Journal of Natural Products
Vol. 51, No. 5, pp. 996-998, Sep-Oct 1988

CHEMISTRY OF *HYPTIS MUTABILIS*: NEW PENTACYCLIC TRITERPENOIDS[1]

ROGELIO PEREDA-MIRANDA[2,*] and MARIANO GASCÓN-FIGUEROA

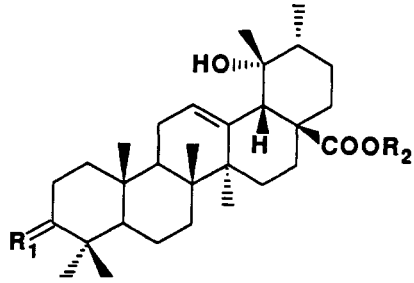
Departamento de Química Orgánica, Escuela Nacional de Ciencias Biológicas del Instituto Politénico Nacional, Prol. de Carpio y Plan de Ayala, México, DF 11340

In continuation of our studies on the constituents of Mexican Labiatae species (1–5), the present investigation deals with some triterpenoids isolated from *Hyptis mutabilis* (Rich.) Briq., a widely distributed shrub in the intertropical and tropical Americas, which is used as an indigenous drug in various gastrointestinal ailments and malaria (6–8).

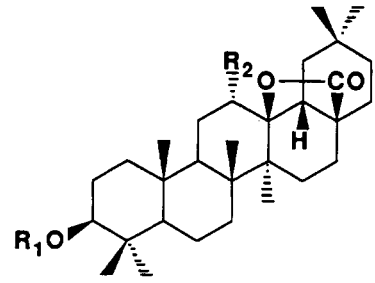
Chromatographic separation of the Me_2CO extract of dried aerial parts allowed the isolation of methyl betulinate, oleanolic acid acetate, and ursolic, oleanolic, and maslinic acids, as well as two new triterpenoids **1** and **5**.

Compound **1** had the molecular composition $C_{30}H_{48}O_4$ (elemental analysis and ms). Its ir showed bands for hydroxyl (3427 cm^{-1}), carbonyl (1695 cm^{-1}), and olefin (1630 cm^{-1}) absorptions. On alkylation with CH_2N_2, it formed an amorphous methyl ester, **2**. The mass spectrum of **2** showed diagnostically important peaks at *m/z* 278 (retro-Diels-Alder fragmentation around ring C), 260 $[278-H_2O]^+$, 250 $[278-C_2H_4]^+$, 219 $[278-CO_2Me]^+$, 218 $[278-HCO_2Me]^+$, 207, 201 $[219-H_2O]^+$, 179, and 146, which are consistent with the fragmentation pattern characteristic for the amyrin series (9). From the above mass spectral fragments, it was also evident that the secondary hydroxyl group was present in the A/B ring portion and its location at C-3 was highly probable on a biogenetic basis (10). The ^{1}H-nmr spectrum of **2** exhibited signals for six tertiary methyl groups, one proton singlet at δ 2.59 (H-18), and one proton multiplet at δ 5.35 (H-12), as expected for an urs-12-ene skeleton bearing an α-hydroxyl at C-19 (11–13). Also, it showed a triplet-like signal centered at δ 3.39, whose chemical shift and splitting pattern were typical of a 3α-hydroxyl group (14). Therefore, these results led us to formulate the structure for this natural triterpene as 3α,19α-dihydroxyurs-12-en-28-oic acid [**1**].

Further chemical evidence supporting the proposed stereochemistry was pro-



1 R_1=β-H, α-OH; R_2=H
2 R_1=β-H, α-OH; R_2=Me
3 R_1=O; R_2=Me
4 R_1=β-OH, α-H; R_2=Me



5 R_1=Ac, R_2=H
6 R_1=R_2=H
7 R_1=H, R_2=Br

[1]Part 1 in the series "Chemical Studies on Mexican *Hyptis* Species." Taken in part from the B.S. research work of M. Gascón-Figueroa.

[2]Present address: Departamento de Química Farmacéutica y Productos Naturales, División de Estudios de Posgrado de la Facultad de Química, Universidad National Autónoma de México, Circuito Escolar, Ciudad Universitaria, Coyoacán 04510, México, DF.

vided by the conversion of the derivative **2** to pomolic acid methyl ester **4** by Jones oxidation of this compound, to yield the oxo product **3**, followed by borohydride reduction. All physical and spectral data for compounds **3** and **4** were in agreement with those values previously reported (15).

The second new triterpenoid **5** analyzed for $C_{32}H_{50}O_4$ ($[M]^+$ at *m/z* 498) and was shown to be a γ-lactone with an acetoxyl function (ir ν max 1766 and 1725 cm^{-1}). There was no additional ir evidence for the presence of olefinic and hydroxylic functionalities. The ms features of this triterpene were common to some naturally occurring pentacyclic derivatives and suggestive of an oleanan-13β,28-olide system (16,17). The ^{1}H-nmr and ^{13}C-nmr spectra of **5** confirmed the structure of this metabolite as the 3β-acetoxy-oleanan-13β,28-olide, whose physical and spectroscopic properties were identical with those previously described (18,19). This is the first report of **5** as a natural product, although it has been synthesized during the course of the chromatographic isolation of the oleanolic lactone **6** (18,19).

The chemistry of *H. mutabilis* does not differ from the profile outlined for Labiatae, because it contains pentacyclic triterpenes that are widely distributed metabolites among other species of this family.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. The ir spectra were taken on a Perkin-Elmer 283B instrument. ^{1}H-nmr and ^{13}C-nmr spectra were determined on Varian FT-80 apparatus. Mass spectra were recorded on a Hewlett-Packard 5985-B spectrometer by direct inlet probe at 70 eV.

PLANT MATERIAL.—The aerial parts of *H. mutabilis* were collected in February 1987, km 9 Jalapa-Puerto de Veracruz High-road, Veracruz, México. A sample is maintained in the National Herbarium Instituto de Biología, UNAM (voucher 8520 M).

EXTRACTION.—Dried and finely powdered plant material (1.2 kg) was extracted with Me_2CO at room temperature for 5 days. After filtration, the solvent was evaporated, yielding a gum (58 g).

ISOLATION OF TRITERPENOIDS.—The crude extract was chromatographed on a column over Si gel (1.2 kg deactivated with 10% H_2O) using $CHCl_3$/EtOAc gradient elution system. Fractions of 500 ml were collected.

The low polarity fractions 32–53, eluted with $CHCl_3$-EtOAc (9:1), contained a solid residue, which upon crystallization from hexane-MeOH (2:1) yielded 38 mg (0.003% of the dry wt) of methyl betulinate (20). Fractions 67–82, eluted from the original column with the same solvent system, were rechromatographed on 120 g of Si gel. Elution with hexane-EtOAc (95:5) afforded 85 mg (0.007% of the dry wt) of the oleanolic lactone acetate **5** (19): mp 292–294°; $[\alpha]^{20}D$ +18.8° (*c*= 6.9, $CHCl_3$), whereas hexane-EtOAc (9:1) eluate yielded 33 mg (0.0027% of the dry wt) of oleanolic acid acetate, which was identical to an authentic sample.

Fractions 86–105, eluted with $CHCl_3$-EtOAc (4:1), afforded 1 g of a mixture of oleanolic and ursolic acids; this material was dissolved in MeOH (150 ml) and treated with Br_2 (50 mg). After 3 h, the solution was cooled in an ice bath to give a crystalline mixture of two substances. The binary mixture was separated by cc on Si gel using as eluents $CHCl_3$-EtOAc (8:2). After usual workup, of 12α-bromo,3β-hydroxy-oleanan-13β,28-olide [**7**] (168.04 mg), mp 225° (21), and ursolic acid (832 mg, 0.069% of the dry wt) were separated. Oleanolic acid was finally obtained by heating the bromolactone **7** with HOAc (30 ml) and zinc dust (0.5 g) on a steam bath for 4 h. The reaction mixture was worked up as previously described (21) to yield 56 mg of crude oleanolic acid (0.0046% of the dry wt). Both ursolic and oleanolic acids, as well as their methyl ester derivatives, were identical to standard samples.

Rechromatography of the subsequent fractions 107–112, from the original column, allowed the separation of an additional 28 mg of ursolic acid, which was closely followed in the chromatographic elution with $CHCl_3$-Me_2CO (9:1) by 3-*epi*-pomolic acid **1** (34.3 mg; 0.0028% of the dry wt): oil, ir ν max (Nujol) cm^{-1} 3427, 2950, 2872, 1695, 1458, 1377, 1164, 1048, 960; ^{1}H nmr (80 MHz, $CDCl_3$/DMSO-d_6) δ 0.70 (3H, s, 26-Me), 0.83 (3H, s, 23-Me), 0.91 (3H, s, 24-Me), 0.97 (3H, s, 25-Me), 1.22 (3H, s, 27-Me), 1.25 (3H, s, 29-Me), 3.40 (1H, t, *J*= 3 Hz, H-3), 5.34 (1H, m, H-12); eims *m/z* (% rel. abundance), $[M]^+$ 472 (1.3), 454 (2.8), 439 (2.2), 426 (9.2), 408 (1.6), 393 (1.5), 354 (4.8), 219 (9.5), 218 (9.2), 146 (48.5). Found C 76.35, H 10.26; $C_{30}H_{48}O_4$ requires C 76.27, H 10.23%.

Finally, the polar fractions 120–132, eluted with $CHCl_3$-EtOAc (7:3), afforded 47 mg (0.003% of the dry wt) of maslinic acid, which

was identified by comparison with an authentic sample.

METHYL ESTER **2** OF COMPOUND **1**.—Compound **1** (30 mg) dissolved in EtOH was alkylated with an excess of CH_2N_2 in Et_2O at 5° to give **2** (28.7 mg): oil; ir ν max ($CHCl_3$) cm^{-1} 3625, 2936, 1718, 1462, 1390, 1150, 1067, 928; ^1H nmr (80 MHz, $CDCl_3$) δ 0.68 (3H, s, 26-Me), 0.85 (3H, s, 23-Me), 0.92 (3H, s, 24-Me), 0.95 (3H, s, 25-Me), 1.22 (3H, s, 27-Me), 1.25 (3H, s, 29-Me), 2.59 (1H, s, H-18), 3.39 (1H, t, J = 3 Hz, H-3), 3.60 (3H, s, MeOCO-), 5.35 (1H, m, H-12); eims *m/z* (% rel. abundance), $[M]^+$ 486 (2.3), 471 (0.7), 468 (3.2), 453 (2.8), 426 (6.5), 408 (2.0), 393 (1.5), 369 (1.0), 354 (2.5), 278 (2.9), 260 (6.2), 250 (6.8), 220 (6.0), 219 (6.1), 218 (6.1), 207 (8.5), 201 (20.1), 190 (23.8), 179 (55.7), 146 (33.5), 55 (39.3), 43 (100).

OXIDATION OF **2**.—Derivative **2** (25 mg) was dissolved in Me_2CO (3 ml), and 0.5 ml of Jones reagent was added at room temperature. After shaking 5 min, the solution was diluted with H_2O and extracted with $CHCl_3$. Removal of the excess of solvent left a residue which was purified by cc over Si gel with $CHCl_3$-Me_2CO (9:1) to yield a crystalline residue which upon recrystallization from MeOH afforded 15.3 mg of **3**: mp 201–203° [lit. (15) mp 204°]; $[\alpha]^{20}D$ +50.2° (c = 2.0, $CHCl_3$); ir ν max ($CHCl_3$) cm^{-1} 3625, 2936, 1720, 1460, 1378, 1260, 1176, 1032, 984, 925; ^1H nmr (80 MHz, $CDCl_3$) δ 0.75 (3H, s, 26-Me), 1.07 (6H, s, 24-Me, 25-Me), 1.10 (3H, s, 23-Me), 1.21 (3H, s, 27-Me), 2.59 (1H, s, H-18), 3.60 (3H, s, MeOCO-), 5.35 (1H, m, H-12); eims *m/z* (% rel. abundance), $[M]^+$ 484 (2.5), 469 (0.2), 466 (1.0), 451 (1.5), 424 (2.0), 352 (0.8), 278 (5.4), 260 (12.0), 250 (8.3), 219 (15.4), 218 (14.8), 201 (22.4), 179 (100), 146 (45.7), 55 (39.4), 43 (79.4).

REDUCTION OF **3**.—The oxo derivative **3** (15 mg) was refluxed with $NaBH_4$ (0.1 g) in THF (20 ml) for 25 min. Workup in the usual way gave 11 mg of white needles of **4**. The spectral and physical properties of this compound were identical to those previously described for pomolic acid methyl ester (15).

ACKNOWLEDGMENTS

The authors would like to thank Dr. Guillermo Delgado, Instituto de Química de la Universidad Nacional Autónoma de México, for his continuous support; thanks are also due to the ms and nmr staff, from the same institution, for the recording of the spectra. Finally, we wish to thank Biol. Esteban Manuel Martínez, National Herbarium, Instituto de Biología de la Universidad Nacional Autónoma de México, for the identification of the plant material.

This work was supported in part by the Dirección de Estudios de Posgrado e Investigación del Instituto Politécnico Nacional through the research grant No. 872334.

LITERATURE CITED

1. G. Delgado, R. Pereda-Miranda, and A. Romo de Vivar, *Heterocycles*, **23**, 1869 (1985).
2. R. Pereda-Miranda, G. Delgado, and A. Romo de Vivar, *J. Nat. Prod.*, **49**, 225 (1986).
3. G. Delgado, X. Cárdenas, L. Alvarez, A. Romo de Vivar, and R. Pereda-Miranda, *J. Chem. Res.*, (S) 268, (M) 2565 (1986).
4. R. Pereda-Miranda, G. Delgado, and A. Romo de Vivar, *Phytochemistry*, **25**, 1931 (1986).
5. R. Pereda-Miranda and G. Delgado, *J. Nat. Prod.*, **49**, 1160 (1986).
6. M. Martínez, "Las Plantas Medicinales de México," 4th ed., Ed. Botas, México, 1959, p. 289.
7. H. García Barriga, "Flora Medicinal de Colombia. Botánica Médica," Ed. Instituto de Ciencias Naturales, Colombia, Vol. 3, 1975, p. 11.
8. D. Alzugaray and C. Alzugaray, "Plantas que curan," Ed. Grupo de Comunicão Três, Brazil, Vol. 1, 1984, p. 41.
9. L. Ogunkoya, *Phytochemistry*, **20**, 121 (1981).
10. S. Seo, Y. Tomita, and K. Tori, *J. Am. Chem. Soc.*, **103**, 2075 (1981).
11. P. Potier, B.C. Das, A. Bui, M. Janot, A. Pourrat, and H. Pourrat, *Bull. Soc. Chim. Fr.*, 3458 (1986).
12. J. Bermejo, J.L. Bretón, G. de la Fuente, and A.G. González, *Tetrahedron Lett.*, 4649 (1967).
13. G. Romeo, P. Giannetto, and M.C. Aversa, *Org. Magn. Reson.*, **9**, 29 (1977).
14. N.S. Bhacca and D.H. Williams, "Application of NMR Spectroscopy in Organic Chemistry," Holden-Day, San Francisco, 1964, p. 78.
15. C.H. Brieskorn and H. Wunderer, *Chem. Ber.*, **100**, 1252 (1967).
16. T. Murata, S. Imai, M. Imanishi, M. Goto, and K. Morita, *Tetrahedron Lett.*, 3215 (1965).
17. P.L. Majumber and M. Chakraborty, *Tetrahedron*, **35**, 2397 (1979).
18. V. Bellavista, F. Schiafella, and T. Mezzetti, *Phytochemistry*, **13**, 289 (1974).
19. H.M.G. Al-Hazimi, G.A. Miana, and M.S.H. Deep, *Phytochemistry*, **26**, 1091 (1987).
20. P. Monaco and L. Previtera, *J. Nat. Prod.*, **47**, 673 (1984).
21. K.G. Lewis and D.L. Tucker, *Aust. J. Chem.*, **36**, 2297 (1983).

Received 2 February 1988