STEROIDs, A LIGNAN AND A FLAVONOID FROM CENTAUREA MELITENsIS L.

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KEY WORDS

Centaurea melitensis L., Asteraceae, β-sitosterol 3- O- β-D-glucoside, β-sitosterol, stigmasterol, arctin, hispidulin.

SUMMARY

β-Sitosterol 3- O- β-D-glucoside, β-sitosterol, stigmasterol, the lignan arctin, and the 6-methoxylated flavone hispidulin were isolated from a chloroform extract of the aerial parts of Centaurea melitensis L. (Asteraceae). None of these compounds had been reported previously as constituents of this plant.

INTRODUCTION

Centaurea melitensis L. (Asteraceae) is a European weed which has become fairly common in Chile, where it is known by the name «cizaña» (in this context = "weed", cf. Zizania, Poaceae), presumably as a reference to its lack of medicinal or other uses [1]. Nevertheless, in Spain this plant is supposedly useful as a stomachic and diuretic [2] and in the treatment of hypoglycemia (sic[3]). A weak hypoglycemic action has been demonstrated for its extracts [4]. Spanish workers studying C. melitensis...
isolated three eleanolide sesquiterpene lactones: melitensin [5, 6, 7], its 
β-hydroxy-isobutyrate, and 11 (13)-dehydromelitensin β-hydroxyisobuty-
rate [3]. Much more recent work failed to confirm these results but on the 
contrary proved the presence of the germacranolides saloinitenolide, 
onopordopicrin and artiopicrin, which led to the suggestion that the 
eleanolides might be artefacts arising by Cope rearrangement under the 
extration conditions [8]. Furthermore, an ethyl acetate extract of the 
flowers afforded the 7-O-glucosides of luteolin, 6-methoxyquercetin, and 
6-methoxyluteolin [9] and the flavone C-glucosides homo-orientin, orientin, 
isovitexin, vicenin II, and schaftoside [10].

Continuing with our studies on the chemistry of Centaurea species 
growing in Chile [11, 12, 13, 14], we have now examined the chloroform 
extract of the aerial parts of C. melitensis (stems, leaves, and flowers, 
combined). In this paper we report the isolation of β-sitosterol 3-O- 
β-D-glucoside, β-sitosterol, stigmasterol, the lignan arctiin, and the 6-
methoxylated flavone hispidulin.

CHEMISTRY

STEROIDS.

A mixture of sterols which could not be resolved by TLC was acetylated. 
The acetates also showed identical TLC behavior, but could be separated by 
GLC and were eventually identified by GLC-MS as β-sitosterol and 
stigmasterol. The identification of the widely distributed β-sitosterol 3-
O-β-D-glucoside was trivial, but a combination of 2D-NMR methods 
(\(^1H/\(^1H\) COSY and \(^1H/\(^13C\) HETCOR) has now made a complete assign-
ment of the \(^1H\) and \(^13C\) resonances possible (Table I, Figs. 1 and 2). It 
should be pointed out that although the physical properties of the 
compound isolated by us agree well with those of β-sitosterol 3-O-
β-D-glucoside, the NMR data do not rule out the possibility that it is the 
hitherto unknown glucoside of the relatively rare 24 S epimer of sitosterol, 
elionasterol [15].

HISPIDULIN.

Hispidulin (6-methoxy-4',5,7-trihydroxyflavone) was identified on the 
basis of its UV spectra employing the usual shift reagents [16] and the \(^1H\) 
NMR spectrum of its triacetate. TLC comparison showed it to be identical 
with the compound isolated previously from C. chilensis [13].

ARCTIIN.

The chemical ionization (N.H.) mass spectrum showed a pseudomolecul-
lar ion at \(m/z\) 552 (100 %) suggesting a molecular weight of 534 attributable
to a lignan glycoside \((\text{C}_{27}\text{H}_{34}\text{O}_{11})\). Peaks at \(m/z\) 390 (26.1\%) and 372 (6.9\%) correspond to the aglycone plus two or one ammonium ions, respectively. The sugar residue is evidenced by peaks at \(m/z\) 198 (0.28\%, \([\text{Gl + NH}_4]^+\)) and 180 (67.5\%). The IR spectrum indicated the presence of intramolecularly hydrogen bonded hydroxyls, aromatic rings, a \(\gamma\)-lactone carbonyl group, methyl groups and aryl ether functions.

The \(^1\text{H}/^1\text{H}\) COSY spectrum (Fig. 3) showed a weak correlation between the aromatic ring proton resonating at 7.04 ppm \((\text{H}-5')\) with the anomeric H-1", which demonstrates that the sugar is bonded to ring I and more specifically to C-4'. The assignment of the signal due to this quaternary carbon atom is based on its correlations with the H-6' (6.64 ppm) and H-2' (6.73 ppm) resonances in the LR-HETCOR (Fig. 4). The methoxyl proton resonance at 3.78 ppm is correlated with the quaternary C-3' and H-2' signals in the LR \(^1\text{H}/^{13}\text{C}\) HETCOR and COSY spectra, respectively, showing that this group is bonded to the same ring as the sugar moiety. The signal due to the quaternary C-1' is correlated on one hand with the H-5' resonance (7.04 ppm), and on the other with the C-7' methylene proton signature at 2.81 and 2.90 ppm. The latter resonances are correlated with the \(\gamma\)-lactone carbonyl peak, showing that this methylene group binds ring I to the \(\alpha\) carbon of the lactone ring. The placement of the remaining methoxyl groups on aromatic ring II and the bonding of this ring through an identified methylene group to the \(\beta\) carbon of the lactone function was deduced similarly from the COSY and LR-HETCOR spectra. The \(^1\text{H}\) and \(^{13}\text{C}\) NMR signal assignments (400/100 MHz in methanol-\(d_4\)) are comparable with slightly less complete published values (400 MHz \(^1\text{H}\) in pyridine-\(d_5\) [17]; 15.0 MHz \(^{13}\text{C}\) in DMSO-\(d_6\) [18]) and are summarized in Table 2.

**EXPERIMENTAL**

1. **Plant material.**

Aerial parts of *Centaurea melitensis* L. were collected in flower (November) in the Renca hills, Metropolitan Region (33° S latitude), Chile. Voucher specimens are retained in the herbarium of the Faculty of Chemical and Pharmaceutical Sciences of the University of Chile, Santiago.

2. **Extraction and isolation.**

Stems, leaves and flowers, combined, were dried in the shade. The ground plant material (3.7 kg) was defatted (Soxhlet) with light petrol and then extracted with chloroform.

The residue of the chloroform extract (123 g) was chromatographed on a
silica gel G column, dry-packed and moistened with light petrol, eluting first with this solvent, then with light petrol-EtOAc mixtures, increasing the concentration of the latter solvent in 5% increments to pure EtOAc, then similarly with EtOAc-MeOH mixtures, and finally with pure MeOH.

The light petrol-EtOAc (95:5) fraction afforded crystals of a product (A, 0.001%) which showed a single spot upon TLC on silica gel G, developing with light petrol-EtOAc (70:30), spraying with Liebermann-Burchard reagent and heating to 10°. TLC of A under similar conditions, after acetylation with Ac₂O-pyridine, also gave a single purplish-pink spot. The light petrol-EtOAc (45:55) fraction provided a product designated as B, which after further purification by chromatography on a polyamide column weighed 20 mg. TLC as described above showed a single bright yellow spot which also became visible after exposure to NH₃ vapors or spraying with AlCl₃ solution. The (25:75) to (15:85) light petrol-EtOAc fractions yielded a third crystalline product (C) which after being purified by recrystallization in CHCl₃-EtOAc weighed 40 mg. The light petrol-EtOAc (5:95), EtOAc, and EtOAc-MeOH (95:5) fractions gave a fourth product (D) which, purified by chromatography on several successive silica gel G columns and crystallization in CHCl₃-EtOAc, weighed 70 mg. Purification of C and D was monitored by TLC on silica gel G, developing with CHCl₃-MeOH (80:20) and spraying with Liebermann-Burchard reagent, which gave purplish pink spots changing to brown and grey, respectively, after several hours at room temperature.

3. Identification.

β-Sitosterol and Stigmasterol. Product A, after acetylation, was subjected to GLC on an SE-30 capillary column which showed that it consisted of an approximately equimolar mixture of two components. GLC/MS analysis with electron impact ionization and comparison with standards led to the identification of β-sitosterol and stigmasterol acetates.

Hispidulin (6-methoxy-5,7,4'-trihydroxyflavone). Product B, crystallized in MeOH, melted at 293° TLC behavior identical with material isolated from C. chilensis [13]. UVλmax (MeOH) 277,335 nm; (NaOMe) 282, 324, 390 nm; (AlCl₃) 263 sh, 291 sh, 300, 360 nm; (AlCl₃/HCl) 265 sh, 301, 356 nm; (NaOAc) 279, 305 sh, 320, 390 nm; (NaOac/H₃BO₃) 277, 340 nm. Acetylation with Ac₂O/pyridine afforded a triacetate: mp 169°; ¹H NMR δ ppm from TMS (ir CDCl₃, 60 MHz) 7.78 (2H, d, J = 9 Hz, H-2'/H-6'), 7.27 (1H, s, H-8), 7.21 (2H, d, J = 9 Hz, H-3'/H-5'), 6.57 (1H, s, H-3), 3.84 (3H, s, OMe), 2.46 (3H, s, AcO), 2.39 (3H, s, AcO), 2.33 (3H, s, AcO).

Sitosterol 3-O-β-D-glucoside. Product C, crystallized in CHCl₃/EtOAc, melted at 282-284°. TLC behavior identical with material isolated from Zea
mays [19]. CIMS (CH$_4$) $m/z$ (%) 415 (10) [M-C$_6$H$_{10}$O$_5$ + 1]$^+$, 414 (20) [M-C$_6$H$_{10}$O$_5$]$^+$, 413 (23) [M-C$_6$H$_{10}$O$_5$ - 1]$^+$, 399 (24) [M-(C$_6$H$_{10}$O$_5$ + CH$_3$)]$^+$, 397 (100) [M-(C$_6$H$_{10}$O$_5$ + OH)]$^+$, 396 (44) [M-(C$_6$H$_{10}$O$_5$ + H$_2$O)]$^+$, 382 (17) [M-(C$_6$H$_{10}$O$_5$ + CH$_3$ + OH)]$^+$, 381 (37) [M-(C$_6$H$_{10}$O$_5$ + CH$_3$ + H$_2$O)]$^+$, 303 (7) [M-(C$_6$H$_{10}$O$_5$ + C$_7$H$_{13}$O)]$^+$, 273 (10) [M-(C$_6$H$_{10}$O$_5$ + C$_{10}$H$_{21}$)]$^+$, 255 (22) [M-(C$_6$H$_{10}$O$_5$ + C$_{10}$H$_{21}$ + H$_2$O)]$^+$.

$^1$H NMR δ ppm from TMS (in pyridine-$d_5$, 400 MHz) 5.35 (1 H, br. s), 5.05 (1 H, d, $J = 8$ Hz), 4.57 (1 H, br. d, $J = 12$ Hz), 4.40 (1 H, dd, $J = 12$ Hz, $J' = 4$ Hz), 4.28 (2 H, m), 4.06 (1 H, dd, $J = 8$ Hz), 3.97 (1 H, m), 3.95 (1 H, m), 3.82 (6H, s), 3.78 (3H, s), 3.74 (3H, s), 3.68 (1H, m), 3.64 (1H, m), 3.61 (1H, m), 2.81 (1 H, br. s), 2.46 (1 H, br. t, $J = 12$ Hz), 2.12 (1 H, dd, $J = 12$ Hz, $J' = 4$ Hz), 1.87 (1 H, m), 1.85 (1 H, m), 1.74 (1 H, m), 1.69 (1 H, m), 1.67 (1 H, m), 1.54 (2 H, m), 1.40 (2 H, m), 1.38 (2H, m), 1.34 (1 H, m), 1.25 (2H, m), 1.23 (1 H, m), 1.20 (2 H, m), 1.12 (1 H, m), 0.94 (5H, m), 0.91 (1 H, m), 0.90 (1 H, m), 0.87 (6H, s), 0.85 (3H, s), 0.84 (3H, s), 0.64 (3H, s). $^{13}$C NMR δ ppm from TMS (off-resonance decoupled and DEPT, in pyridine-$d_5$, 100 MHz) 140.88 (C), 121.91 (CH), 102.54 (CH), 78.55 (CH), 78.45 (CH), 78.07 (CH), 75.29 (CH), 71.62 (CH), 62.78 (CH$_2$), 56.81 (CH), 56.22 (CH), 50.32 (CH), 46.01 (CH), 42.46 (C), 39.93 (CH$_2$), 39.31 (CH$_2$), 37.46 (CH$_2$), 36.90 (C), 36.38 (CH), 34.18 (CH$_2$), 32.16 (CH$_2$), 32.03 (CH), 30.23 (CH$_2$), 29.43 (CH), 28.54 (CH$_2$), 26.34 (CH$_2$), 24.50 (CH$_2$), 23.73 (CH$_2$), 21.27 (CH$_2$), 19.98 (CH$_3$), 19.41 (CH$_3$), 19.21 (CH$_3$), 19.01 (CH$_3$), 12.15 (CH$_3$), 11.97 (CH$_3$).

$^1$H/$^{13}$C HETCOR 2D-NMR spectra (in pyridine-$d_5$, 400 and 100 MHz), Figures 1 and 2. For assignments, see Table 1.


CIMS (NH$_3$) $m/z$ (%) 552 (100) [M + NH$_4$]$^+$, 390 (26), 372 (6.9), 198 (0.28), 180 (67). $^1$H NMR δ ppm from TMS (in methanol-$d_4$, 400 MHz), 7.04 (1 H, d, $J = 8.5$ Hz), 6.81 (1 H, d, $J = 8.5$ Hz), 6.73 (1 H, d, $J = 2$ Hz), 6.64 (1 H, dd, $J = 8.5$ Hz, $J' = 2$ Hz), 6.59 (1 H, dd, $J = 8.5$ Hz, $J' = 2$ Hz), 6.58 (1 H, d, $J = 2$ Hz), 4.83 (1 H, d, $J = 8$ Hz), 4.17 (1 H, dd, $J = 9$ Hz, $J' = 7.5$ Hz), 3.92 (1 H, dd, $J = 9$ Hz, $J' = 8$ Hz), 3.85 (1 H, d, $J = 12$ Hz), 3.78 (6H, s), 3.74 (3H, s), 3.68 (1H, dd, $J = 12$ Hz, $J' = 4$ Hz), 3.47 (2H, m), 3.39 (2H, m), 2.90 (1 H, dd, $J = 14$ Hz, $J' = 5.5$ Hz), 2.81 (1 H, dd, $J = 14$ Hz, $J' = 7$ Hz), 2.67 (1 H, ddd, $J = 7$ Hz, $J' = 7$ Hz, $J'' = 5$ Hz), 2.55 (2H, m), 2.48 (1 H, br. s). $^{13}$C NMR δ ppm from TMS (off-resonance decoupled and DEPT, in methanol-$d_4$, 100 MHz), 181.30 (C), 150.55 (C), 150.35 (C), 149.03 (C), 146.76 (C), 134.14 (C), 132.61 (C), 122.92 (CH), 122.02 (CH), 117.75 (CH), 114.67 (CH), 113.46 (CH), 112.91 (CH), 102.81 (CH), 78.08 (CH), 77.72 (CH), 74.81 (CH), 72.85 (CH$_2$), 71.24 (CH), 62.44 (CH$_2$), 56.64 (CH$_3$), 56.45 (CH$_3$), 56.40 (CH$_3$), 47.56 (CH), 42.41 (CH), 38.84 (CH$_2$), 35.33 (CH$_3$). $^1$H/$^1$H COSY and long-range $^1$H/$^{13}$C HETCOR 2D-NMR spectra (over 2-3 bonds, in methanol-$d_4$, 400 and 100 MHz), Figures 3 and 4. For assignments, see Table 2.
Fig. 1. $^1$H-$^1$C HETCOR spectrum of $\beta$-sitosterol 3-O-$\beta$-D-glucoside in pyridine-$d_5$, 400/100 MHz; upfield region.
Fig. 2. — $^1$H/$^13$C HETCOR spectrum of β-sitosterol 3-O-β-D-glucoside in pyridine-$d_6$, 400/100 MHz, downfield region.
**DISCUSSION**

Although no quantitative data are available, the presence of sitosterol glucoside (1) may account at least in part for the hypoglycemic properties of this plant [4]. The pharmacology of arctiin (2) does not seem to have been studied, but is should be kept in mind that several butanolide lignans have quite recently been examined as putative endogenous digitaloids [20, 21] and are also known to exhibit antihypertensive [22] and natriuretic [23] properties. Arctiin seems to be a good chemotaxonomic characteristic of the genera pertaining to the «genuine» tribe Cynareae [24]; its aglycone
arctigenin has been isolated from C. regia (which also contains sitosterol glucoside) [25], and the presence of the glucoside in an additional Centaurea species confirms this impression. The occurrence of the 6-methoxylated flavone hispidulin in C. chilensis [13] and C. melitensis is of interest in view
Fig. 4. -- Long-range $^1$H/$^{13}$D HETCOR spectrum (over 2-3 bonds) of arctiin in methanol-$d_4$, 400/100 MHz.
of the fact that this plant also contains the 7-O-glucosides of 6-methoxyluteolin (a flavone) and 6-methoxyquercetin (a flavonol) [9]. Quite recently, C. cineraria subsp. umbrosa was shown to contain the O-methylated 6-methoxyluteolin derivatives salvigenin, 5-hydroxy-3',4',6,7-tetramethoxyflavone, eupatilin, and jaceosidin [26].

ACKNOWLEDGEMENTS

We wish to express our sincere thanks to Profs. A. San Martin, University of Chile, and E. Gros, University of Buenos Aires, for the GLC-MS study; to Prof. A. Cavé, University of Paris-Sud, for the MS of arctinin; and to Prof. M. Tempesta; University of Missouri, for the MS of 3-O-β-sitosterol β-D-glucoside. We especially value the assistance of the IOCD service programme, and funding by the DTI, University of Chile, Project No. Q-1882-8855.
REFERENCES