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WEDELIN, A SAPONIN FROM *WEDELIA SCABERRIMA*

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ABSTRACT.—A new saponin, wedelin, identified as (3-β)-28 (β-*D*-glucopyranosyloxy)-28-oxo-olean-12-en-3-yl-β-D-galacto-pyranosiduronic acid, has been isolated from the molluscicidal ethanolic extract of the leaves of *Wedelia scaberrima* Bent. Structure elucidation was performed by spectroscopic and chemical methods. The crude ethanolic extract of *W. scaberrima* showed activity (against *Biomphalaria glabrata*) at a concentration of 13 ppm; and the saponin mixture, at a concentration of 8 ppm.

Naturally occurring molluscicides isolated from various plant sources are currently receiving considerable attention, since the discovery of very active saponins from *Phytolacca dodecandra* L. Hert (1) and *Hedera helix* L. (2).

In continuation of our screening studies for biologically active compounds from the Compositae family (3), we now report our findings on a new saponin, wedelin, from *Wedelia scaberrima*.

RESULTS AND DISCUSSION

The leaves of *W. scaberrima* were extracted with ethanol and the extract partitioned between chloroform and water. The latter phase produced abundant foam on shaking and, after lyophilization, afforded a gummy material that decomposed at 220°. After treatment with methanol and acetone, a solid was obtained, which was again partitioned in a chloroform, methanol, water system, and the aqueous fraction was freeze-dried. The residue gave a positive, colored reaction with acetic anhydride and concentrated sulfuric acid for the genin moiety of the molecule. The tlc examination showed the presence of three spots. Attempts to separate the constituents by Celite 545 and Sephadex were unsuccessful. Acid hydrolysis (4) furnished oleanolic acid as the only genin from the saponin mixture. The pentacyclic triterpene acid (5) was obtained as white crystals (mp 302-304°) by recrystalization from ethanol. On methylation with CN_2N_2, the genin furnished methyl oleanolate, which was fully characterized by comparison of its physicochemical and spectroscopic proprieties (5-7).

Peracetylation and subsequent methylation of the saponins afforded an acetylated and methylated mixture which, on silica gel column chromatography, gave wedelin derivative **1** as a pure compound, after recrystalization from chloroform-ethyl acetate.

Mass spectral data, together with ^{1}H- and ^{13}C-nmr data and vlc suggested a 3-28-*O*-diglycosidic attachment to the genin. Despite the absence of $M^{+\cdot}$ in the mass spectrum, the following peaks, represented in figure 1 are conclusive. in the structural elucidation of the saponin: *m/z* 785, 5% (*a*); 667, 20% (*b*); 331, 95% (*c*); 257, 23% (*d*) and 248, 48% (*e*). The latter peak resulted from the retro Diels-Alder mechanism typical of a $\Delta^{11,12}$ unsaturated triterpene fragmentation (8), confirming that the carboxylic acid group at C-28 is attached to a sugar moiety and is not part of a methyl ester. The ion at *m/z* 785 (*a*) confirmed the sugar attached at C-28. The presence of a glucose derivative was further supported by an ion at *m/z* 331 (*c*) and by partition vapor chromatography of the sugar obtained after hydrolysis, when compared with standard samples of the alditol acetates prepared according to the Sawardeker and Crowell proce-

dures (9,10). The ion at *m/z* 257 (*d*) showed the presence of an uronic acid derivative, which is attached at the C-3 hydroxy group of the genin, as evidenced by the ion at *m/z* 667 (*b*). All these data pointed to structure **1** for the wedelin derivative.

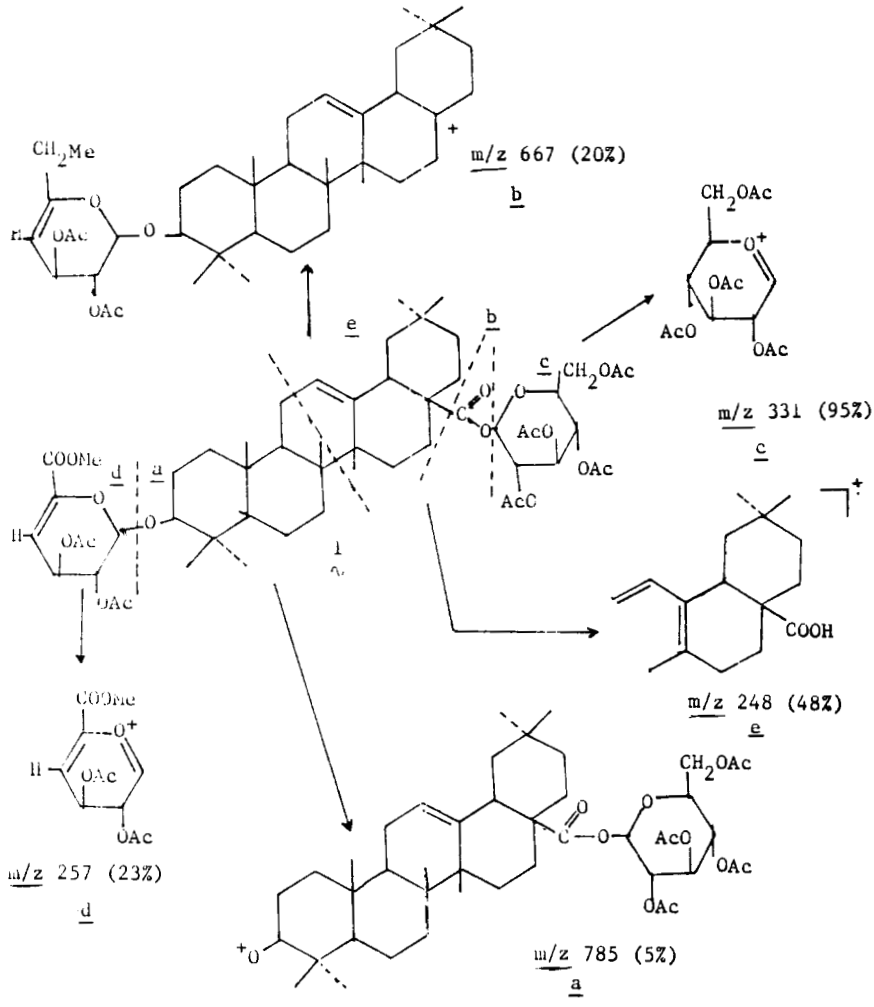

FIGURE 1. Partial mass spectrum *m/z* and ion assignments for the peracetylated and methylated derivative of Wedelin **1**.

The attributions of the C atom signals in the ^{13}C-nmr spectrum of **1** (see table 1) were determined in the light of recent studies of the ^{13}C-nmr spectra of several oleanolic acid derivatives (11,12). The unusual absorption seen as a doublet at 6.18 δ (H-4′, 1H, J=5 Hz) in the ^{1}H-nmr spectrum, the resonances at 107.13 and 142.95 δ for C-4′ and C-5′, respectively, in the ^{13}C-nmr spectrum, and the absorption at λ max (EtOH) 234 nm proved the uronic acid to be unsaturated, which is extremely rare in nature. This led us to investigate further the sugar moiety at C-3 of the genin, because the dehydration might have occurred during the acetylation step.

According to Lindberg (13) and Boyd and Turvery (14), base treatment of an uronic acid promoted elimination yielding an unsaturated uronate residue. Therefore, the sugar at position C-3 could be glucuronic or galacturonic acid. In order to prove the sec-

TABLE 1. ^{13}C-nmr spectral data of compound **1**[a]

Carbon	ppm	Carbon	ppm
1	38.56(t)	16	23.61(t)
2	25.38(t)	17	47.07(s)
3	90.70(t)	18	41.29(d)
4	39.13(s)	19	45.95(t)
5	55.59(d)	20	30.80(s)
6	18.44(t)	21	33.99(t)
7	33.15(t)	22	Oleanolic 32.02(t)
8	39.61(s)	23	acid 28.32(q)
9	47.79(d)	24	16.36(q)
10	36.91(s)	25	15.47(q)
11	23.09(t)	26	17.19(q)
12	123.16(d)	27	25.86(q)
13	143.11(s)	28	175.87(s)
14	41.95(s)	20	33.15(q)
15	27.94(t)	30	23.61(q)
1′	99.03(d)		−OCOMe; 169,12(1);
2′	65.03(d)		169, 60(1); 169.67(1);
3′	68.72(d)	galacturonic	170.29(2); 170.75(1);
4′	107.13(d)	methyl ester	
5′	142.95(s)	acetylated	−OCO*Me*: 20.69(3);
6′	162.67(s)	derivative	20.79(1); 20.93(2)[b]
Me ester	52.65(q)		
1″	91.83(d)		
2″	70.27(d)	glucose	
3″	73.15(d)	acetylated	
4″	68.97(d)	derivative	
5″	72.73(d)		
6″	61.84(t)		

[a]Run in $CDCl_3$, TMS as internal standard.
[b]Letters in parentheses refer to signal multiplicities and numbers to integration.

ond hypothesis, partially hydrolyzed olaxoside (12) received the same treatment as our saponin mixture. In these conditions, elimination did not occur. The inference of galacturonic acid is based on the favorable position of the hydroxyl group at C-4, this being *trans* to the hydrogen at C-5, allowing an easy elimination (15).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. The uv spectra were prepared in ethanol on a Beckman OB-GT instrument; ir spectra were taken in KBr pellets or in chloroform on a Perkin-Elmer 137 model instrument. ^{1}H-nmr spectra were determined at 100 MHz on a Varian XL-100 apparatus, in $CDCl_3$, using TMS as internal standard. ^{13}C-nmr spectra were carried out in $CDCl_3$ at 25.2 MHz (Varian XL-100) and 62.68 MHz (Gif-sur-Yvette, France). The partially decoupled spectra (SFORD) were obtained with −2 δ decoupling. Chemical shifts (δ) are reported in ppm; and coupling constants (*J*), in Hz. Mass spectra were obtained on a Varian MAT-85-100 MHz by direct inlet probe, at 70 eV. Silica gel G,H, H_{254}, GF_{254}, and PF_{254} were from E. Merck, Darmstadt; Sephadex LH-20, from Pharmacia Fine Chemicals; and Celite 545, from Queel Tenant Chemistry Analytical. Gas chromatography was carried out with the alditol acetates in a Varian Aerograph 1400 using a NPGS column programmed to 215°. Injection temperature was held at 250° with a N_2 flow rate of 12 ml/h. Elementary analysis was conducted by Alfred Bernhardt Laboratories, West Germany.

EXTRACTION.—The defatted, powdered plant material (3 kg) was extracted with ethanol (5 liters) in a Soxhlet apparatus for 72 h. After concentration, this extract (295 g) was partitioned between $CHCl_3$ (6 liters) and water (1:1). The aqueous layer was kept in the refrigerator for 36 h, when a brown precipitate was obtained. It was separated by filtration and kept in the desiccator for 24 h to yield a solid (6.6 g). This was dissolved in methanol (250 ml), filtered, and the filtrate concentrated under reduced pressure to 70 ml,

which was treated with excess acetone to furnish an insoluble material (2.0 g), which was left in a desiccator for 48 h. The solid was then dissolved and partitioned in chloroform, methanol, and water (35:65:40). The aqueous layer was lyophilized, furnishing a solid residue (1.42 g), which, on tlc examination using EtOAc, MeOH, and water (60:15:15) as the development system, was shown to be a mixture of saponins. The Rf values were 0.40, 0.55, and 0.61, after being sprayed with ceric sulfate solution.

PERACETYLATION AND METHYLATION OF THE SAPONIN MIXTURE.—To pyridine (15 ml) and acetic anhydride (10 ml) was added the saponin mixture (1.36 g). The reaction was stirred for 72 h at room temperature, diluted with cold water (40 ml), acidified to pH 2 with HCl, and extracted with chloroform (3×50 ml). The chloroform extracts were neutralized with sodium bicarbonate, dried with $MgSO_4$ (anhydrous), and filtered. The filtrate was evaporated under reduced pressure to give an oil (1.60 g). The oil was chromatographed on a silica gel column, and the eluate (*n*-hexane-ethyl acetate, 40:60) afforded a crystalline material shown to be a mixture of acetates by tlc (development system *n*-hexane, ethyl acetate 30:70). The solid (1.48 g) was dissolved in ether (20 ml) and treated with diazomethane at room temperature for 18 h. Work-up in the usual way gave the peracetylated and methylated saponin derivatives. The yellow, solid material was separated by plc using $CHCl_3$-ethyl acetate (90:10) as the development system. Colorless crystals (0.040 g) of **1** were obtained on crystallization from chloroform-EtOAc (1:1), mp 108-110°, uv λ max (EtOH) 234 nm (ε=5,000); ir ν max ($CHCl_3$) 1751 (C=O), 1653, 1357, 1222, 1215, 905 (=CH−) cm^{-1}; ^{1}H-nmr (100 MHz, $CDCl_3$) δ 0.74 (s, 3H, CH_3); 0.77 (s, 3H, CH_3); 0.90 (s, 9H, 3×CH_3); 0.97 (s, 3H, CH_3); 1.13 (s, 3H, CH_3); 2.03 (s, 9H, 3×$OCOCH_3$); 2.08 (s, 9H, 3×$OCOCH_3$); 2.82 (m, 1H); 3.22 (s, 1H); 3.76 (s1, 1H); 3.85 (s, 3H, OCH_3); 3.95-4.39 (m, 3H); 5.0-5.40 (m, 7H); 5.57 (d, 1H, CH=C, J=8 Hz); 6.18 (d, 1H, CH=C, J=5 Hz); ^{13}C-nmr see table 1; ms, no molecular ion, fragmentations at *m/z* (% rel. abundance), 785 (5), 769 (3), 667 (20), 439 (23), 438 (12), 393 (47), 331 (95), 289 (8), 257 (23), 248 (48), 247 (14), 229 (65), 169 (75), 127 (65), 109 (79).

Anal. calcd for: $C_{55}H_{78}O_{19}\cdot 5H_2O$; C, 58.30; H, 7.70. Found: C, 57.92; H, 7.50.

ACID HYDROLYSIS.—The saponin mixture (0.34 g) was dissolved in an ethanolic solution of 1 N HCl (25 ml) and refluxed for 24 h (4). After concentration under reduced pressure, the genin was precipitated by adding water (30 ml). The precipitate was filtrated and the residue washed with water. The genin (0.070 g), after plc using *n*-hexane-EtOAc (65.35), furnished an amorphous material (0.55 g), which, after recrystallization from ethanol, yielded white crystals of oleanic acid (0.048 g), mp 300-302° (15). The methyl ester derivative, prepared by treatment with diazomethane, after working up in the usual way, gave methyl oleanate, mp 198-200° (5).

IDENTIFICATION OF SUGARS.—Wedelin (**1**) (0.0017 g) was hydrolized with 6 N trifluoracetic acid (1 ml) and a 1.5 mg/ml solution of alditol (25 μl) by treating it in a sealed tube at 100° for 3 h. The mixture was evaporated under reduced pressure and reduced with sodium borohydride (0.0016 g) in an ammonium hydroxide solution 0.1 N (1 ml) for 1 h at room temperature. The excess sodium borohydride was destroyed with 20% aqueous solution of AcOH. Boric acid was eliminated by co-evaporation with MeOH (3×5 ml). The residue was dissolved in a mixture of acetic anhydride and pyridine, and heated for 1 h in a sealed tube. Distilled water (5 ml) was added to the yellow liquid obtained, and the reaction medium was extracted with $CHCl_3$ (3×5 ml). The chloroform extracts were combined and evaporated to give a residue, which was dissolved in dichloromethane (150 μl). From this solution, an aliquot (2 μl) was chromatographed on a column of neopentylglycol succinate 3%, 100/120 Gaschrom Q (NPGS), isothermic 215°, N_2 flow rate 12 ml/h against standard alditol acetates. Retention time for the sample: 1.33, and Rt for glucose standard: 1.34.

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