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THE TOXIC EXTRACTIVES FROM *WEDELIA ASPERRIMA*, III.[1] STRUCTURES OF TWO NATURALLY OCCURRING RHAMNOSYL ANALOGUES OF WEDELOSIDE

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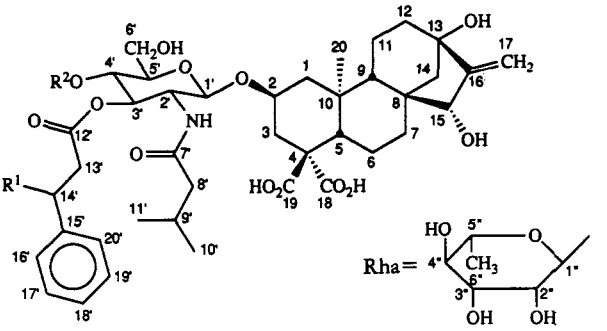
ABSTRACT.—The structures of two new toxic 4′-*O*-rhamnosyl analogues of wedeloside [**1**], from *Wedelia asperrima*, have been determined by a combination of chemical, nmr, and mass spectrometric analysis.

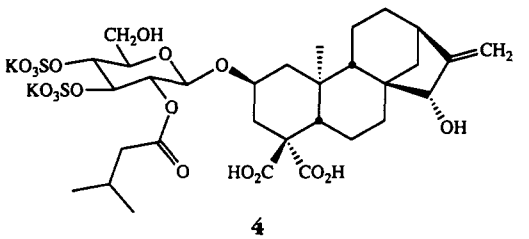
In previous papers, we described the structural elucidation of the diterpene aminoglycoside wedeloside [**1**], the major toxic constituent of *Wedelia asperrima* Benth. (Compositae) (1,2). Subsequent biochemical studies (3) have shown wedeloside to be a powerful inhibitor of ADP/ATP transport across the mitochondrial membrane, with a binding affinity to the carrier protein comparable to that of the related diterpene glycoside, carboxyatractyloside [**4**] (4). We now report the structures of two additional toxic components **2** and **3** from *W. asperrima*.

RESULTS AND DISCUSSION

For the structure of *O*-α-L-rhamnosyl-(1″↦4′)-wedeloside [**2**], the fab mass spectrum of **2** showed an $[MH]^+$ ion at *m/z* 904 and an $[MNa]^+$ ion at *m/z* 926, which mass-



1 $R^1=R^2=H$
2 $R^1=H$, $R^2=Rha$
3 $R^1=OH$, $R^2=Rha$



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[1]For Part II, see Lewis *et al.* (2).

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measured for $C_{46}H_{66}NO_{17}$ and $C_{46}H_{65}NO_{17}Na$, respectively, corresponding to wedeloside [**1**] plus an additional $C_6H_{10}O_4$ unit. After permethylation of **2**, the eims of the product **5** showed an $[M]^+$ at *m/z* 911 ($C_{47}H_{77}NO_{16}$) with prominent ions at *m/z* 476 ($C_{23}H_{42}NO_9$), 419 ($C_{24}H_{35}O_6$), and 189 ($C_9H_{17}O_4$) corresponding to the glycoside ($[G]^+$), aglycone ($[A]^+$), and terminal sugar ($[S]^+$) fragments (Table 1). As was the case with wedeloside [**1**] (1), the 3-phenylpropanoyl group was lost from **2** during the permethylation procedure. On pertrideuteromethylation, the $[M]^+$, $[G]^+$, $[A]^+$, and $[S]^+$ ions shifted to *m/z* 941 (10 × Me), 494 (6 × Me), 431 (4 × Me), and 198 (3 × Me), respectively, in accordance with the expected number of active hydrogens (OH, NH) in the different portions of the molecule.

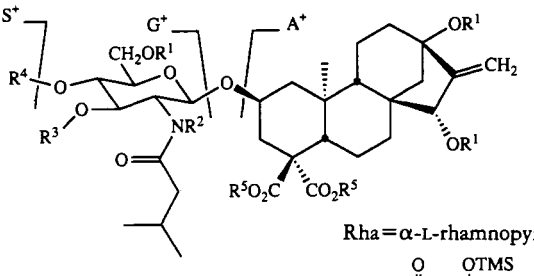
TABLE 1. Structurally Significant Ions (*m/z*) in the Mass Spectra of Derivatized Toxins **1–3**.

Compound	Derivative	$[M]^+$	$[A]^+$	$[G]^+$	$[S]^+$
5	per Me **2**	911	419	476	189
6	per TMSi **3**	1567	651	900	363
7	per TMSi **1**[a]	1189	651	522	—

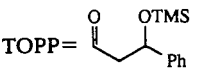
[a]Data are from Lewis *et al.* (2).

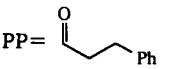
Acid hydrolysis of **2** in aqueous MeOH followed by trimethylsilylation and subsequent gc-ms of the product mixture showed the presence of TMSi-3-phenylpropanoate [**9**] (1), the tri-TMSi derivative **11** of methyl 2-deoxy-2-(3-methyl-1-oxobutyl)amino-α-D-glucopyranoside (1), and a 6-deoxyhexose derivative. The latter was compared with standard TMSi-6-deoxyhexoses and showed an identical mass spectrum and gc retention time to those of tetra-TMSi-L-rhamnopyranoside [**13**].

Methanolysis of **5**, the permethyl derivative of **2**, gave three major products. One was a compound with $[M]^+$ at *m/z* 436 and diagnostic fragment ions at *m/z* 128, 145, and 165, confirming its structural identity with 13,15,18,19-tetra-*O*-methylwedeligenin [**16**] (2). The second was a compound with $[M]^+$ at *m/z* 319 corresponding to a tetramethyl derivative of the de-esterified 2-deoxy-2-acylaminoglucoside unit in **2** (i.e., structure **12**). The location of the free hydroxy substituent at C-4 in this compound (corresponding to the position of attachment of the rhamnosyl moiety in **2**)

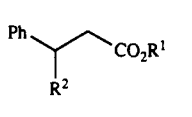


Rha=α-L-rhamnopyranosyl

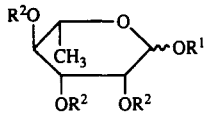




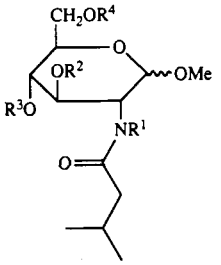
5 $R^1=R^2=R^3=R^5=Me$, $R^4=Rha(Me)_3$
6 $R^1=R^5=TMSi$, $R^2=H$, $R^3=TOPP$, $R^4=Rha(TMSi)_3$
7 $R^1=R^4=R^5=TMSi$, $R^2=H$, $R^3=PP$
8 $R^1=R^2=R^3=H$, $R^4=Rha$, $R^5=Me$



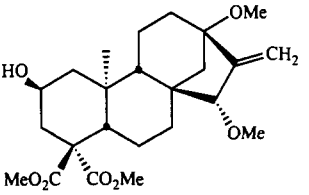
9 R^1=TMSi, R^2=H
10 R^1=Me, R^2=OH



13 R^1=R^2=TMSi
14 R^1=R^2=Me
15 R^1=Me, R^2=H



11 R^1=H, R^2=R^3=R^4=TMSi
12 R^1=R^2=R^4=Me, R^3=H



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could be deduced from the presence of well documented cleavage ions at *m/z* 87, 170, and 171 in its mass spectrum (5). The third was a compound corresponding to a tetra-*O*-methyl-6-deoxyhexose, with structure **14**.

The basic skeletal structure of **2** was initially deduced by comparison of its ^{13}C-nmr spectrum with that of wedeloside [**1**] (6) (Table 2). With the exception of C-4′, the position of attachment of the rhamnosyl moiety, which showed the expected downfield shift of ca. 7 ppm, and the 3′- and 5′-carbons which exhibited small (2–3 ppm) upfield shifts, the chemical shift values for the carbon atoms of the aglycone (C-1 to C-20) and acylaminoglucoside (C-1′ to C-20′) moieties were directly comparable. The remaining six carbon resonances C-1″ to C-6″ corresponded to the values reported for a 1-α-linked L-rhamnopyranosyl residue (7). Verification of the structure of **2** was obtained using various 2D nmr experiments (COSY, long-range COSY, HETCOR, long-range HETCOR, and phase-sensitive double-quantum-filtered COSY). These experiments allowed direct correlations between geminally and vicinally coupled and long-range coupled protons as well as analogous proton-to-carbon correlations yielding complete and unambiguous chemical shift assignments. Most notable were the ^{13}C chemical shift assignments of the glycosidic carbons and C-2. Severe overlap in the ^{1}H-nmr spectrum of the protons associated with these carbons and the proximity of their ^{13}C chemical shifts necessitated the use of these nmr spectral techniques. The quaternary carbons, as well as the C-5 and C-9 methines, were distinguished via their long-range ^{1}H-^{13}C couplings to their neighboring methylenes.

In the ^{1}H-nmr spectrum of **5**, the two C-17 olefinic methylene protons were present at δ 5.13 and 5.24, while the anomeric protons on C-1′ and C-1″ appeared at δ 4.6 (J = 8 Hz) and δ 4.99 (J = 1 Hz), respectively, the former supporting the assignment of a 1-β-linkage for the 2-deoxy-2-acylaminoglucosyl group (1).

The fab spectrum of **3** showed a weak $[MH]^+$ ion at *m/z* 920 together with a stronger $[MNa]^+$ ion at *m/z* 942 ($C_{46}H_{65}NO_{19}Na$) and ions for CO_2 loss at *m/z* 876 and 898, respectively. The per-TMSi derivative of **3** exhibited, in its eims, an $[M]^+$ at *m/z* 1567 and an $[A]^+$ ion at *m/z* 651, indicating the presence of the same aglycone moiety as in **7**, the per-TMSi derivative of wedeloside [**1**] (Table 1). Further, the $[S]^+$ ion at *m/z* 363 suggested that compound **3** also possessed a rhamnose sugar but differed from

TABLE 2. ^{13}C Chemical Shift Assignments[a] for Compounds **1**, **2**, and **3**.

Carbon	**1**[b]	**2**	**3**
C-1	48.1	47.2	46.4
C-2	73.5	75.3	73.3
C-3	41.0	42.9	41.2
C-4	59.0	61.0	59.1
C-5	49.8	52.2	51.7
C-6	24.0	23.4	24.0
C-7	35.8	35.1	35.9
C-8	46.7	47.2	46.9
C-9	53.5	51.9	53.8
C-10	40.0	40.4	41.2
C-11	20.9	20.2	21.0
C-12	41.0	40.5	40.2
C-13	79.5	79.8	79.7
C-14	43.9	45.8	45.4
C-15	82.2	81.5	82.4
C-16	160.4	159.0	160.9
C-17	108.9	109.3	108.8
C-18	175.5	179.0	175.8
C-19	175.5	179.4	175.3
C-20	17.6	17.6	17.7
C-1′	101.2	99.7	101.0
C-2′	55.2	54.7	55.7
C-3′	77.0	75.4	76.7
C-4′	69.6	76.5	76.3
C-5′	77.4	74.7	75.9
C-6′	62.2	61.0	62.0
C-7′	175.5	177.3	175.3
C-8′	46.7	46.2	46.7
C-9′	27.2	26.7	27.2
C-10′	22.9	22.3	23.0
C-11′	22.9	22.4	23.0
C-12′	174.2	175.4	172.1
C-13′	31.5	35.8	48.2
C-14′	36.6	38.9	71.2
C-15′	141.7	141.3	145.2
C-16′, -20′	129.2	129.1	127.1
C-17′, -19′	129.2	129.7	129.4
C-18′	127.1	124.1	128.6
C-1″	—	102.0	102.8
C-2″	—	70.7	72.0
C-3″	—	71.2	72.4
C-4″	—	72.7	73.9
C-5″	—	70.3	70.8
C-6″	—	17.6	18.1

[a]δ, ppm from TMS, in CD_3OD (**1** and **3**) and D_2O (**2**).
[b]Data are from Lewis and MacLeod (6).

2 in having an extra hydroxy substituent located on the aminoglucoside moiety. The presence in the spectrum of an ion at *m/z* 179 (C_7H_6OTMSi) placed the hydroxy group on either the phenyl ring or C-3 of the phenylpropanoate group. Treatment of **3** with CH_2N_2 followed by NaOMe/MeOH yielded, after hplc purification, methyl 3-hydroxy-3-phenylpropanoate [**10**], identical by ms and ^1H nmr to an authentic racemic sample, while its [α]D value established the absolute stereochemistry of the chiral center as *S* (8). The residual compound **8** on methanolysis gave 1-*O*-methyl α-L-rhamnopy-

ranoside [**15**], which had a ^{13}C-nmr spectrum and optical rotation identical to authentic 1-*O*-methyl α-L-rhamnopyranoside (7,9).

The ^{13}C-nmr spectrum of **3** (Table 2) provided confirmation of its assigned structure. Apart from the carbon atoms at the 13′, 14′, and 15′ positions, which showed the expected downfield shifts compared to those of **2** due to the additional hydroxy substituent on C-14′, the spectra of **2** and **3** were comparable, any slight differences being attributable to solvent effects (MeOH vs. H_2O).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Partial details of these have been reported previously (1). Additionally, some gc-ms were recorded on a VG7070F mass spectrometer equipped with a Varian 1400 gc using a 2 m × 2 mm glass column packed with 2% OV-17; some ^{1}H- and ^{13}C-nmr spectra were run on either a JEOL FX-200 or a Bruker HFX270 spectrometer. The COSY, long range COSY, HETCOR and long-range HETCOR experiments were carried out on a Varian VXR-300S spectrometer, while the phase sensitive double-quantum-filtered COSY and the long range version of the same experiment were performed on a Varian VXR-500 nmr spectrometer. Optical rotations were measured using a Perkin-Elmer 241 spectropolarimeter. Fabms accurate mass measurements were obtained on a VG ZAB-SEQ mass spectrometer using glycerol as matrix and polyethylene glycol as internal reference.

ISOLATION OF **2** AND **3**.—The extraction and isolation procedure for **2** and **3** from *W. asperrima* follows that reported for wedeloside [**1**] (1) up to the point of elution of the toxic fractions from a column of High Flow Super Cell. The dried fraction containing **2** and **3** was dissolved in MeOH-H_2O-HOAc (40:60:1) saturated with 1,2-dichloroethane and applied to a column of micronized XAD-2 resin (50 g). Elution with the same solvent mixture (500 ml) was followed by stepwise elution with solvent mixtures (100 ml each) containing an increasing proportion of MeOH to H_2O but maintaining the same proportion of HOAc. Fractions from the column were monitored by tlc and after drying provided **2** and **3** as amorphous powders (60 mg and 200 mg, respectively). Compound **2** was further purified by chromatography on a Sephadex LH-20 column (2 m × 1 cm) using MeOH as eluent followed by a second chromatographic step on a Sephadex G-10 column (1 m × 1 cm) again using MeOH as eluent.

Compound **2**.—$[\alpha]^{20}_D$ −34° (*c* = 1.5, MeOH); fabms *m/z* $[MH]^+$ 904.4410 (calcd for $C_{46}H_{66}NO_{17}$, 904.4331), $[MNa]^+$ 926.4140 (calcd for $C_{46}H_{65}NO_{17}Na$, 926.4150); ^{1}H nmr δ (D_2O, 100 MHz) 1.36 (d, *J* = 6 Hz, Me), 5.32 and 5.41 (both bs, $=CH_2$), 7.63 (bs, C_6H_5); ^{13}C nmr (D_2O) see Table 2.

Compound **3**.—Fabms $[MNa]^+$ 942.416 (calcd for $C_{46}H_{65}NO_{18}Na$, 942.410); ^{13}C nmr see Table 2.

TOXICITY TESTING.—This was carried out as previously reported (10). Both compounds **2** and **3** were toxic to mice and rats down to the 1 mg/kg level.

PERMETHYLATION AND PERTRIDEUTEROMETHYLATION OF **2**.—Compound **2** (9.8 mg) was permethylated according to the procedure described previously for wedeloside (1). The permethylated derivative was purified by preparative tlc on silica [MeCN-C_6H_6, (40:60), R_f 0.39], giving **5** (6 mg). Using the same procedure, but substituting CD_3I, the pertrideuteromethylated derivative of **2** was also prepared.

Compound **5**.—^{1}H nmr δ ($CDCl_3$, 100 MHz) 4.60 (d, *J* = 8 Hz, anomeric H), 4.99 (bs, *J* ~ 1 Hz, anomeric H), 5.13 and 5.24 (both bs, $=CH_2$); ^{13}C nmr ($CDCl_3$, 15 MHz) 16.7, 17.8, 20.4, 22.9, 25.2, 28.3, 34.8, 36.8, 39.5, 39.9, 42.2, 43.7, 46.5, 47.2, 50.3, 50.7, 51.1, 52.1, 53.0, 57.2, 58.0, 58.9, 59.5, 60.8, 62.8, 68.5, 71.5, 72.2, 77.9, 80.6, 81.1, 82.3, 84.6, 90.4, 98.0, 98.6, 109.3, 151.8, 172.5, 174.4; eims *m/z* $[M]^+$ 911.5242 (calcd for $C_{47}H_{77}NO_{16}$, 911.5276), 896, 880, 706, 560, 476 (found 476.2850, calcd for $C_{23}H_{42}NO_9$, 476.2860), 444, 419 (found 419.2433, calcd for $C_{24}H_{35}O_6$, 419.2434), 387, 224, 189 (found 189.1128, calcd for $C_9H_{17}O_4$, 189.1127), 171, 165, 128.

Pertrideuteromethylated **2**.—Eims *m/z* $[M]^+$ 941, 926, 907, 727, 575, 494, 459, 431, 396, 230, 198, 177, 171, 134.

HYDROLYSIS OF **2**.—Compound **2** (1.0 mg) in a mixture of MeOH (1 ml), H_2O (0.5 ml), and 4 M HCl (0.5 ml) was heated in a sealed tube at 90° for 2 h. After evaporation to dryness, the residue was silylated with TRISIL and analyzed by gc-ms (100–250°, Δ10). Three major hydrolysis products were identified: TMSi-3-phenylpropanoate [**9**], by comparison of its ms and retention time with the TMSi derivative of an authentic sample (Aldrich); compound **11**, by comparison with its published mass spectrum (1); and tetra-TMSi-rhamnose, by comparison of its ms and retention time with the TMSi derivative of an authentic sample (Sigma).

METHANOLYSIS OF **5**.—The permethylated derivative **5** (1.0 mg) was dissolved in MeOH (1 ml) and heated at 60° over Dowex 50W resin (H^+ form) for 2 h. An aliquot, analyzed directly by gc-ms (100–

200°, Δ10), showed three major peaks: compound **16** ($[M]^+$ *m/z* 436) identified as 13,15,18,19-tetra-*O*-methylwedeligenin [**16**] by comparison with its published mass spectrum (2); compound **12**, eims *m/z* (rel. int.) $[M]^+$ 319 (0.5), 304 (1.5), 288 (1), 287 (2), 274 (4), 272 (4), 244 (10), 224 (20), 171 (21), 170 (27), 141 (30), 140 (79), 117 (100), 87 (>100); and compound **14**, identified by comparison of its ms and retention time with those of authentic tetra-*O*-methyl rhamnose.

SILYLATION OF **3**.—Compound **3** (1.0 mg) was suspended in dry MeCN (10 μl) and treated with BSTFA-TMCS (9:1) (50 μl) at 70° for 20 min to give the per-TMSi derivative **6**. An aliquot was subjected to direct probe eims. For significant ions, see Table 1. Accurate mass measurement on *m/z* 179 gave 179.0894, $C_{10}H_{15}OSi$ requires 179.0892.

HYDROLYSIS OF **3**.—Compound **3** (40 mg) in MeOH (2 ml) was first treated with CH_2N_2 in Et_2O, followed by addition of a small amount of sodium to the MeOH solution after removal of the Et_2O. After 18 h at room temperature, the solvent was removed and the residue triturated with $CHCl_3$. Hplc of the $CHCl_3$ washings on a Zorbax SIL column (25 cm × 4.6 mm) using CH_2Cl_2-MeCN (9:1) as eluent afforded (*S*)-methyl 3-hydroxy-3-phenylpropanoate [**10**] (2.6 mg), $[\alpha]^{25}D$ −15° (*c* = 1.3, EtOH) [lit. (8) (*R*)-form +18.3°]. ^1H-, ^{13}C-nmr, and mass spectra were identical to an authentic sample of racemic **10**.

The residue from alkaline hydrolysis was taken up in MeOH (2 ml). Dowex 50W resin (H^+ form) was added and the mixture was heated at 60° for 2 h. After filtration and removal of solvent, the hydrolysate was chromatographed [plc; EtOAc-MeCN (9:1)] giving (R_f 0.23) 1-*O*-methyl α-L-rhamnopyranoside [**15**] (5 mg) identical (^{13}C nmr, optical rotation) to an authentic sample.

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