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Sesquiterpene lactones from *Centaurea tweediei*

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Abstract

Aerial parts of *Centaurea tweediei* from Argentina afforded as the main constituent the sesquiterpene lactone onopordopicrin and minor amounts of a new heliangolide, a new guaianolide, a new eudesmanolide, a new eudesmane acid and the lignans arctigenin and matairesinol. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Centaurea tweediei*; Centaureinae; Cardueae; Asteraceae; Sesquiterpene lactones; Lignans

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1. Subject and source

Aerial parts of *Centaurea tweediei* Hook. et Arn. (Asteraceae, Cardueae, Centaureinae) were collected at the flowering stage on November 11, 1998 near Estacion Paul Groussac, Road M, km 856, Santa Fé Province, Argentina. A voucher specimen (C.L. Cristobal and A. Krapovickas #2487) is deposited in the herbarium of the Instituto de Botanica del Nordeste (CTES), Corrientes, Argentina.

2. Previous work

Numerous publications have dealt with secondary metabolites of the large genus *Centaurea*, sesquiterpene lactones being the most characteristic constituents (Nowak et al., 1994). Of the mere handful of *Centaurea* species endemic to the New World all of which have been assigned to *Centaurea* group Plectocephalus II (Wagenitz and

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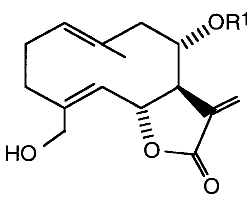
Hellwig, 1996) three have been studied so far. North American *C. americana* contained the guaianolide cynaropicrin (Ohno et al., 1973) and *C. chilensis* Hook. et. Arn. yielded several related guaianolides (Negrete et al., 1988, 1989), simple sesquiterpenes (Negrete et al., 1984) and a costic acid derivative (Sepulveda-Bozq and Breitmeier, 1987) while flavonoids have been reported from both *C. chilensis* (Negrete et al., 1988) and another Chilean endemic (Wagenitz, 1955) *C. floccosa* Hook. et Arn. (Negrete et al., 1987).

3. Present study

Centaurea tweediei Hook. et Arn. is a rare South American endemic whose distribution is limited to northeastern Argentina, Uruguay and southeastern Brazil (Cabrera, 1974). It has not been studied previously. Aerial parts (1500 g) were extracted with $CHCl_3$ (2×7 l) at RT for 7 days to give 90.9 g (6.06%) of crude extract which was suspended in EtOH (800 ml) at 55°C, diluted with H_2O (500 ml) and extracted successively with *n*-hexane (3×600 ml) and $CHCl_3$ (3×600 ml). The $CHCl_3$ extract on evaporation at reduced pressure furnished 42.76 g of residue a portion of which (30 g) was subjected to CC over Si gel (910 g, 70–230 mesh) using 21 fractions of $CHCl_3$ containing increasing amounts of EtOAc (0% frs 1–14, 20% frs 15–26, 33% frs 27–36, 50% frs 37–49, 100% frs 50–61). All fractions were monitored by TLC. Frs 24–27 (0.23 g) were combined and a portion (91 mg) was processed by HPLC (MeOH–H_2O 2 : 1, 1.5 ml min^{-1}) using a Beckman ultrasphere column C-18 (10 mm i.d. $\times$ 250 mm—column A) giving a mixture (mixture A) of poorly resolved peaks (12 mg, R_t 2.5–6.1 min), 12.8 mg of arctigenin (R_t 6.2 min) and mixtures. Rechromatography of mixture A using a Beckman ultrasphere column C-8 (column B, MeOH-H_2O 6 : 4, 1.5 ml min^{-1}) furnished an additional 2 mg of arctigenin (R_t 10.4 min). Frs. 28–33 (0.17 g) were combined; a portion (70 mg) on HPLC (column B, MeOH-H_2O 2 : 1, 1.5 mil min^{-1}) gave 5.7 mg of matairesinol (R_t 11.5 min) and 15.8 mg of arctigenin (R_t 15.4 min). Frs 43–45 (0.29 g) were combined; a portion (20 mg) on preparative TLC (Si gel, $CHCl_3$–EtOAc 3 : 2) gave a mixture of **2** and **3**; HPLC (column A, MeOH–H_2O 11 : 9, 2 ml min^{-1}) of another portion (106 mg) gave mixtures one of which (15.5 mg, R_t 9–15 min) resubmitted to HPLC gave 0.9 mg of **3** (R_t 11.5 min), 0.9 mg of **2** (R_t 17.5 min) and 1.4 mg of **4** (R_t 23.5 min). Frs 46–51 (19.8 g) and frs 52–62 (2.7 g) contained more than 90% onopordopicrin (**1**) as shown by ^{1}H NMR spectrometry. A portion (304 mg) of frs 46–51 on HPLC (column A, MeOH–H_2O 1 : 1, 1.5 ml min^{-1}) gave mixtures, 4.8 mg of **5** (R_t 29.9 min) and 232 mg of onopordopicrin.

(1*R**, 4*R**, 5*R**, 6*R**, 7*R**, 8*S**, 10*R**)-1-Hydroxy-8-methacryloxy-15-oxoeudesm-11(13)en-6,12-olide (**2**). Gum which decomposed on standing before the MS could be run; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.66 (*s*, H-15), 6.12 (*brs*, H-3′a), 6.11 (*d*, $J = 3.5$ Hz, H-13a), 5.63 (*t*, $J = 1.5$ Hz, H-3′b), 5.52 (*d*, $J = 3$ Hz, H-13b), 5.24 (*ddd*, $J = 11, 11, 4.5$ Hz, H-8), 3.97 (*t*, $J = 11$ Hz, H-6), 3.42 (*dd*, $J = 11, 4$ Hz, H-1a), 2.87 (*tt*, $J = 11, 3$ Hz, H-7), 2.57 (*dd*, $J = 12.5, 4.5$ Hz, H-9a), 2.57 (*dd*, $J = 12.5, 4.5$ Hz, H-9a), 2.50 (*dddd*, $J = 11, 11, 4, 3$ Hz, H-4), 1.95 (*brs*, 3p, Me), 1.87 (*t*, $J = 11$ Hz,



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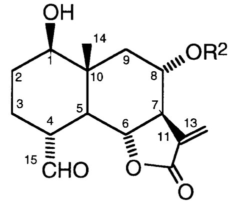

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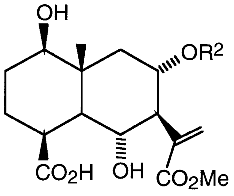

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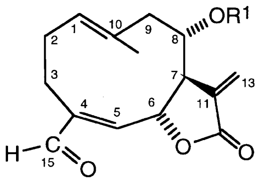

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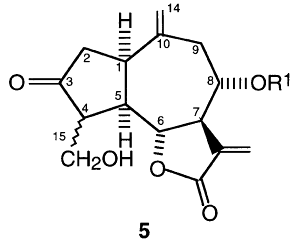

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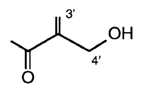

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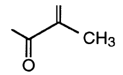

3p, H-5), 1.76 *m*, 1.65 *m*, 1.5 *c* (4 p, H-2a, b, 3a, b), 1.29 (*dd*, $J = 13$, 12 Hz, H-9b), 1.03 (*s*, 3p, H-14). The stereochemistry at C-1 and C-4 followed from the coupling constants and by comparison with the spectrum of the 4′-hydroymethacryl analog (Rustaiyan et al., 1986; see also García et al., 1996).

($1R^*$, $4S^*$, $5R^*$, $6R^*$, $7R^*$, $8S^*$, $10R^*$ Methyl 1,6-dihydroxy-8-methacryloxyeudesm-11(13)-en-15-oic acid-12-oate (**3**). Gum; Ms m/z PCI (rel. int.) 397 ($M + H^+$, 18), 379 ($M + H^+$ -H_2O, 67), 311 (100), 293 (58); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.29 (*s*, H-13a), 5.97 (*t*, $J = 1$ Hz, H-3′a), 5.70 (*s*, H-13b), 5.48 (*t*, $J = 1.5$ Hz, H-3′b), 5.33 (*ddd*, $J = 11.5$, 11.5, 5 Hz, H-8), 4.00 (*t*, $J = 10$ Hz, H-6), 3.75 (*s*, 3p, OMe), 3.36 (*dd*, $J = 11.5$, 4.5 Hz, H-1), 2.62 (*brt*, $J = 11$ Hz, H-7), 2.41 (*dd*, $J = 12$, 5 Hz, H-9a) 2.3 (*m*, H-4 and H-3a), 1.84 (*s*, 3p, H-4′) 1.82 (*dd*, $J = 11.5$, 5 Hz, H-5), 1.63 (*m*, H-2a, H-2b obscured) 1.47 (*m*, H-3b), 1.23 (*m*, H-9b), 1.03 (*s*, 3p, H-4′). The stereochemistry at C-1, C-4, C-6 and C-8 followed from the coupling constants.

($6S^*$, $7R^*$, $8S^*$)-8-(4′-Hydroxymethacryloxy)-15-oxohelianga-1(10), 4, 11 (13)-trien-6, 12-olide (**4**). Gum; MS m/z PCI (rel. int) 347 ($M + H^+$, 57), 245 (100); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.43 (*s*, H-15), 6.39 (*d*, $J = 1.5$ Hz, H-13a), 6.22 (*brs*, H-3′a), 6.21 (*d*, $J = 9.5$ Hz, H-5), 5.88 (*d*, $J = 1$ Hz, H-3′b), 5.76 (*d*, $J = 1.5$ Hz, H-13b), 5.26 (*t*, $J = 8$ Hz, H-1), 5.11 (*ddd*, $J = 10$, 10, 4 Hz, H-8,), 4.92 (*brd*, $J = 9.5$, H-6), 4.29 (*brs*, 2p, H-4′a,b), 3.07 (*dq*, $J = 10$, 1.5 Hz, H-7), 2.75 (*ddd*, $J = 12$, 3.5, 3.5 Hz, H-3a), 2.64 (*brdd*, $J = 12.5$, 4.5 Hz, H-9a), 2.46 (*m*, H-2a), 2.32 (*t*, $J = 11$, H-9b), 2.14 (*brtd*, 12, 12, 5, H-3b) 1.91 (*dddd*, $J = 13$, 13, 5 Hz, H-2b), 1.85 (*brs*, 3p, H-14). The values of $J_{6,7}$ and $J_{7,13a,b}$ showed that the substance was a heliangolide, the coupling constants involving H-8 that the ester side chain was α-orientated and the chemical shift of H-5 that the formyl group was on C-15.

($1R^*$, $4S^*$ or R^*, $5R^*$, $6S^*$, $7R^*$, $8S^*$)-15-Hydroxy-8-(4′-hydroxymethacroyloxy)-10 (14), 11 (13)-guaiadien-6,12-olide (**5**). Gum; MS m/z PCI (rel. int.) 363 ($M + H^+$, 21), 345 (35), 261 (100); ^{1}H NMR ($CDCl_3$, 500 HMz) δ 6.33 (*brs*, H-3a′), 6.26 (*d*,

$J = 3.5$ Hz, H-13a), 5.97 (*brs*, H-3′b), 5.67 (*d*, $J = 3$ Hz, H-13b), 5.18 (sl. split, H-14a), 5.16 (*ddd*, $J \sim 9.5, 5, 3$ Hz, H-8), 5.05 (*brs*, H-14b), 4.38 (*brs*, 2p, H-4′ a,b), 4.27 (*dd*, $J = 11, 9$ Hz, H-6), 3.98 (*dd*, $J = 11, 4.5$ Hz, H-15a), 3.94 (*dd*, $J = 11, 5.5$ Hz, H-15b), 3.28 (*dddd*, $J = 11, 9, 3, 3$ Hz, H-7) superimposed on multiplet of H-1, 3.00 (*c*, H-5), 2.98 (*c*, H-4), 2.65 (*dd*, $J = 15, 5.5$ Hz, H-9a), 2.56 ((*dd*, $J = 19.5, 8.5$ Hz, H-2a), 2.42 (*dd*, $J = 15, 3.5$ Hz, H-9b), 2.35 ((*dd*, $J = 19, 5$ Hz, H-2b). The stereochemistry at C-8 followed from the values of $J_{7,8}$ and $J_{8,9}$. Since the signals of H-4 and H-5 practically coincided the stereochemistry at C-4 could not be established with confidence although H-5 was clearly *alpha* ($J_{5,6} = 11$ *Hz*) as was H-1 ($J_{1,2a} = 8.5$ Hz, $J_{1,2b} = 5$ Hz).

4. Chemotaxonomic significance

The predominant secondary metabolite of *C. tweediei* was onopordopicrin (**1**) which has so far not been found in other New World *Centaurea* endemics and has in fact been reported previously from only four Old World *Centaurea* taxa out of 99 studied so far for sesquiterpene lactone content (El-Masry et al., 1985, Lonergan et al., 1992, Bruno et al., 1996, Youssef, 1998). On the other hand onopordopicrin is a characteristic constituent of *Onopordon* (Carduae, subtribe Carduinae), having been reported from seventeen out of nineteen *Onopordon* species studied so far for sesquiterpene lactone content (Drozdz et al., 1968, Drozdz and Piotrowski, 1973, Khafagy et al., 1977, Rustaiyan et al., 1979a, b, 1986, Gonzalez Collado et al., 1984, Miski et al., 1988, Eid and El-Dahmy, 1989, Meriçli and Tuzlaci, 1989, Cardona et al., 1992, García et al., 1996, Lazari et al., 1998, Braca et al., 1999). Lactones or acids of types **2** and **3** have been encountered in both genera, although again more commonly in *Onopordon* and so far not in New World *Centaurea* endemics, while heliangolides and melampolides are rare in the entire tribe. The taxonomic implications, if any, of these findings are not clear.

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