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Sesquiterpene lactones and other constituents of *Centaurea diffusa*

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1. Subject and source

Aerial parts of *Centaurea diffusa* Lam. were collected at the flowering stage on November 11, 1998 along provincial road No 1 near Las Albahacas, Rio Cuarto Department, Cordoba Province, Argentina. A voucher specimen (C.L. Cristobal, A. Krapovicka and G. Seijo #2487) is on deposit in the herbarium of the Instituto de Botanica del Nordeste (IBONE), 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., 1996). Reports are sparse on the chemistry of *C. diffusa*, (diffuse knapweed), an invasive Eurasian species which has infested grasslands and crop areas of both North and South America and is of concern to agricultural interests in Central and

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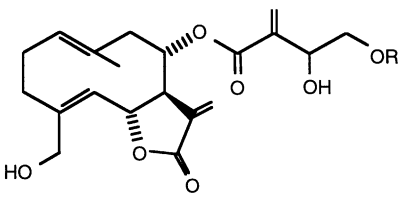
Northeastern Argentina. Isolation of the sesquiterpene lactone cnicin (**1a**) from the herb was described as early as 1966 (Drozdz, 1966) while a later report (Milkova et al., 1986) mentioned taraxerol and its acetate. Muir and Majak (1983) associated the allelopathic potential of Canadian *C. diffusa* with sesquiterpene lactone fractions from both roots and shoots, but while cnicin was a major component of the inhibitory fractions it was not a major inhibitor by itself. A recent article (Callaway and Ascheshong, 2000) has provided evidence for the view that *C. diffusa* produces chemicals to which its long term and familiar Eurasian neighbors have adapted but to which its new Western hemisphere neighbors have not, but the nature of these chemicals was not discussed.

3. Present study

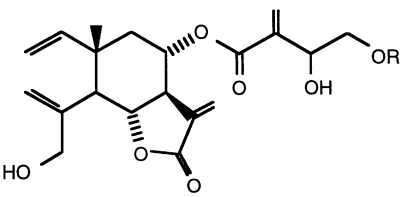
Aerial parts (1150 g, dry weight) of *C. diffusa* were extracted with $CHCl_3$ (2×5 l) at room temperature for 7 days to give 108.3 g of crude extract which was suspended in EtOH (800 ml) at 55°C, diluted with H_2O (550 ml) and extracted successively with *n*-hexane (3×600 ml) and $CHCl_3$ (3×600 ml). The $CHCl_3$ extracts on evaporation at reduced pressure furnished 39.9 g of residue, a portion of which (21 g) was subjected to CC over Si gel (Merck, 840 g, 70–230 mesh) using $CHCl_3$ with increasing amounts of EtOAc (0%, 2.0 l, frs. 1–12; 20%, 2.0 l, frs. 13–27; 25%, 1.0 l, frs. 28–33; 33%, 2.0 l, frs. 34–44; 50%, 2.0 l, frs. 45–57; 65%, 1.0 l, frs. 58–63; 80%, frs. 64–69; 100%, 2.0 l, frs. 70–83). All fractions were monitored by TLC. Frs. 1–38 did not exhibit lactone absorption in the IR and were discarded.

Frs. 39–53 (530 mg) were combined and rechromatographed over Si gel (30 g, 230–400 mesh) using $CHCl_3$–EtOAc 95:4 (frs. 1–33), $CHCl_3$–EtOAc–AcOH 95.5:4:0.5 (frs. 34 and 35) and 95:4:1 (frs. 36–41). CC of frs. 7–10 (15 mg) over Si gel (1 g, $CHCl_3$–MeOH 7:3) gave 7 mg of a 2.5:1 mixture of 5-hydroxy-6,7,3′,4′-tetramethoxyflavone and cirsimaritin, frs. 11–17 (149 mg) gave a solid residue which after washing with Et_2O gave 97 mg of slightly impure cirsilineol. Frs. 18–28 (42 mg) on HPLC (Beckman ultrasphere C 18 column, 10 mm i.d. × 250 mm, MeOH–H_2O 4:3, 1.5 ml min^{-1}) gave 8.6 mg of dihydrosyringenin (R_t 2.75 min) (Rustaiyan et al., 1991), mixtures of sesquiterpene lactones and 8.8 mg of impure cirsilineol (R_t 42 min). Frs. 29–31 (31 mg) on HPLC as above furnished 3.8 mg of dihydrosyringenin (R_t 3.5 min), 3.6 mg of loliolide (R_t 6.5 min), 2.6 mg of impure loliolide (R_t 7 min) and 2.2 mg of **2b** (R_t 23 min) (Bruno et al., 1994). Frs. 54–62 (4.6 g) of the mother column showed a single spot on TLC and were identified as **1b** (Jakupovic et al., 1986). Frs. 63–67 (1.43 g) were reunited; a portion (100 mg) on HPLC (MeOH–H_2O 4:3, 1.5 ml min^{-1}) gave fractions containing mainly **3** (Cardona et al., 1994). Frs. 68–71 (397 mg) were also reunited; a portion (108 mg) on HPLC (MeOH–H_2O 4:3, 1.5 ml min^{-1}) gave more **3** (10.3 mg, R_t 8.0 min), mixtures containing mainly **4a** (2.9 mg, R_t 20 min) and **4b** (1.6 mg, R_t 31.5 min) (Bruno and Herz, 1988). Frs. 72–78 (10.09 g) on washing with Et_2O afforded 9.35 g of pure cnicin (**1a**, mp 144°C). Known compounds were identified by ^1H NMR, and by comparison with spectra in our files and MS.

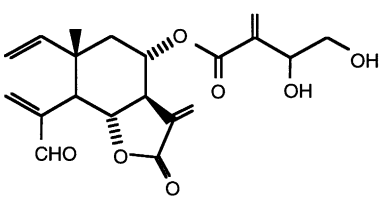
1,2-Diangelyloxyglucose (**4a**). Gum; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.17(qq, *J*'s=7.5, 1.5 Hz, H-3′ A), 6.13 (*J*'s=7.5, 1.5 Hz, H-3′ B), 5.83 (d, *J*=8.5 Hz, H-1), 5.01 (dd, *J*=9, 8.5 Hz, H-2) 3. 94 (dd, *J*'s =12, 3 Hz, H-6a), 3.84 (dd, *J*'s=12, 4.5 Hz, H-6b), 3.76 (ddd, *J*'s=9, 9, 4 Hz, H-3), 3.73 (ddd, *J*'s=9, 9, 3 Hz, H-4), 3.57 (ddd,*J*'s=9, 4, 4 Hz, H-5), 2.92 and 2.75 (both broad, –OH), 1.96 (dd, 3p, *J*'s=7, 1.5 Hz, H-4′ A), 1.93 (dq, 3p, *J*=7, 1.5 Hz, H-4′B), 1.84 (q, 6p, *J* =1.5 Hz, H-5′ A, B); MS PCI 327 (M+H−H_2O, 2.3), 301 (M+H−CO−H_2O, 5.6), 245 (M+H−$C_5H_8O_2$, 100). Acylation at C-1 and C-2 followed from the chemical shifts of H-1 and H-2.



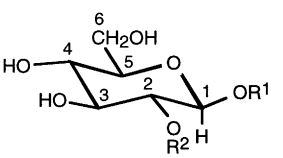
1a R = H
b R = Ac



2a R = H
b R = Ac



3



4a R^1, R^2 = Ang
b R^1 = iVal, R^2 = Ang

1-(3-Methylbutanoyloxy),2-angelyloxyglucose (**4b**). Gum; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.16 (qq, *J*'s=7.5, 1.5 Hz H-3′ of angelate), 5.78 (d, *J*=8.5 Hz, H-1), 4.94 (dd, *J*'s=9, 8.5 Hz, H-2), 3.92 (brdd, *J*'s=11.5, 2.5 Hz, H-6a), 3.83 (dd, *J*=11.5, 4 Hz, H-6b), 3.72 (m, H-3), 3.70 (m, H-4), 3.53 (ddd, *J*'s=9, 4.5, 4 Hz, H-5), 2.89 and 2.68 (both broad, –OH), 2.22 and 2.18 (both m, H-2′a,b of isovalerate), 2.06 (approx. sept, *J*=7 Hz, H-3′ of isovalerate), 1.97 (dd, 3p, *J*'s=7.5, 1.5 Hz, H-4′ of angelate), 1.85 (t, 3p *J*'s=1.5 Hz, H-5′ of angelate), 0.907 and 0.905 (each d and 3p, *J*=6.5 Hz, H-4′ and H-5′ of isovalerate); MS PCI 329 (M+H−H_2O, 2), 301 (M+H−H_2O−CO, 8), 245 (M+H−$C_5H_{10}O_2$, 100). Acylation at C-1 and C-2 followed from the chemical shifts of C-1 and C-2. That the isovaleryl residue was attached to C-1 rather than to C-2 of the glucose unit followed from the facile loss of $C_5H_{10}O_2$ in the CI MS compared with the equally facile loss of $C_5H_8O_2$ in the CI MS of **4a**. A closely related 1,2-diacylglucose derivative has been isolated from *Centaurea napifolia* (Bruno et al., 1995) while a 1,6-diisobutyrate has been reported from *Centaurea aspera* var. *subinermis* (Fernández et al., 1993).

4. Chemotaxonomic significance

C. diffusa has been placed in section Acrolophus (Cass.) DC. of *Centaurea* (Wagenitz, 1955; Wagenitz and Hellwig, 1996) relatively few members of which have been investigated. Among these are *Centaurea cuneifolia* (Õksûz et al., 1988; Aslan and Õksûz, 1999) and *Centaurea paniculata* ssp. *castellana* (Bruno et al., submitted for publication) both of whose sesquiterpene lactone chemistry resembles that of *C. diffusa*, but whose habitat differs significantly. The chemical basis for the invasive behavior of *C. diffusa* remains to be elucidated.

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