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ATRACTYLOSIDE, TOXIC COMPOUND FROM *WEDELIA GLAUCA*

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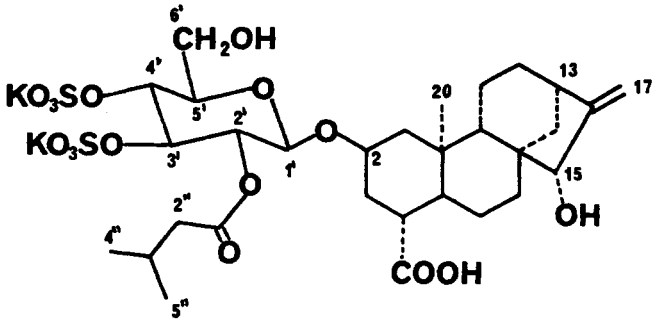
Wedelia glauca (Ort.) Hoffmann ex Hicken (Family: Compositae, tribe: Heliantheae, subtribe: Ecliptinae) is a plant indigenous to Argentina, Uruguay, and southern Brazil, and it is well known for its lethality to cattle (1).

Upon partition of the methanolic extract of *W. glauca* between *n*-BuOH and H_2O, only the aqueous layer showed lethality (ip) in mice. This layer was chromatographed over Sephadex LH-20 leading to a lethal polar diterpenoid (**1**), which was identified as atractyloside by spectral and chemical methods as well as by comparison with a standard compound (ir, ^{1}H-nmr, and ^{13}C-nmr).



1

The ^{1}H-nmr spectrum showed a singlet at 0.93 ppm (CH_3-20), a multiplet at 2.58 ppm (H-13), and two broad singlets at 4.90 and 5.10 ppm (H-17 and 17′). Sugar protons H-3′ and H-4′ appeared as two triplets (J=8 Hz) at 4.42 and 4.05 ppm, respectively, downfield ($\sim$+0.6 ppm) to those of the free sugar due to the effect of the sulfate groups attached to C-3′ and C-4′. Proton H-2′ was also shifted because of the acylation with isovaleric acid, but its signal was superimposed with that of the anomeric proton in the region 4.50-4.70 ppm. A doublet at 0.90 ppm (J=6 Hz) was assigned to the isopropyl group of the isovaleroyl moiety.

^{13}C-nmr signals were assigned by comparison with atractyligenin 2-O-β-(2′-O-isovaleroyl)-D-glucopyranoside (2). Deshielding of C-3′ and C-4′ (+4.5 and +3.5 ppm, respectively) and shielding of C-2′ and C-5′ (−1.9 and −2.9 ppm, respectively) were observed in agreement with the sulfate shift rules (3).

Isolation of **1** in small quantities was efficiently achieved by chromatography on Sephadex LH-20, while previous isolations, for example from rootstocks of *Atractylis gummifera* (4) and *Callilepis laureola* (5) were carried out by direct crystallization.

Injection (ip) of **1** into mice caused their death by respiratory arrest, in agreement with reported pharmacological actions for atractyloside (6).

It is noteworthy that *Wedelia asperrima* from Australia (7) is also toxic, and its active principle, wedeloside, is an acylaminoglycoside of a diterpene structurally related to atractyloside.

EXPERIMENTAL

PLANT MATERIAL.—*W. glauca* was collected

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in March in Córdoba City, Province of Córdoba, Argentina. A voucher specimen (BAFC 1297) was deposited at the Departamento de Biología, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina.

ISOLATION OF ATRACTYLOSIDE (**1**).—Dried ground aerial parts of *W. glauca* (970 g) were successively extracted in a Soxhlet with petroleum ether (60-80°) (40.0 g of extract, 4.1%) and MeOH (125.8 g of extract, 12.9%). A portion of the MeOH extract (5.94 g) was partitioned between *n*-BuOH (20 ml) and H_2O (20 ml). The upper layer was extracted with H_2O (10 ml), and the combined aqueous layers were evaporated under vacuum to give a syrup (4.56 g) that was treated twice with MeOH (20 ml) leading to a partially desalted extract (2.3 g). The residue mainly contained KCl.

The desalted methanolic extract was chromatographed on a Sephadex LH-20 column (33 g) using MeOH as eluent. Three fractions were obtained. Fraction 1 contained sugars and terpenes, fraction 2: phenolic compounds and KCl, fraction 3: 51 mg of **1** (1.1 g/kg dry plant). Compound **1** was purified by dissolution in H_2O and precipitation with KCl to saturation and by further recrystallization from EtOH-H_2O (1:1).

CHARACTERIZATION OF ATRACTYLOSIDE (**1**).—Tlc: Rf 0.52 (Silicagel, *n*-BuOH-HOAc-H_2O, 30:10:8, anisaldehyde-H_2SO_4: pink→violet); $[\alpha]^{25}D$ −49° (c 0.36, H_2O); ir (5); ^{1}H-nmr (DMSO-d_6, 100 MHz) ppm: 0.90 (6H, d, J=6 Hz, CH_3-4″ and 5″), 0.93 (3H, s, CH_3-20), 2.1-2.3 (3H, H-2″ and H-4), 2.58 (1H, m, H-13), 3.5-4.8 (H-2, H-15, H-5′ and H-6′), 4.05 (t, J=8 Hz, H-4′), 4.42 (t, J=8 Hz, H-3′), 4.5-4.7 (H-1′ and H-2′), 4.90 and 5.10 (2H, two br s, H-17 and 17′); ^{13}C-nmr (D_2O, 25.2 MHz) ppm: 16.8 (C-20), 18.7 (C-11), 22.71 and 22.85 (C-4″ and C-5″), 25.7 (C-3″), 26.0 (C-6), 32.9 (C-12), 34.6[a] (C-3), 35.4[a] (C-7), 36.6 (C-14), 41.1 (C-10), 42.9 (C-13), 44.0 (C-4 and C-2″), 47.3 (C-1), 48.1 (C-8), 49.2 (C-5), 52.9 (C-9), 61.5 (C-6′), 72.9 (C-2), 74.9[b] (C-2′), 75.3[b] (C-4′ and C-5′), 79.5 (C-3′), 83.2 (C-15), 99.4 (C-1′), 109.2 (C-17), 159.8 (C-16), 175.6 (C-1″), 180.4 (C-19); ms *m/z* (rel. int., %): 302 (A-H_2O, 1), 284 (A-2H_2O, 3), 269 (A-2H_2O-15, 1), 256 (A-H_2O-HCOOH, 2), 241 (A-H_2O-15-HCOOH, 2), 240 (2), 239 (A-2H_2O-COOH, 2), 238 (A-2H_2O-HCOOH, 2), 228 (1), 225 (2), 223 (A-2H_2O-15-HCOOH, 3), 143 (18), 91 (12), 87 (12), 85 ($C_4H_9CO^+$, 41), 69 (55), 60 (73), 57 ($C_4H_9^+$, 68), 55 (20), 45 (31), 43 (72), 41 (100), (A=aglycone).

[a,b]Assignments bearing the same superscript may be interchanged. Superscripts ′ and ″ refer to the glucose and isovaleroyl moieties, respectively.

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