

SESQUITERPENE LACTONES FROM *STEVIA OVATA* AND CRYSTAL STRUCTURE OF 11,13-DEHYDROERIOLIN

JOSÉ S. CALDERÓN, LEOVIGILDO QUIJANO, FEDERICO GÓMEZ-GARIBAY, DONATO M. SANCHEZ, TIRSO RIOS and FRANK R. FRONCZEK*

Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Coyoacán 04510, México, D.F.; *Department of Chemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

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Abstract—The aerial parts of *Stevia ovata* afforded three sesquiterpene lactones, of which one was new. Its structure was established by spectroscopic methods and chemical reactions. One of the compounds, 4 α ,5 β -epoxy-8-epi-inunolide was chemically correlated with 11,13-dehydroeriolin. The structure of the latter was confirmed by single crystal X-ray diffraction. The conformation of 11,13-dehydroeriolin in the solid is chair-chair [1D_5 , $^1D^{14}$].

INTRODUCTION

The genus *Stevia* (tribe Eupatoriaceae, subtribe Piqueriinae) [1] with about 150–200 species is abundant in Mexico [2]. So far phytochemical investigations have shown that this genus is chemically heterogeneous [3].

As part of our chemical systematic study of the tribe Eupatoriaceae, we have previously analysed *Stevia monardaefolia* [4, 5] and *Stevia rhombifolia* H.B.K. [6], which according to Grashoff [7] is synonymous with *Stevia ovata* Willd. Previous studies on this species afforded the paniculoides I–V [8], a longipinene derivative [9] and two bisabolene derivatives [10]. We now report the isolation and structure elucidation of the new sesquiterpene lactone 4-acetyl-8-epi-inunviscolide (1) and the known lactones 4 α , 5 β -epoxy-8-epi-inunolide (5) and inunviscolide (7) [11].

RESULTS AND DISCUSSION

4-Acetyl-8-epi-inunviscolide (1) had the molecular formula $C_{17}H_{22}O_4$ and was shown to be a sesquiterpene lactone of the guaianolide type. IR absorptions at 1730 cm^{-1} indicate the presence of an acetate group (m/z 230 due to $[M - 60]^+$) and at 1765 cm^{-1} which are typical of an α,β -unsaturated γ -lactone. This is supported by the presence of two doublets typical of the lactonic exocyclic methylene at $\delta 6.27$ ($J = 2.2$) and 5.66 ($J = 2.0$) in the ^1H NMR spectrum of 1. Also the ^1H NMR of 1 exhibited two broad singlets at $\delta 4.92$ and 4.99 which were assigned to an exocyclic methylene group (IR absorption at 900 cm^{-1}). The doublet of doublet of doublets at $\delta 4.53$ ($J = 10, 7.5, 5.0\text{ Hz}$) was assigned to H-8, since irradiation of this signal affected H-9 and converted a broad signal at $\delta 3.07$ into a broad triplet assigned to H-7. Conversely, irradiation at $\delta 3.07$ (H-7) affected H-8 and collapsed the conjugated exocyclic methylene proton signals to singlets. The *cis*-lactone ring fusion was deduced from the allylic

coupling constants of the C-13 methylene according to Samek's rule [12] and the chemical shift of H-8 ($\delta 4.53$) shifted to lower field compared with that of inunviscolide ($\delta 4.33$) (7) which possesses *trans* annelation [11]. We have observed that in C-8 closed γ -lactones, the H-8 α (equatorial) signal in *cis*- γ -lactones has a higher chemical shift than the H-8 β (axial) signal in *trans*- γ -lactones, for instance tomentosin ($\delta 4.67$) [13] and its 8-epimer xanthosin ($\delta 4.24$) [13]; helenalin acetate ($\delta 4.90$) [14] and its 8-epimer bigelovin ($\delta 4.66$) [15]; ivangustin ($\delta 4.86$) [16] and 8-epi-ivangustin ($\delta 4.09$) [11]; pleniradin acetate ($\delta 5.39$) [17] and its 8-epimer gaillardin ($\delta 4.48$) [18]. Therefore the chemical shift of H-8 seems to be sufficiently characteristic for assignment of the C-8 lactone ring closure. The acetoxy group in 1 was placed at C-4 in the following manner. Acid hydrolysis of 1 afforded an elimination product characterized as zinniolide (2) [19]. Selective epoxidation of 2 with *m*-chloro perbenzoic acid produced the α -epoxide 3. Its stereochemistry was deduced on the reasonable assumption that the peracid attacked from the less hindered α -face. Treatment of epoxide 3 with boron trifluoride furnished the ketone 4 ($\nu_{\text{max}} 1740\text{ cm}^{-1}$), thus establishing the acetoxy group at C-4 and the methylene group at C-10. Since the acetoxy group undergoes in high yield bimolecular elimination toward C-3 rather than toward C-5, as in pseudoivalin [20], the stereochemistry of the acetoxy group is very likely α [20]. The stereochemistry of H-1 and H-5 must be the same as in zinniolide (2). Based on all these facts we propose 1 as the more likely structure for 4-acetyl-8-epi-inunviscolide.

The second compound isolated, 4 α ,5 β -epoxy-8-epi-inunolide (5), $C_{15}H_{20}O_3$, mp $95\text{--}98^\circ$, exhibited IR and ^1H NMR features which closely resembled those of a substance isolated from *Inula* species [13], to which structure 8 has been assigned only with a different stereochemistry at C-4, C-5 and a *cis*-fused lactone ring, in spite of the chemical shift of H-8, which was markedly different from that of inunolide [21], for which the stereochemistry of the lactone ring was established as *cis*.

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The same compound with the structure **8** was also isolated from *Telekia speciosa* [22] but the stereochemistry of the 4,5-epoxy group was changed without any argument. Furthermore in a recent paper [23], the stereochemistry of compound **8** was proposed as the same as our compound **5**, which we have previously established based on the cyclization of **5**, which afforded the guaianolide inuviscolide (**7**) [24]. Final confirmation of the structure of **5** was achieved by epoxidation with *m*-chloroperoxybenzoic acid which furnished the epoxy derivative **6**, which was identical with an authentic sample of 11,13-dehydroeriolin (**6**), previously isolated from *Schkuhria virgata* [24].

In order to eliminate structural and stereochemical uncertainties single crystal X-ray data on 11,13-dehydroeriolin (**6**) were obtained. The solid state structure of 11,13-dehydroeriolin is depicted in Fig. 1. The torsion angles in Table 1 demonstrate that the conformation of the 10-membered ring is the chair-chair form [$^{15}\text{D}_5$, $^{14}\text{D}_4$], with crossed epoxidized double bonds, typical of *trans,trans*-germacrolides. The distance between the bond centres C1-C10 and C4-C5 is 3.108 Å. The conformation of the lactone ring is half chair with C12 and the C7-C8 midpoint on the local two-fold axis. Bond distances (esds 0.003–0.004 Å) and angles (esds 0.2–0.3°)

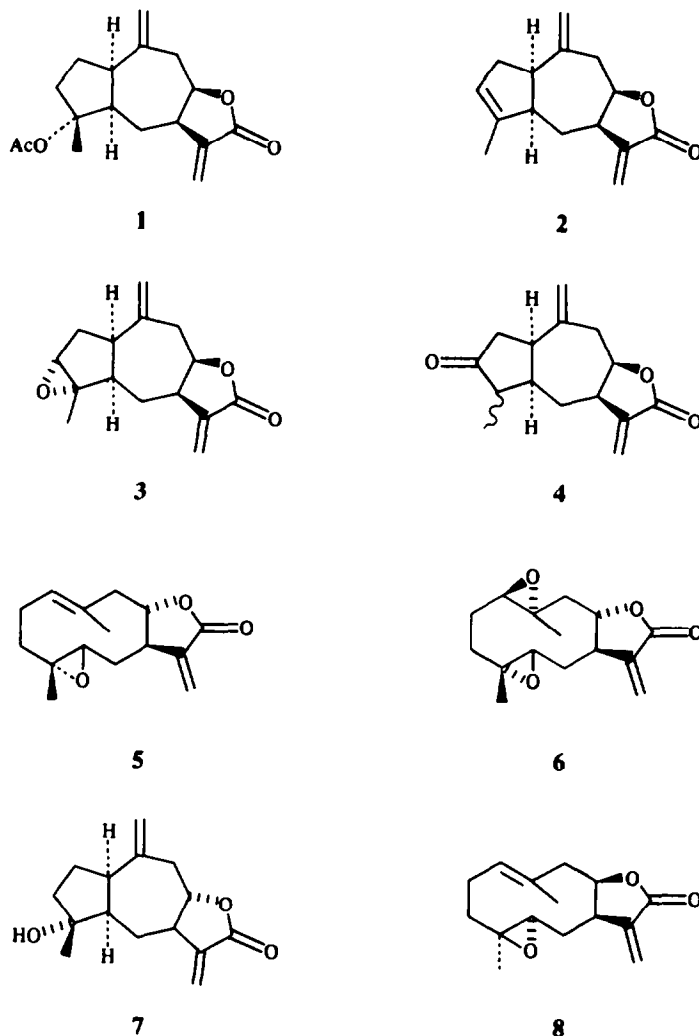
are normal, and have been deposited with the Cambridge Crystallographic Data Centre. The two epoxides are unsymmetrical, with C–O distances involving the methyl-substituted carbon atom averaging 1.460 Å, and those involving the unsubstituted carbon atom averaging 1.437 Å. The epoxide C–C distances average 1.473 Å. C–H distances for refined hydrogen atoms range 0.89(4)–1.08(3) Å and average 0.99 Å.

The third compound was the inuviscolide (**7**) which is an artefact originated by cyclization of **5** during the purification on silica gel, therefore it is not a constituent of *S. ovata*.

EXPERIMENTAL

Aerial parts of *Stevia ovata* Willd were collected 5 km W of Oaxtepec on road Cuautla–Cuernavaca, State of Morelos, Mexico, in November, 1980. A voucher specimen Calderon no. 66 is on deposit at the National Herbarium UNAM, (MEXU).

The air-dried plant material, leaves and flowers (100 g) were extracted 2 × with boiling petrol (3 l) and the solvent removed *in vacuo*. The petrol extract (20 g) was chromatographed over 250 g silica gel (Grace 204–325 mesh) using petrol–CHCl₃, CHCl₃ and CHCl₃–Me₂CO as eluants. Fractions eluted with petrol–CHCl₃ (1:1) and CHCl₃ (5:15 g) were combined and rechromatographed



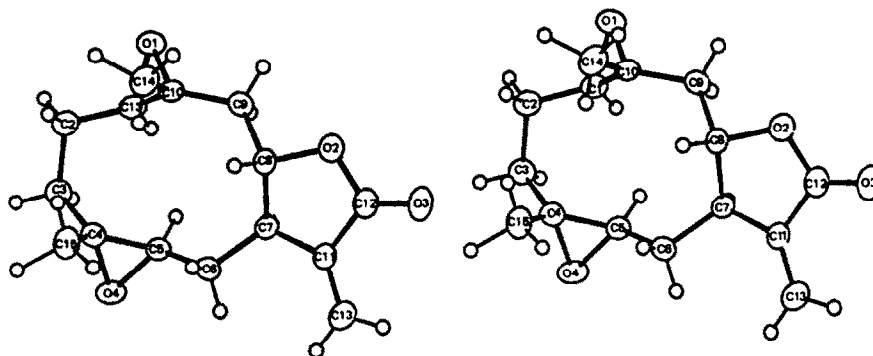


Fig. 1. Stereoscopic representation of 11,13-dehydroeriolin (6).

Table 1. Selected torsion angles in 11,13-dehydroeriolin

Atoms	Angle (deg)	Atoms	Angle (deg)
C1–C2–C3–C4	56.3	C9–C10–C1–C2	152.6
C2–C3–C4–C5	–85.2	C10–C1–C2–C3	–109.0
C3–C4–C5–C6	154.4	C7–C8–O2–C12	–24.1
C4–C5–C6–C7	–126.3	C8–O2–C12–C11	9.6
C5–C6–C7–C8	78.1	O2–C12–C11–C7	9.4
C6–C7–C8–C9	–89.5	C12–C11–C7–C8	–22.6
C7–C8–C9–C10	90.3	C11–C7–C8–O2	27.4
C8–C9–C10–C1	105.4		

on 120 g silica gel using CHCl_3 – Me_2CO mixtures of increasing polarity, to yield 14 fractions of 125 ml. Fractions 6–9 (1.68 g) were combined and purified by prep. TLC (CHCl_3 – Me_2CO , 9:1) yielding 675 mg 1 and 768 mg 5. Fractions 11–13 (205 mg) were purified by prep. TLC (CHCl_3 – Me_2CO , 9:1) affording 45 mg 7.

4-Acetyl-8-*epi*-inuvicolide (1). Colourless oil IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1765, 1730, 940, 900. EIMS 70 eV m/z (rel. int.): no $[\text{M}]^+$, 230 $[\text{M} - \text{HOAc}]^+$ (39), 215 $[\text{M} - \text{HOAc} - \text{Me}]^+$ (15), 145 (49), 119 (89), 91 (97), 79 (54), 43 $[\text{MeCO}]^+$ (100). $^1\text{H NMR}$ (200 MHz, CDCl_3): δ 1.54 (3H, s, H-15), 2.01 (3H, s, Ac), 2.50 (1H, dd, $J_{9a,9b} = 13$, $J_{8a,9b} = 10.0$ Hz, H-9 β), 2.67 (1H, dd, $J_{9a,9a} = 13$, $J_{8a,9b} = 5.0$ Hz, H-9 α), 3.07 (1H, m, H-7), 4.53 (1H, ddd, $J_{8a,9b} = 5.0$ Hz, $J_{8a,9b} = 10$, $J_{7a,8a} = 7.5$ Hz, H-8), 4.92 (1H, br s, H-14), 4.99 (1H, br s, H-14'), 5.67 (1H, d, $J = 2.0$ Hz, H-13), 6.27 (1H, d, $J = 2.2$ Hz, H-13').

$$[\alpha]_{\text{D}}^{25} = \frac{589}{+10.0} \quad \frac{578}{+11.7} \quad \frac{546}{+12.6} \quad \frac{435}{+22.2} \quad \frac{365}{+28.9}$$

(CHCl_3 ; c 0.239).

Compound 2. To a soln of 350 mg of 1 in 125 ml MeOH, 1 ml of HCl was added. After usual work-up, the residue was purified by prep. TLC (CHCl_3 – Me_2CO , 9:1) giving 175 mg of 2 as an oil. $[\alpha]_{\text{D}}^{25} = +107^\circ$ (CHCl_3 , c 0.216) reported $[\alpha]_{\text{D}}^{24} = +140.2^\circ$ (CHCl_3 , c 1.64) [19], IR, MS and $^1\text{H NMR}$ spectra identical with those previously published [19].

Epoxidation of 2. To a soln of 2 (120 mg) in CHCl_3 , *m*-chloroperbenzoic acid (120 mg) was added and the reaction monitored by TLC. Work-up as usual yielded 65 mg of 3 as a crystalline compound, mp 107–109° (CHCl_3 –petrol). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1760, 1050, 1000, 950, 910; EIMS 70 eV m/z (rel. int.): 246 $[\text{M}]^+$ (9), 231 $[\text{M} - \text{Me}]^+$ (8), 131 (50), 107 (44), 105 (46), 91 (100). $^1\text{H NMR}$ (60 MHz, CDCl_3): δ 1.45 (3H, s, H-15), 4.50 (1H, td, $J = 6.5$,

9.0 Hz, H-8), 4.95 (2H, br s, H-14), 5.65 (1H, d, $J = 2.0$ Hz, H-13), 6.25 (1H, d, $J = 2.0$ Hz, H-13').

Reaction of 3 with $\text{BF}_3 \cdot \text{Et}_2\text{O}$. Epoxide 3 (55 mg) was dissolved in C_6H_6 (30 ml) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.3 ml) was added. After usual work-up, the residue was purified by TLC (CHCl_3 – Me_2CO , 9.5:0.5) to give 40 mg 4. Colourless oil. IR $\nu_{\text{max}}^{\text{film}}$ cm^{-1} : 1765, 1740, 1055. EIMS 70 eV m/z (rel. int.): 246 $[\text{M}]^+$ (17), 231 $[\text{M} - \text{Me}]^+$ (5), 174 (47), 91 (100), 79 (76), 77 (82), 55 (79). $^1\text{H NMR}$ (60 MHz, CDCl_3): δ 1.16 (3H, d, $J = 6.5$ Hz, H-15), 4.85 (1H, td, $J = 5.5$, 8.0 Hz, H-8), 5.0 (1H, br s, H-14), 5.06 (1H, br s, H-14'), 5.67 (1H, d, $J = 2.2$ Hz, H-13), 6.33 (1H, d, $J = 2.5$ Hz, H-13').

4 α ,5 β -Epoxy-8-*epi*-inuvicolide (5). Crystalline compound, mp 95–98°, $[\alpha]_{\text{D}}^{25} = -6.2^\circ$ (CHCl_3 , c 0.193). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1} : 1765, 1140, 990, 890; EIMS 70 eV m/z (rel. int.): 248 $[\text{M}]^+$ (0.5), 230 $[\text{M} - \text{H}_2\text{O}]^+$ (0.8), 95 (56), 81 (65), 68 (100), 43 (62). $^1\text{H NMR}$ (100 MHz, CDCl_3): δ 1.15 (3H, s, H-15), 1.77 (3H, s, H-14), 2.82 (1H, m, H-7), 2.64 (1H, br d, $J_{5-6} = 10$ Hz, H-5), 4.07 (1H, ddd, $J_{8\beta-9\beta} = 2$, $J_{7a-8\beta} = 7.0$, $J_{8\beta-9a} = 10.5$ Hz, H-8), 5.26 (1H, m, H-1), 5.62 (1H, d, $J = 3.0$ Hz, H-13), 6.28 (1H, d, $J = 3.5$ Hz, H-13').

Epoxidation of compound 5. To a soln of 5 (76 mg) in 30 ml Me_2CO , 300 mg K_2CO_3 and 100 mg *m*-chloroperbenzoic acid were added, and the mixture allowed to react for 1.5 hr. Usual work-up gave 44 mg 6. Identical in all respects with authentic 11,13-dehydroeriolin 6 [24].

Cyclization of compound 5. *p*-Toluenesulphonic acid (50 mg) was added to a soln of 5 (50 mg) in C_6H_6 . Work up as usual yielded 30 mg 7 which was identical to inuvicolide [11].

X-Ray data for 11,13-Dehydroeriolin. A crystal of dimensions 0.24 \times 0.40 \times 0.64 mm was used for data collection on an Enraf-Nonius CAD4 diffractometer equipped with MoK α radiation ($\lambda = 0.71073$ Å) and a graphite monochromator. Crystal data are: $\text{C}_{15}\text{H}_{20}\text{O}_4$, $M_r = 264.3$, orthorhombic space group $P2_12_12_1$, $a = 6.706(1)$, $b = 13.236(4)$, $c = 15.273(3)$ Å, $Z = 4$, $d_c = 1.295$ g cm^{-3} , μ (MoK α) = 0.87 cm^{-1} . Data were collected by ω - 2θ scans of variable speed, designed to yield $I = 50\sigma(I)$ for all significant reflections. One octant of data having $1^\circ < \theta < 30^\circ$ was collected at 23°, yielding 2264 unique reflections, of which 1428 had $I > 3\sigma(I)$ and were used in the refinement. Data reduction included corrections for background, Lorentz, and polarization effects; absorption effects were negligible.

The structure was solved by direct methods and refined by full matrix least squares based on F with weights $W = \sigma^{-2}(F_o)$. Nonhydrogen atoms were treated anisotropically; hydrogen atoms were located by difference maps and refined isotropically, except for those of methyl groups, which were included as fixed contributions with isotropic $B = 5.0$ Å². Convergence was

achieved with $R = 0.045$, $R_w = 0.056$, and maximum residual density 0.19 eÅ^{-3} for 228 variables.

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REFERENCES

- Robinson, H. and King, R. M. (1977) *The Biology and Chemistry of the Compositae* (Heywood, V. H., Harborne, J. B. and Turner, B. L., eds) p. 437. Academic Press, London.
- Matuda, E. (1958) *Bol. Soc. Bot. Mex.* **23**, 55.
- Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1982) *Phytochemistry* **21**, 2021.
- Quijano, L., Calderon, J. S., Gomez, G. F., Vega, J. L. and Rios, T. (1982) *Phytochemistry* **21**, 1369.
- Gomez, G. F., Quijano, L., Calderon, J. S., Perales, A. and Rios, T. (1983) *Phytochemistry* **22**, 197.
- Rios, T., Romo de Vivar, A. and Romo, J. (1967) *Tetrahedron* **23**, 4265.
- Grashoff (1972) Doctoral thesis, University of Texas, Austin, Tx.
- Kaneda, N., Kohda, H., Yamasaki, K., Tanaka, O., Nishi, K. (1978) *Chem. Pharm. Bull.* **26**, 2266.
- Román, L. U., Del Rio, R. E., Hernández, J. D., Jopeph-Nathan, P., Zabel, V. and Watson, W. H. (1981) *Tetrahedron* **16**, 2769.
- Bohlmann, F., Suwita, A., Natu, A. A., Czerson, H. and Suwita, A. (1977) *Chem. Ber.* **110**, 3572.
- Bohlmann, F., Czerson, H. and Schoneweiß, S. (1977), *Chem. Ber.* **110**, 1330.
- Samek, Z. (1978), *Collect. Czech. Chem. Commun.* **43**, 3210.
- Bohlmann, F., Mahanta, P. K., Jakupovic, J., Rastogi, R. C. and Natu, A. A. (1978) *Phytochemistry* **17**, 1165.
- Diaz, E., Nathan, P. J., Romo de Vivar, A. and Romo, J. (1965) *Bol. Inst. Quim. Univ. Nacl. Auton. Mex.* **17**, 122.
- Parker, B. A. and Geissman, T. A. (1962) *J. Org. Chem.* **27**, 4127.
- Herz, W., Sumi, Y., Sudarsanam, V. and Raulais, D. (1967) *J. Org. Chem.* **32**, 3658.
- Herz, W., Murari, R. and Blount, J. F. (1979) *J. Org. Chem.* **44**, 1873.
- Dullforce, T. A., Sim, G. A. and White, D. N. J. (1969) *Tetrahedron Letters* 973.
- Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 1343.
- Herz, W., Romo de Vivar, A. and Lakshmikantham, M. V. (1965) *J. Org. Chem.* **30**, 118.
- Ravindranath, K. R., Raghavan, R., Parknikar, S. K., Trivedi, G. K. and Bhattacharyya, S. C. (1978) *Indian J. Chem. Sect. B* **16B**, 27.
- Bohlmann, F., Jakupovic, J. and Schuster, A. (1981) *Phytochemistry* **20**, 1891.
- Bohlmann, F., Jakupovic, J., Ahmed, M. and Schuster, A. (1983) *Phytochemistry* **22**, 1623.
- Romo de Vivar, A., Perez, A. L., Leon, C. and Delgado, G. (1982) *Phytochemistry* **21**, 2905.