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SESQUITERPENE LACTONES AND OTHER CONSTITUENTS OF *EIRMOCEPHALA MEGAPHYLLA* AND *CYRTOCYMURA CINCTA*

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Key Word Index—*Eirmocephala megaphylla*; *Cyrtocymura cincta*; Compositae; Vernonieae; Vernoniinae; sesquiterpene lactones; glaucolides; piptocarphols; 7-epiloliolide.

Abstract—Aerial parts of *Eirmocephala megaphylla* afforded one known and one new glaucolide, four known piptocarphols, 3,6-dimethoxy-5,3′-4′-trihydroxyflavone, loliolide and 7-epiloliolide. Aerial parts of *Cyrtocymura cincta* furnished two known glaucolides and two known piptocarphols, while the roots contained costunolide and two eudesmanes.

In continuation of our study of Vernoniinae of northern Argentina and adjacent regions [1–7] we have examined *Eirmocephala megaphylla* (Hieron.) H. Robinson (old binomial *Vernonia megaphylla* [8]) and *Cyrtocymura cincta* (Griseb.) H. Robinson (*V. scorpioides* (Lam.) Pers. var. *cincta* Griseb. [8]). Aerial parts of *E. megaphylla* gave glaucolide B (**1a**) [9], the major lactone constituent, and the new analogue **1b**. The latter, like other lactones of this type, exhibited only broad signals at room temperature, some of which did not sharpen significantly at 76°. Decoupling in benzene-d_6 (see Experimental) established the structure. Further lactones found in this species were **2a** [10], **2b** [6, 7, 11], piptocarphin D (**2c**) [1, 2, 7, 12] and **2d** [1, 7]. Other constituents were 3,7-dimethoxy-5,3′,4′-trihydroxyflavone, loliolide and the previously unreported 7-epiloliolide (**3**), which differed from loliolide only in chemical shift and coupling constants of H-7 (see Experimental).

Aerial parts of *C. cincta* contained **1a**, **1c**, previously [13] isolated only from *C. lanuginosa* (Gardn.) H. Robinson (old binomial *V. lanuginosa* Gardn. [8]), **2b** and **2e** [14]. The roots contained costunolide, the eudesmane **4** also found earlier in the roots of *C. lanuginosa* [13], as well as the corresponding alcohol **5** (see Experimental). The similarity between the two *Cyrtocymura* species is noteworthy.

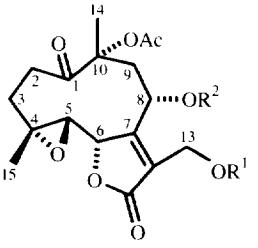
EXPERIMENTAL

General. For sepn of mixts, HPLC with a differential refractometer was used. Columns employed were (A) a Beckman Ultrasphere C8 (5 mm, 10 × 250 mm) and (B) a Phenomenex C18 (5 mm, 10 × 250 mm). Retention times were measured from the solvent peak.

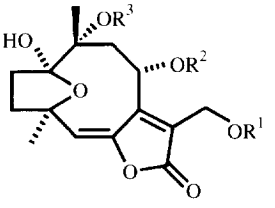
Plant material. Aerial parts of *E. megaphylla* (Hieron.) H. Robinson were collected at the flowering stage in October 1992 near Buena Vista, Departamento Santa Cruz, Bolivia. A voucher specimen LIL No. 595771 is deposited in the herbarium of the Fundación Miguel Lillo, Tucumán. Aerial parts and roots of *C. cincta* (Griseb.) H. Robinson were collected at the flowering stage in October 1994 near S. M. de Tucumán, Tucumán province, Argentina. A voucher specimen LIL No. 599841 is deposited in the herbarium of the Fundación Miguel Lillo, Tucumán.

Extraction and fractionation. Finely ground flowers and leaves of *E. megaphylla* (1100 g) were extracted with EtOAc free of HOAc (2 × 5 l) at room temp. for 4 days in a shaker. Evapn of the solvent gave 63 g of residue (5.7% yield), which was suspended in 540 ml EtOH at 55°, diluted with H_2O (405 ml) and extracted successively with hexane (2 × 300 ml), C_6H_6 (2 × 300 ml), CH_2Cl_2 (2 × 300 ml) and EtOAc (2 × 200 ml). Evapn of the C_6H_6 extract at red. pres. gave 6.2 g of residue, which was defatted by stirring with MeOH. Filtration and evapn of solvent furnished 5 g of residue, which was chromatographed over silica gel (eluent C_6H_6 and increasing amounts of EtOEt, 0–100%), 15 frs being collected. Frs containing sesquiterpene lactones as indicated by an IR band at 1765 cm^{-1} were purified further as follows.

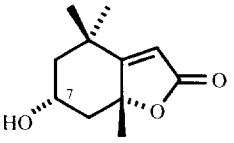
Frs 8–10 (combined wt 2.5 g) were taken up in $CHCl_3$. The insoluble material (60 mg) was identified as 3,7-dimethoxy-5,3′,4′-trihydroxyflavone by MS, ^1H NMR and NOE spectrometry. Evapn of the supernatant



1a R^1, R^2 = Ac
b R^1 = H, R^2 = Ac
c R^1 = Ac, R^2 = CH_2CH_3



2a R^1; R^2 = H, R^2 = Ac
b R^1, R^2 = Ac, R^3 = H
c R^1 = Ac, R^2, R^3 = H
d R^1 = Et, R^2 = Ac, R^3 = H
e R^1 = Ac, R^2 = Prop, R^3 = H



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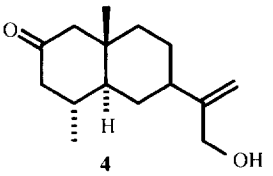

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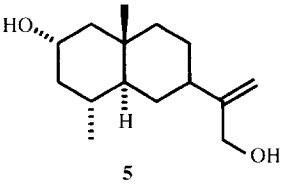

5

furnished 1.2 g of residue, which was subjected to HPLC (column B, MeOH–H_2O, 4:3, 2 ml min^{-1}) to give four peaks. The material in peak 3 (188 mg, R_t 21 min) was **1a**. Since TLC showed that the material in peaks 1, 2 and 4 represented mixts they were combined and reprocessed by HPLC (column A, MeOH–H_2O, 1:1, 2 ml min^{-1}) to give 4.3 mg of a complex mixt. of lactones (R_t 12 min), 4.8 mg **2a** (R_t 14 min) 7.9 mg of a mixt. of lactones (R_t 24 min), 6.6 mg **2b** (R_t 27 min), 53 mg **1a** (R_t 35 min) and 2.6 mg **2d** (R_t 60 min). Known compounds were identified by MS and ^1H NMR spectrometry, which included decoupling.

Fr. 11 (170 mg) of the original chromatogram on HPLC (column B, MeOH–H_2O, 1:1, 2 ml min^{-1}) yielded 3.0 mg **2a** (R_t 14 min), 1.8 mg of a mixt. containing loliolide (R_t 16 min), 21 mg **2b** (R_t 24 min) and 4.9 mg **1a** (R_t 42 min). Frs 12–15 of the original chromatogram (combined wt 520 mg) on HPLC (column B, MeOH–H_2O, 1:2, 2 ml min^{-1}) gave 6 peaks. Peak 3 (9 mg, R_t 18 min) was **1b**, peak 4 (11.4 mg, R_t 26 min) was **2b**, peak 5 (6.5 mg, R_t 32 min) was **1a** and peak 6 (R_t 35 min) was a mixt. of lactones. Rechromatography of peaks 1 and 2 (column A, MeOH–H_2O, 1:1, 1.7 ml min^{-1}) gave mixts (3.6 mg, R_t 15 min and 2.4 mg, R_t 18 min) and 2.8 mg **2c** (R_t 21 min).

Evapn of the CH_2Cl_2 extract gave 1.3 g of residue, a portion of which (325 mg) was subjected to HPLC (column B, MeOH–H_2O, 2:3, 3 ml min^{-1}) to give mixts and 6.9 mg **2b** (R_t 42 min).

Flowers and leaves of *C. cincta* (600 g) were extracted with $CHCl_3$ (2 × 4 l) at rom temp. for 4 days with shaking to give 20.1 g of residue (3.3%), which was suspended in 170 ml EtOH at 55°, diluted with H_2O (130 ml) and extracted successively with hexane (2 × 150 ml), C_6H_6 (2 × 150 ml), $CHCl_3$ (2 × 150 ml) and EtOAc (2 × 150 ml). Evapn of the C_6H_6 extract at red. pres. gave 7 g of residue, which was flash chromatographed on silica gel with C_6H_6 and increasing amounts of EtOAc (0–50%) and finally MeOH to give 28 frs. Frs containing sesquiterpene lactones (IR band at 1765 cm^{-1}) were further purified as follows.

Fr. 9 (73.1 mg) processed by HPLC (column B, MeOH–H_2O, 3:2, 2 ml min^{-1}) gave 2.5 mg **2b** (R_t 8 min) and 3 mg of unidentified material (R_t 48 min).

Frs. 10 and 11 (combined wt 119 mg) processed by HPLC (column A, MeOH–H_2O, 3:2, 2.2 ml min^{-1}) gave 84 mg **1c**. An 866 mg portion of frs 12–18 (combined wt 1.70 g) was processed by HPLC (column B, MeOH–H_2O, 1:1, 2 ml min^{-1}) to give 41 mg **1a** (R_t 14 min) and 196 mg **1c** (R_t 29 min). A third peak representing a lactone mixt. was reprocessed by HPLC (column A, MeOH–H_2O, 6:5, 2.5 ml min^{-1}) to give 2.9 mg **2e** (R_t 43 min). A 999 mg portion of frs 19–26 (combned wt 1.367 g) was also processed similarly (column B, MeOH–H_2O, 1:1, 2 ml min^{-1}) to give 1.2 mg **2b** (R_t 20 min), 6.1 mg **1a** (R_t 29 min), 159 mg **2e** (R_t 38 min) and 1.5 mg of a lactone mixt. (R_t 54 min). HPLC of frs 27 and 28 (combined wt 803 mg) in the same manner gave 156 mg **2b** (R_t 19 min), 59 mg **2e** (R_t 33 min), a further 2 mg **2e** (R_t 37 min) and 12 mg of a lactone mixt.

Evapn of the $CHCl_3$ extract gave 1.2 g of residue, which was subjected to HPLC (column B, MeOH–H_2O, 1:1, 2 ml min^{-1}) to give 24 mg **2b** (R_t 14 min), 17 mg **2e** and 8.7 mg **1c** (R_t 39 min).

The ground roots of *C. cincta* (280.5 g) were extracted successively with hexane (2 × 1.5 l), C_6H_6 (2 × 1.5 l). Evapn of the C_6H_6 extract at red. pres. gave 723 mg of residue, a portion of which (453 mg) was subjected to HPLC (column B, MeOH–H_2O, 2:1, 2 ml min^{-1}) to give 10 mg **4** (R_t 12 min) and 3 mg costunolide. Evapn of the CH_2Cl_2 extract gave 689 mg of residue, a portion of which (408 mg) was processed by HPLC (column B, MeOH–H_2O, 3:2, 2 ml min^{-1}) to give a further 4.5 mg **4** (R_t 21 min) and 1.5 mg **5** contaminated by **4** (R_t 22 min), MS PCI (NH_3) of **5** *m/z* (rel. int.): 256 $[M + NH_4]^+$ (32), 238 (45), 223 (100). ^1H NMR ($CDCl_3$, 500 MHz): δ 5.03 (*d*, J = 1 Hz, H-13a), 4.91 (*br s*, H-13b), 3.90 (*tt*, J = 11.5, 4.5 Hz, axial H-2), 4.14 (*br s*, 2H, H-12a,b), 0.88 (*d*, J = 6.5 Hz, 3H, H-15), 0.85 (*s*, 3H, H-14). Known compounds were identified by MS and ^1H NMR spectrometry, with decoupling.

3-Deacylglaucolide B (**1b**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 397 $[M + H]^+$ (100, $C_{19}H_{24}O_9$), 355 (7), 269 (37), IR ν_{max} cm^{-1}: 3400, 1770, 1730, 1720. ^1H NMR (500 MHz, $CDCl_3$, 60°): δ 4.84 (*br d*, $J \simeq$ *ca* 9 Hz, H-8), 4.82 (*br*, H-6), 4.47 (*br*, 2H, H-13a,b), 2.75 (*dd*, J = 16, 7 Hz, H-9a), 2.73 (*br*, 2H), 2.68 (*dd*, J = 15, 4.5 Hz), 2.50 (*br*), 2.40–2.35 (*br*, 2-3H), 2.09, 2.08 (each *s*, 3H, Ac), 1.64 (*s*, 3H, H-14) 1.59 (*br s*, 3H, H-15) 1.57 (*m*, H-9b); (C_6D_6, 76°): δ 4.77 (*dd*, J = 8, 1.5 Hz, H-8), 4.69 (*br d*, J = 10 Hz, H-6), 4.21 (*br d*, J = 15 Hz, H-13a), 4.19 (*br d*, J = 15 Hz, H-13b), 2.40 (*dd*, J = 16, 8 Hz, H-9a), 2.34 (*br ddd*, J = 17, 11.5, 5, 1 Hz, H-2a) 2.19 (*br d*, J = 10 Hz, H-5), 2.25 *br t* (7), 2.15 *m*, 1.95 *m*, 1.88 (*m*, 2H, H-2b, 3a), 1.60, 1.54 (each *s*, 3H, Ac), 1.47, 1.44 (each *s*, 3H, H-14, H-15), 1.28 (*m*, H-3b).

7-Epiloliolide (**3**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 197 $[M + H]^+$ (100, $C_{11}H_{16}O_3$). ^1H NMR ($CDCl_3$, 500 MHz): δ 5.71*s* (H-3), 4.13 (*tt*, J = 11.5, 4 Hz, H-7), 2.53 (*ddd*, J = 12, 4, 2 Hz), 2.04 (*ddd*, J = 13, 4, 2 Hz), 1.58, 1.31, 1.26 (each *s*, 3H); ^1H NMR (C_6D_6): δ 5.32 (*s*, H-3), 3.35 (*tt*, J = 11.5, 4 Hz), 2.02 (*ddd*, J = 12, 4, 2 Hz), 1.40 (*ddd*, J = 13, 4, 2 Hz), 1.07 (*t*, J = 12 Hz), 0.74 (*dd*, J = 13, 12 Hz), 1.04, 0.69, 0.60 (each *s* and 3H).

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