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DESACETYLEUPASERRIN AND NEVADENSIN FROM *HELIANTHUS PUMILUS**

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Key Word Index—*Helianthus pumilus*; Compositae; germacranolides; sesquiterpene lactones; antitumor compounds; flavonoids.

Abstract—Extraction of *Helianthus pumilus* L. furnished the antileukemic germacradienolide desacetyleupaserrin which was isolated in crystalline form and the relatively rare flavone nevadensin (5,7-dihydroxy-6,8,4′-trimethoxyflavone).

INTRODUCTION

In 1960 one of us isolated a previously unknown crystalline sesquiterpene lactone from *Helianthus pumilus* L., a very abundant sunflower of the mesas and lower foothills on the Eastern slopes of the Rocky Mountains in Colorado and Wyoming [1], but the material decomposed before structure studies could be undertaken. Reinvestigation has now resulted in identification of this substance as the antileukemic germacradienolide desacetyleupaserrin (**1a**) which was reported more recently [2] as a 'brittle foam' from *Eupatorium semiserratum* DC. The flavone nevadensin (5,7-dihydroxy-6,8,4′-trimethoxyflavone, **2a**) [3] was also isolated.

DISCUSSION

The difference between the properties of our crystalline lactone, mp 134–135°, $C_{20}H_{26}O_6$, IR bands at 3480, 1740 and 1655 cm^{-1}, CD curve λ_{max} 262 nm, $[\theta]$ −4980 and those reported [2] for desacetyleupaserrin did not give us cause to suspect their identity until we had established its complete structure by NMR spectrometry at 270 MHz. Chemical shifts and coupling constants of our material are given in Table 1, but details of the decoupling procedure which allowed deduction of the gross formula and stereochemistry identical with that assigned to desacetyleupaserrin will not be detailed. Acetylation with acetic anhydride–potassium carbonate afforded **1b**, mp 159–160°, which is somewhat higher than the value of 153–154° reported for eupaserrin [2], but whose IR and NMR spectrum corresponded to the values given for **1b** in ref. [2]. A small amount of the previously unreported isomeric monoacetate **1c** was also obtained by acetylation of **1a**.

Table 1. 270 MHz ^{1}H-NMR spectrum of **1a***

H-1	5.0 *m*	H-9a	2.86 *dd* (6, 15)
H-2	4.74 *dt* (5.5, 9.5)	H-9b	2.36 *dd* (2, 15)
H-3a	2.72 *d* (9.5, 5.5)	H-13a	6.33 *d* (3.5)
H-3b	2.10 *t* (9.5)	H-13b	5.61 *d* (3)
H-5	5.0 *m*	H-14	1.77 (3p)
H-6	5.13 *dd* (10, 7)	H-15	1.53 (3p)
H-7	2.94 *m* (7, ~4, 3.5, 3)	H-3^1	6.4 *q* (7.5)
H-8	5.85 *br* (~4, 6, 2)	H-4^1	2.01 *d* (3p, 7.5)
		H-5^1	4.21 (2p, AB quartet, 7)

* Run in $CDCl_3$. Unmarked signals are singlets.

EXPERIMENTAL

Helianthus pumilus L. 25 kg collected on July 16, 1972 in Larimer County, 10 miles west of US Highway 287 on the Livermore–Red Feather road northwest of Fort Collins, Colorado

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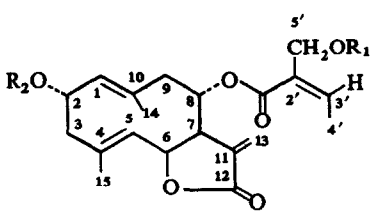
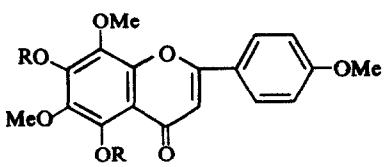


1a R_1, R_2 = H
1b R_1 = Ac, R_2 = H
1c R_1 = H, R_2 = Ac



2a R = H
2b R = Ac
2c R = OMe

(altitude 2200 m.), (FRS-42), was extracted with $CHCl_3$ and worked up in the usual fashion [4]. One half of the crude gum, 73.4 g, was chromatographed over 1.3 kg of silicic acid (Mallinckrodt 100 mesh), 500 ml fractions being collected. Fractions 8, 9 and 10 (C_6H_6–$CHCl_3$ 9:1) solidified. Recrystallization from MeOH and preparative TLC gave 90 mg of a flavone, mp 185° which gave a green color reaction with $FeCl_3$ and a red color with Mg and HCl, NMR signals (DMSO) at 12.59 (C-5 OH), 7.83 *d* and 6.94 *d* (J = 10 Hz, AB *q* of H-2, H-3, H-5 and H-6), 6.66 (H-3), 3.66, 3.66, 3.57 ppm (3 OMe), MS *m/e* 344 (M^+ 72.2%), 329 (base peak), 314, 197, 169, 133: UV in MeOH, MeOH–NaOAc and MeOH–$AlCl_3$, as reported [3]. Acetylation and recrystallization from C_6H_6 afforded a diacetate, mp 165°, lit. mr 170–173 [3], NMR signals as reported [3]. Methylation with Me_2SO_4/K_2CO_3 gave a mixture which was separated by TLC. The pentamethoxyflavone was recrystallized from hexane–C_6H_6 and melted at 149–150°, lit. mp for tangeretin **2c** 152–153.5° [3], mmp undepressed. Direct comparison of the flavone and its diacetate with authentic samples of nevadensin and diacetylnevadensin showed that they were identical. Fractions 19–28 eluted with $CHCl_3$–MeOH (4%) were evaporated and the residue, wt 13 g, was rechromatographed over 560 g of silicic acid. The yield of pure crystalline desacetyleupaserrin, mp 134–135° after recrystallization from EtOAc–hexane, was 1.5 g. Calcd for $C_{20}H_{26}O_6$: C, 66.28: H, 7.23: O, 26.49; MW, 362.1728. Found: C, 66.55; H, 7.24: O, 26.28; MW (MS), 362.1738. Its other properties are presented in the discussion and in Table 1. Acetylation with Ac_2O/K_2CO_3 for 0.5 hr at 40° followed by the usual work-up, separation by preparative TLC and recrystallization from MeOH of the less polar fraction gave eupaserrin (**1b**) mp 159–160°, in whose NMR spectrum the AB quartet of H-4[1] centered 4.21 ppm was shifted downfield to doublets at 4.82 and 4.50 ppm (J = 12). A second more polar monoacetate **1c** was isolated in small quantity only: recrystallization from MeOH afforded needles, mp 174–176°, IR bands at 1755 and 1655 cm^{-1}. The high resolution MS of this substance did not exhibit a peak corresponding to the molecular ion, but strong peaks at 344 ($C_{20}H_{24}O_5$), (M^+-$MeCO_2H$), 326 ($C_{20}H_{22}O_4$, M^+-$MeCO_2H$-H_2O), 288 ($C_{17}H_{20}O_4$, M^+-$C_5H_8O_3$) and 228 ($C_{15}H_{16}O_2$, M^+-$C_5H_8O_3$-$MeCO_2H$) and 99 ($C_5H_7O_2$). These results indicated that the location of the acetate residue was on the germacradiene nucleus as in **1c**, not on the five carbon side chain.

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THE OCCURRENCE OF ATRACTYLOSIDE IN *CALLILEPIS LAUREOLA*

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Key Word Index—*Callilepis laureola*; Compositae; oxeye daisy; diterpene glycoside; atractyloside; hypoglycemic nephrotoxin.

Callilepis laureola has been used as a herbal medicine by the Zulus and other African people [1–3] and is reported to contain a toxic resin [1]. Our attention was drawn to this plant by Prof. J. Wainwright who found that many African patients who had used the enlarged subterranean rootstock as a medicine suffered serious and often fatal results due to severe liver lesions.

The dried, powdered rootstock was extracted in succession with hexane, ether, acetone and methanol. Biological tests showed that the toxic agent(s) were confined to the acetone and particularly to the methanol extracts. On TLC [Si gel EtOAc–MeOH–H_2O–HOAc (25:5:2:1); spray, anisaldehyde, H_2SO_4 and EtOH] the dark hygroscopic methanolic extract separated into a large number of component spots, three of which were contiguous and red in colour (R_f 0.23, 0.38, 0.43). An aqueous solution of the methanol extract on dilution with an equal volume of methanol slowly deposited a dark granular precipitate over a period of five days. Repeated crystallisation of this material from water provided a white powder which, by TLC, was shown to consist of three components, one being predominant. Further recrystallisation from aqueous methanol yielded a pure product of the major constituent as microcrystals, mp 225–226° (dec.), ν_{max}^{KBr} 3400–3600, 2950, 2870, 1720, 1250, 1035, 1000, 800 cm^{-1}. The two minor constituents were not examined further.

Ignition of this material left a white powder which gave a positive reaction for K^+ with sodium cobaltinitrite–silver nitrate solution, and this was confirmed by atomic absorption spectroscopy. Mild base hydrolysis provided isovaleric acid and SO_4^{2-}. Glucose was obtained on drastic base hydrolysis with aqueous KOH (20%, refluxed for 8 hr) producing an acid aglycone, mp 147–149°, M^+ 320, ν_{max}^{KBr} 3415, 2925, 2850, 1705, 1690, 1655, 1445, 1245, 1190, 1035, 995, 905, 775 cm^{-1}.

The identity of the complex glucoside and its aglycone as atractyloside and atractyligenin respectively was established by direct comparison (TLC, mmp, NMR, IR and MS) with authentic samples of each. The hypoglycemic agent, atractyloside, has previously been isolated from the rootstock of a Mediterranean plant, *Atractylis gummifera* L. (Compositae) [4].

Wainwright [5] confirmed the hypoglycemic activity