3.65, $J = 11$ Hz), which shifted downfield on acylation in **1b**, indicative of the presence of an equatorial hydroxy methylene group attached to an asymmetric

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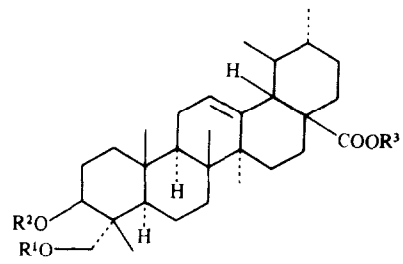
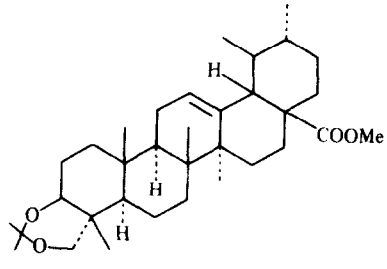


1 $R^1 = R^2 = R^3 = H$
1a $R^1 = R^2 = H$, $R^3 = Me$
1b $R^1 = R^2 = Ac$, $R^3 = H$



1c

centre; finally, one signal at δ 3.57 (*dd*, $J = 8$, 6 Hz) was assignable to H-3 [4, 8].

On biogenetical grounds it was highly probable that the secondary hydroxyl group was on C-3 with a β-disposition. In favor of its β-orientation was the chemical shift and the splitting pattern observed for the signal at δ 4.74 (*dd*, $J = 8$, 6 Hz) in the ^{1}H NMR spectrum of compound **1b** [4–8].

The placement of the primary hydroxyl group at C-23 was unequivocally ascertained from the ^{1}H NMR analysis of the acetonide **1c**. As with other acetonides previously reported for other pentacyclic triterpenes exhibiting 3β and 23-hydroxyl functionalities, the two isopropylidene methyls appeared as two peaks with slight differences in chemical shift at δ 1.41 and 1.44. H-3, with an axial orientation showed a signal at δ 3.6 (*m*). Finally, the resonances due to the C-23 hydrogens appeared as a broad signal at δ 3.45 [8].

Cucurbitacin E, isocucurbitacin B, *epi*-isocucurbitacin B and their correspondant acetyl derivatives showed identical spectrometric parameters (UV, IR, ^{1}H NMR and MS) to those described in the literature [9–12].

It is interesting to point out that the presence of cucurbitacins in Rubiaceae is no longer restricted to *Hintonia latiflora* (Sesse *et* Moc *ex* DC) Bullock and, as it has been previously suggested these secondary metabolites seem to be a common phytochemical character to those species related to *Hintonia*, which posses bitter stem barks [13].

Table 1. ^{1}H NMR spectra of compounds **1**–**1c** (80 MHz, TMS as int. standard)*

Compound	H-12	H-18	H-3	H-23		H-24	H-25	H-26	H-27	H-29	H-30	Me–C(O)–O	CO_2Me	OAc
1†	5.20*m*	2.17*d* (11)	3.57*dd* (8.6)	3.29*d* (11.5)	3.60*d* (11.5)	0.84*s*	1.08*s*	0.84*s*	1.13*s*	0.90*d* (6)	0.96*d*§	—	—	
1a†	5.21*m*	2.18*d* (11)	3.59*dd* (8.6)	3.29*d* (11.5)	3.60*d* (11.5)	0.85*s*	1.05*s*	0.83*s*	1.13*s*	0.90*d* (6)	0.96*d*§	—	3.59*s*	—
1b‡	5.25*m*	2.17*d* (11)	4.74*dd* (8.6)	3.65*d* (11.5)	3.90*d* (11.5)	0.81*s*	1.05*s*	0.75*s*	1.22*s*	0.89*d*§	0.96*s*§	—	—	2.00*s* 2.03*s*
1c‡	5.24*m*	2.14*d* (11)	3.6*m*	3.45*brs*		0.83*s*	1.05*s*	0.74*s*	1.10*s*	0.90*d*§	0.95*d*§	1.41*s* 1.44*s*	3.58*s*	—

*Coupling constants (Hz) in parentheses.
†$CDCl_3$–DMSO-d_6.
‡$CDCl_3$.
§The signals are overlapped.

EXPERIMENTAL

Plant material. Cigarrilla mexicana (Zucc *et* Martius *ex* DC) Aiello was collected in La Barranca de Tolantongo, Estado de Hidalgo, Mexico, on 23 April 1986. A Voucher (DL5040) is deposited at the National Herbarium, Instituto de Biologia, UNAM.

Extraction and preliminary fractionation. The dried and ground aerial parts of the plant (3.4 kg) were first macerated (×2) for 3 days periods with hexane at room temp. The marc was then refluxed with MeOH (3×). Evaporation of the MeOH extract under red. pres. led to a viscous (1.228 kg) residue. Part of the extract (588.52 g) was then subjected to CC on silica gel (3 kg) using as eluants hexane, hexane–$CHCl_3$ in different proportions, $CHCl_3$, $CHCl_3$ with increasing amounts of EtOAc, EtOAc, and EtOAc–MeOH increasing from 9:1 to 1:1, 500 ml fractions were collected.

Isolation of oleanolic and ursolic acid. From fractions 223–456 eluted with $CHCl_3$–EtOAc (9:1), 33.19 g of a white powder, mp 270–273°, were separated. This material (1 g) in MeOH (60 ml) was treated with Br_2 (50 mg) in MeOH (15 ml). After 2 hr the soln was cooled in an ice bath to give a crystalline mixture of two substances (1.12 g). The binary mixture was separated by CC on silica gel; elution with $CHCl_3$ afforded 350 mg of 12α-bromo-3β-hydroxy-oleanan-13β,28-olide, mp 225° (lit. [3] mp 225–226°) whose spectral properties were identical to those previously reported [12]. Elution with $CHCl_3$–EtOAc (8:1) yielded 200 mg of ursolic acid, mp 278–279° (lit. [3] mp. 281–283°) which was identical to a standard sample.

Oleanolic acid was finally obtained by heating 300 mg of the bromolactone with AcOH (7.5 ml) and Zn dust (1.8 g) on a steam bath for 3 hr. The reaction mixture was then filtered and worked up as previously described [14] to yield 170 mg of crude oleanolic acid which was identified as the methylester by comparison with an authentic sample. The total yield of oleanolic acid was 0.2069% of dry wt.

Isolation of 3β-24-*dihydroxy-urs*-12-*en*-28-*oic acid* (**1**), *cucurbitacin E, isocucurbitacin B and* epi-*isocucurbitacin B.* Fractions 457–586 (6 g) eluted with $CHCl_3$–EtOAc 8:2, 7:3, 6:4 and 5:5, of the initial column were rechromatographed on silica gel (180 g). Elution was accomplished with hexane with increasing amounts of EtOAc. Fractions 115–122 yielded 21 mg (0.0013% dry wt) of cucurbitacin E, mp 230–231°, (Lit. [9] mp 233–235°). Fractions 123–130 gave a white crystalline powder which upon recrystallization from MeOH yielded 14 mg of isocucurbitacin B, in the form of colourless needles, mp 219° (Lit. mp [12] 220–222°). From fractions 131–135 were obtained 50 mg of a mixture of two components, mp 204–210°. After prep. TLC on silica gel plates using hexane–Et_2O–EtOAc (1:1:1) and $CHCl_3$–Me_2CO–MeOH (14:1:2) an additional 10 mg (0.0014% dry wt) of isocucurbitacin B and 25 mg (0.0014% dry wt) of *epi*-isocucurbitacin B, mp 95° (Lit. mp [12] 92–98°) were obtained. Fractions 136–160 afforded 100 mg (0.006% of dry wt) of **1**, as needles, mp 283–287°. [Calc. for $C_{30}H_{48}O_4$; C, 71.68; H, 9.22. Found: C, 71.50; H, 9.28%]; U.V. λ_{max}^{MeOH} nm (log ε): 204 (3.6060), 280 (2.0497), EIMS *m/z* (rel. int.): 472 $[M]^+$(1), 454 $[M-18]^+$ (0.6), 446 (0.3), 441 $[M-31]^+$ (0.3), 249 (17), 248 (100), 223 (20), 205 (10), 203 (46), 175 (12), 133 (30); IR ν_{max}^{KBr} cm^{-1}: 3450, 3389, 3365, 2944, 2925, 1702, 1463, 1379, 1041; ^1H NMR (80 MHz, $CDCl_3$–DMSO-d_6), see Table 1.

Acetylation of cucurbitacin E, isocucurbitacin B and epi-*isocucurbitacin B.* To a soln of each compound (10 mg) in 0.5 ml of pyridine was added 0.5 ml of Ac_2O; the mixtures were kept at room temp for 24 hr, and after usual work-up the acetyl derivatives of each compound were obtained. Cucurbitacin E (8 mg) colourless needles, mp 190° (Lit. mp [11] 195°); isocucurbitacin B (7.5 mg) mp 119–121° (Lit. mp [12] 120–123°); *epi*-isocucurbitacin B (9 mg) 101–105° (Lit. mp [12] 103–107).

Acetylation of **1**. Compound **1** (13 mg) was dissolved in 0.5 ml of Ac_2O and 0.5 ml of pyridine. After 12 hr the reaction mixture was worked up as usual to yield 10 mg of the diacetate **1b**, mp 118–120°; IR ν_{max}^{KBr} cm^{-1}: 3020, 2950, 1731, 1724, 1605, 1459, 1370, 1260, 1029, 985, 908. ^1H NMR (see Table 1). EIMS *m/z* (rel. int.), 556 $[M^+]$ (0.5), 512 (0.5), 496 (3), 436 (3), 249 (23), 248 (96), 203 (25), 202 (3), 189 (50), 133 (20), 43 (100).

Preparation of the methyl ester **1a**. Compound **1** (30 mg) dissolved in MeOH was treated with an excess of CH_2N_2 to give **1b** (25 mg), mp 205–210°; EIMS *m/z* (rel. int.): 486 $[M^+]$ (1), 468 (0.5), 426 (1), 262 (100), 224 (15), 203 (30), 189 (21), 187 (60), 133 (40); IR ν_{max}^{KBr} cm^{-1}: 3500, 2987, 2957, 1718, 1460, 1380, 1040; ^1H NMR see Table 1.

Preparation of the acetonide **1c**. The methyl derivative **1a** (20 mg) was dissolved in 3 ml of dry Me_2CO and two drops of conc. H_2SO_4 were added. After shaking 1 hr the soln was filtered over a small basic alumina column to yield 15 mg of crystalline compound **1c**. IR ν_{max}^{KBr} cm^{-1} 2928, 2857, 1718, 1454, 1382, 1225, 1192, 1164, 1117, 1064, 1029, 1000, 861; ^1H NMR, see Table 1.

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REFERENCES

1. Aiello, A. A. (1979) *J. Arnold Arboretum* **60**, 38.
2. Budzikiewicz, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 3688.
3. Ogunkoya, L. (1981) *Phytochemistry* **20**, 121.
4. Pereda-Miranda, R., Delgado, G. and A. Romo de Vivar (1986) *J. Nat. Prod.* **49**, 225.
5. Takani, M. Kibota, K., Nozawa, M., Ushiki, T. and Takahashi, K. (1977) *Chem. Pharm. Bull.* **25**, 981.
6. Bruno, M., Savone, G., Hueso-Rodríguez, J. A., Pascual, C. and Rodríguez, B. (1987) *Phytochemistry* **26**, 497.
7. Cheung, H. T. and Willianson, D. G. (1969) *Tetrahedron* **25**, 119.
8. Tsuda, Y., Sano, T., Isobe, K. and Miyauchi, M. (1974) *Chem. Pharm. Bull.* **22**, 2396.
9. Kupchan, S. M., Gray, A. H. and Grove, M. D. (1967) *J. Med. Chem.* **10**, 337.
10. Lavie, D. and Glotter, E. (1971) *Prog. Chem. Org. Nat. Prod.* **29**, 307.
11. Elkhadem, H. and Abdel Rahman, M. M. A. (1963) *J. Chem. Soc.* 4991.
12. Arisawa, M., Pezzuto, J. M., Kinghorn, A. D., Cordell, G. A. and Farnsworth, N. R. (1984) *J. Pharm. Sci.* **73**, 411.
13. Reguero, M. T., Mata, R., Bye, R., Linares, E. and Delgado, G. (1987) *J. Nat. Prod.* **50**, 315.
14. Lewis, K. G. and Tucker, D. J. (1983) *Aust. J. Chem.* **36**, 2297.