Alkaloidal, Megastigmane and Lignan Glucosides from *Antidesma membranaceum* (Euphorbiaceae)

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Two novel alkaloidal glucosides derived from the recently discovered antidesmone (**1**), together with four known megastigmane and three lignan glucosides, two of which had not previously been described, were isolated from 1-butanol extracts of *Antidesma membranaceum* (Euphorbiaceae). The structural elucidation of (17*RS*)-17-(β-D-glucopyranosyloxy)antidesmone (**2**) and (17*RS*)-8-deoxo-17-(β-D-glucopyranosyloxy)antidesmone (**3**) is based on ^{1}H, ^{13}C, COSY, NOESY, HMQC and HMBC NMR spectra, together with LC/ESI-CIDMS and CD data. Determination of the absolute configuration at C-17 was accomplished by comparison with ^{1}H NMR spectroscopic data for alk-2-yl β-D-glucopyranosides, an approach that also proved useful for the megastigmane glucosides blumenyl C β-D-glucopyranoside (**4**), 3-oxo-α-ionyl β-D-glucopyranoside (**5**), blumenyl B β-D-glucopyranoside (**6**) and blumenyl A β-D-glucopyranoside (**7**). The lignan glucosides lyoniresin-4-yl β-D-glucopyranoside (**8**), 4′-*O*-methyllyoniresin-4-yl β-D-glucopyranoside (**9**) and secoisolariciresin-4-yl β-D-glucopyranoside (**10**), featuring an unusual glucosylation position, were investigated with the aid of ^{1}H and 2D NMR, CD and MS data.

Introduction

Antidesma membranaceum (Euphorbiaceae) is a shrub or small tree of equatorial Africa and belongs to the subfamily of the Phyllanthoideae, which is reported to lack interesting secondary metabolites.[1] In the course of our studies on *A. membranaceum* we found secoisolariciny1 9,9′-diferuloylate and (−)-syringaresinol, together with six new benzopyranones[2] and a novel quinolinone alkaloid, antidesmone (**1**).[3,4] The two alkaloids hyeronine A and B have recently been found[5] in *Hyeronima oblonga*, a closely related species. Of these, the former displays striking spectroscopic similarities to antidesmone and might have the same structure. Hyeronimone, from *Hyeronima alchorneoides*,[6] can be regarded as a dihydro form of antidesmone. The unusual biosynthesis of antidesmone proceeds through formation of a C_{16} polyketide chain and insertion of glycine.[7]

This paper describes the isolation and structural elucidation of the alkaloidal glucosides (17*RS*)-17-(β-D-glucopyranosyloxy)antidesmone (**2**) and (17*RS*)-8-deoxo-17-(β-D-glucopyranosyloxy)antidesmone (**3**), the megastigmane glucosides blumenyl C β-D-glucopyranoside (**4**), 3-oxo-α-ionyl β-D-glucopyranoside (**5**), blumenyl B β-D-glucopyranoside (**6**) and blumenyl A β-D-glucopyranoside (roseoside, **7**) as well as the lignan glucosides lyoniresin-4-yl β-D-glucopyranoside (**8**), 4′-*O*-methyllyoniresin-4-yl β-D-glucopyranoside (**9**) and secoisolariciresin-4-yl β-D-glucopyranoside (**10**), isolated from the 1-butanol extracts of *A. membranaceum*.

Results and Discussion

Leaves and bark from *A. membranaceum* were extracted separately with 80% aqueous MeOH, and the solution was concentrated in vacuo to the aqueous phase and then extracted with *n*-hexane, ethyl acetate and 1-butanol. After chromatography on Sephadex LH-20, acidic liquid-liquid partition and preparative HPLC, compounds **2**, **4**, **5**, **6** and **7** were obtained from the 1-butanol extract of the leaves. The 1-butanol extract of the bark was dissolved in ethyl acetate, which was then extracted with aqueous HCl. The acidic solution was neutralized, concentrated to dryness and extracted with MeOH to yield compounds **3**, **8**, **9** and **10** after preparative HPLC.

The UV spectrum of **2** showed the same maxima as **1**, at 248, 276 and 328 nm, these being characteristic of aromatic compounds with a conjugated oxo function.[8] The positive-ion electrospray mass spectrum obtained by LC-MS exhibited peaks at *m*/*z* = 498 ([M + H]$^+$, base peak) and 336 ([M + H − 162]$^+$), as well as at 318 ([M + H − 162 − 18]$^+$) indicating the loss of a hexose. The elemental composition of the [M + H]$^+$ ion of **2** was determined as $C_{25}H_{40}NO_9$ by HR ESI TOF-MS. Antidesmone (**1**) possesses a significant ESI CIDMS spectrum, the fragmentation pattern of which is highly characteristic of the antidesmone bicyclic system (see Figure 1). The LC/ESI CIDMS spectrum of the aglycon ion [A + H]$^+$ of compound **2** (*m*/*z* = 336), corresponding to the [M + H − 162]$^+$ ion, featured a fragmentation pattern very similar to that of **1** (see Figure 1), suggesting the same bicyclic system and an additional hydroxy function in the side chain. All ^{1}H and ^{13}C NMR signals of the tetrahydroquinolinedione moiety of **2** are in excellent agreement with data from **1**. From analysis of the coupling constants, 5-H must be in a pseudoequatorial position in both **2** and **1**.[3,4] Other than the presence of

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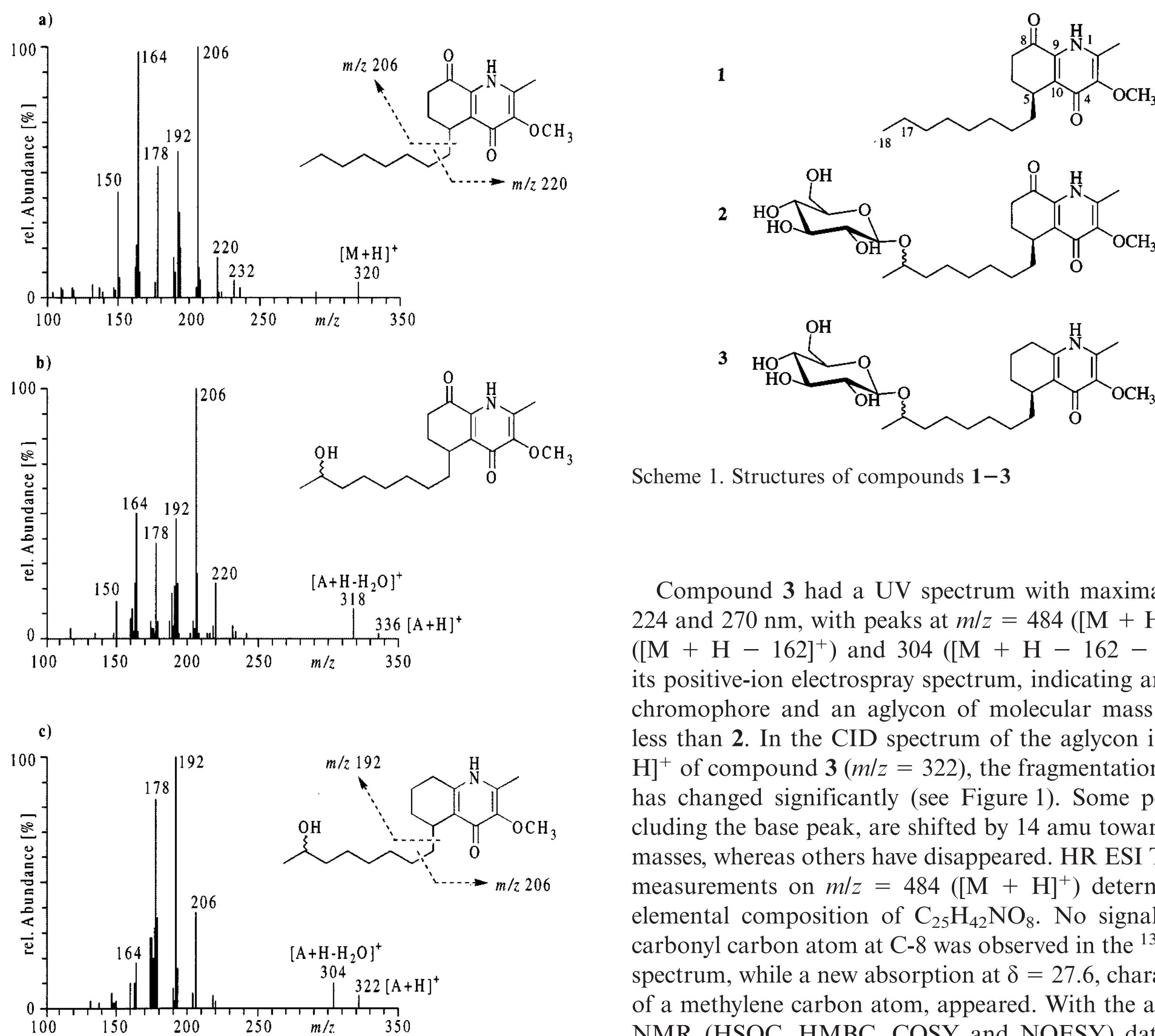


Figure 1. CID mass spectra obtained by LC ESI MS of (a) antidesmone (**1**) and the aglycon ions of (b) **2** and (c) **3**

Scheme 1. Structures of compounds **1−3**

β-D-glucopyranose signals, the most obvious difference from the antidesmone spectrum is the change in the terminal methyl triplet [antidesmone: δ = 0.874 (3 H, t, 7.0 Hz)] to two doublet signals [**2**: δ = 1.162 (2.2 H, d, 6.2 Hz), 1.223 (0.8 H, d, 6.2 Hz)], establishing the linking oxygen position as C-17 (see Scheme 1). The same signal splitting is also observed for the C-16, C-17, C-18, C-1′, 17-H and 2′-H signals. Analysis of 2D NMR (COSY, NOESY, HMQC and HMBC) spectral correlations clearly showed the presence of two molecular species. This can be explained by the fact that glucose, as a chiral molecule, forms local diastereomers with two stereoisomeric forms at C-17. Comparison with reference data on alk-2-yl β-D-glucopyranosides[9,10] allowed the signals to be assigned unambiguously to the (17*R*) or the (17*S*) forms. By integration of the methyl signals the (*R*)/(*S*) ratio at C-17 could be determined as 3:1. Since the CD spectrum of **2** shows the same maxima as that of **1**, the absolute configuration at C-5 must be (*S*).

Compound **3** had a UV spectrum with maxima at 219, 224 and 270 nm, with peaks at *m*/*z* = 484 ($[M + H]^+$), 322 ($[M + H - 162]^+$) and 304 ($[M + H - 162 - 18]^+$) in its positive-ion electrospray spectrum, indicating an altered chromophore and an aglycon of molecular mass 14 amu less than **2**. In the CID spectrum of the aglycon ion $[A + H]^+$ of compound **3** (*m*/*z* = 322), the fragmentation pattern has changed significantly (see Figure 1). Some peaks, including the base peak, are shifted by 14 amu towards lower masses, whereas others have disappeared. HR ESI TOF MS measurements on *m*/*z* = 484 ($[M + H]^+$) determined an elemental composition of $C_{25}H_{42}NO_8$. No signal for the carbonyl carbon atom at C-8 was observed in the ^{13}C NMR spectrum, while a new absorption at δ = 27.6, characteristic of a methylene carbon atom, appeared. With the aid of 2D NMR (HSQC, HMBC, COSY and NOESY) data it was possible to assign all signals from the saturated ring, and so the structure given in Scheme 1 could be derived unambiguously (see Table 1). The protons at C-8 form a multiplet signal at δ = 2.599. The coupling constants of the axial proton at C-6 indicate that the orientation of 5-H must be equatorial, as in **2**. Similarly to compound **2**, signal splitting was observed for a number of signals at the end of the side chain. Integration of the methyl signals and assignment as described above indicated an (*R*)/(*S*) ratio of 1.3:1.

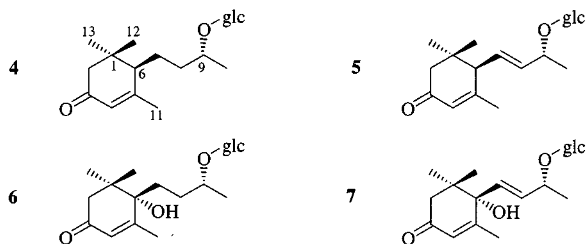
The molecular masses of the megastigmane glucosides **4−7** (see Scheme 2) were derived by positive-ion ESI MS. The identification of **5** and **6** was based on ^1H NMR spectroscopic data, while for **4** and **7** HSQC and HMBC spectra were also recorded. In the case of compound **6**, addition of C_6D_6 to the CD_3OD solvent was necessary to separate the 4-H, 7-H and 8-H signals and hence to establish the (*Z*) conformation of the C-7−C-8 bond.

The determination of the absolute configuration of megastigmane glucosides is usually achieved by cleavage of the glucose and comparison of the rotational strengths of the aglycons with those of synthetic samples. This procedure was not favoured in our case, due to the small sample quantities.

Table 1. ^{1}H and ^{13}C NMR spectroscopic data for compounds **2** and **3** in CD_3OD, 500 MHz and 125.7 MHz, respectively, values for (17*S*) form in parentheses (values in *italics* are chemical shifts of HSQC correlation peaks)

Pos.		**2** (17*R*) [(17S)]			**3** (17*R*) [(17S)]	
	δ (^{13}C)	δ (^{1}H)	mult, *J* [Hz]	δ (^{13}C)	δ (^{1}H)	mult, *J* [Hz]
2	143.1			140.1		
3	148.9			145.7		
4	174.3			174.5		
5	31.9	3.188	br. s	32.3	2.899	br. s
6	25.2	2.213	dddd, 14.5/4.5/2.4/2.4	25.8	1.908	br. d, 13.0
		2.085	dddd, 14.5/14.5/4.5/4.5		1.519	dddd, 13.0/13.0/4.0/4.0
7	33.4	2.539	ddd, 18.2/4.5/2.4	18.0	1.820	m
		2.877	ddd, 18.2/14.5/4.5		1.754	m
8	195.1			27.6	2.599	m
9	134.9			129.5		
10	139.8			144.5		
2-Me	13.7	2.387	s	13.5	2.285	s
3-OMe	59.9	3.827	s	60.1	3.749	s
11	31.8	*1.52*, *1.69*	m	33.7	*1.71*, *1.22*	
12−14	29.4, 26.5, 25.3	*1.40−1.60*	m	28.6, 26.6, 26.3	*1.40−1.60*	
15	30.7	*1.38*, *1.28*		30.9	*1.20−1.40*	
16	38.4 [37.6]	*1.45*, *1.61*		38.4 [37.6]	*1.40*, *1.62*	
17	75.6 [77.6]	3.880	ddd, 6.0/6.0/6.0	75.6 [77.5]	3.877 (3.814)	
18	19.8 [22.0]	1.162 (1.223)[a]	d, 6.2	19.8 [21.9]	1.160 (1.221)[b]	d, 6.0
1′	102.2 [103.9]	4.321	d, 7.8	102.1 [103.8]	4.320 (4.324)	d, 7.8
2′	75.1 [75.3]	3.137 (3.144)	dd, 7.8/9.0	75.1	3.139	dd, 9.0/7.8
3′	78.1 [78.1]	3.346 (3.338)	dd, 9.0/9.0	78.1[a]	3.346	dd, 9.0/9.0
4′	71.8	3.282	dd, 9.0/9.0	71.8	3.278	dd, 9.0/9.0
5′	77.9	3.245	ddd, 9.0/5.5/2.5	77.8	3.24	m
6′	62.9	3.660,	dd, 12.0/2.5	62.8	3.845	dd, 12.5/2.0
		3.846	dd, 12.0/5.5		3.658	dd, 12.5/5.5

[a] Intensities: (17*R*): 2.2 H; (17*S*): 0.8 H. − [b] Intensities: (17*R*): 1.7 H; (17*S*): 1.3 H.



Scheme 2. Structures of compounds **4−7**

As described by Pabst et al.[11] CD spectra of megastigmanes are essentially determined by the configuration at C-6, with only negligible influence from the configuration at C-9. CD data from synthezised compounds are available for the aglycons of **5**,[11] **6** and **7**.[12] Although no CD data for a corresponding synthetic compound were available, the similarity of the CD spectra substantiates the assignment of the configuration at C-6 of **4**.[13] CD data from **4** and from previously isolated blumenyl C β-D-glucopyranoside are also in good agreement.[14] The configuration at C-9 was established by NMR, since general rules for alk-2-yl β-D-glucopyranosides[9,10] can be also applied to the megastigmane glucosides.[15,16]

The absolute configuration of **4** and **5** was therefore assigned as (6*R*,9*R*), thus belonging to the same stereochemical series as compounds **6** and **7**, although the formal descriptor for these is (6*S*,9*R*). Compounds **4**,[14−16] **5**,[11,16] **6**[14,17] and **7**[17−20] represent known constituents, but this is the first report of co-occurrence of these compounds in a higher plant. It is noteworthy that in the symbiotic system *Hordeum vulgare*/*Glomus intraradices* the biosynthetic pathway from blumenol C to blumenin-9-yl 2′-*O*-β-glucuronosyl-β-D-glucopyranoside has been shown to proceed by the glyceraldehyde 3-phosphate/pyruvate pathway, rather than the mevalonate pathway.[21]

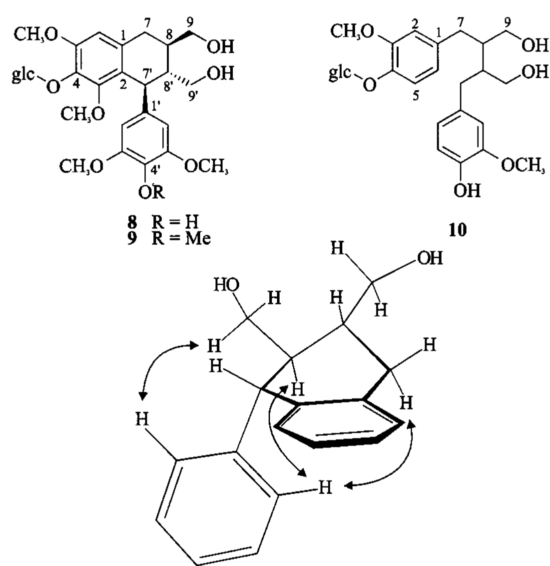
Compounds **8** and **9** were readily identified as glucosylated (+)-lyoniresinol derivatives by their ^{1}H and 2D NMR (see Table 2), MS and CD data.[22−25] From the chemical shift of the anomeric protons (δ = 4.900), together with NOESY and HMBC correlations, however, the position of the glucose was established as at C-4, a previously unknown substitution pattern. Assignment of all NMR signals and HR EIMS data for the aglycon peaks indicated the structures of (+)-lyoniresin-4-yl β-D-glucopyranoside (**8**) and (+)-4′-*O*-methyllyoniresin-4-yl β-D-glucopyranoside (**9**) (see Scheme 3). An artefact as the origin of **9** seems unlikely, as lingueresinol has the same 3′,4′,5′-trimethoxy substitution pattern, the hydroxy groups at C-3 and C-4 still remaining after MeOH extraction.[26]

In **9**, a vicinal coupling constant of 12 Hz between 7-H_{ax} and 8-H indicated an axial position for 8-H and a dihedral angle of ca. 180°. Homonuclear decoupling with irradiation of 9′-H allowed the 8-H/8′-H and 7′-H/8′-H vicinal coupling constants to be determined as 8.5 and 5.5 Hz, respectively. According to the Karplus curve, these coupling constants correspond to dihedral angles of about 150°(8-H/8′-

Table 2. ^{1}H (500 MHz) and ^{13}C NMR spectroscopic data for compounds **8−10** in CD_3OD; ^{13}C NMR values derived from HMQC and HMBC spectra; values in *italics* are chemical shifts of HSQC correlation peaks

Pos.	**8** δ (^{13}C)	δ (^{1}H)	mult., J [Hz]	**9** δ (^{13}C)	δ (^{1}H)	mult., J [Hz]	**10** δ (^{13}C)	δ (^{1}H)	mult., J [Hz]
1	136.6			136.3			137.0		
2	126.7			126.3			113.6	6.588	d, 1.8
3	153.0			153.1			150.5		
4	138.6			138.4			146.0		
5	153.0			153.1			117.6	7.012	d, 8.0
6	109.3	6.696	s	109.2	6.698	s	122.8	6.630	dd, 8.0/1.8
7_{ax}	33.8	2.598	dd, 15.0/11.5	33.6	2.608	dd, 15.0/12.0	32.2	2.546	dd, 13.5/8.0
7_{eq}	-	2.745	dd, 15.0/5.0		2.754	dd, 15.0/4.5		2.660	dd, 13.5/7.0
8	40.6	1.618	m, $w_{1/2}$ = 22	40.4	1.629	m	45.0	1.880	
9A	66.6	3.586	dd, 10.5/5.0	66.3	*3.58*	m	62.0	3.562	dd, 11.0/5.5[a]
9B		*3.47*	m		*3.50*	m		3.584	dd, 11.0/5.5[a]
3-OMe	61.6	3.424	s	61.4	3.446	s	56.2	3.756	s
5-OMe	56.9	3.863	s	56.8	3.863	s			
1′	138.8			144.4			133.5		
2′	106.7	6.371	s	106.6			114.2[a]	6.679	d, 1.8
3′	149.0			154.2			149		
4′	134.4			137.4			145.5		
5′	149.0			154.2			115.8	6.651	d, 8.0
6′	106.7	6.371	s	106.6			122.6	6.526	dd, 8.0/1.8
7′	42.2	4.346	d, 5.5	42.4	4.369	d, 5.5	32.2	2.586	dd, 13.5/8.0
								2.706	dd, 13.5/7.0
8′	48.8	2.000	m, $w_{1/2}$ = 16	48.5	1.995	m[b]	45.0	1.922	m
9′	64.0	*3.48*, *3.47*	m	63.7	*3.50*, *3.41*	m	62.0	3.584	dd, 11.0/5.5[a]
								3.608	dd, 11.0/5.5[a]
3′,5′-OMe	56.8	3.737	s	46.6	3.731	s	56.0[c]	3.736[c]	
1′′	105.2	4.900	d, 8.0	104.7	4.900	d, 7.5	103.2	4.829	d, 7.5
2′′	75.6	*3.42*		75.6	*3.42*		74.5	*3.46*	
3′′	78.2	*3.38*		77.8	*3.38*		77.5	*3.45*	
4′′	71.3	*3.37*		71.1	*3.38*		71.2	*3.38*	
5′′	78.5	3.162	m	78.1	3.152	m	78.0	*3.39*	
6′′A	62.3	3.698	dd, 12.0/2.5	62.3	3.683	dd, 12.0/2.5	62.5	*3.86*, *3.57*	
6′′B		3.622	dd, 12.0/5.0		3.613	dd, 12.0/5.0			

[a] Assignments may be interchanged. − [b] Coupling constants achieved by a homodecoupling experiment with 9′-H: dd 8.5/5.5. − [c] 3′-OMe only.



Scheme 3. Structures of compounds **8−10** and calculated conformation of compound **9**, based on analysis of coupling constants and observed NOEs (substituents on the aromatic rings are omitted for clarity)

H) and 130° (8′-H/7′-H). These angle conditions cannot be fulfilled in either the half-chair or half-boat conformations described in,[23] but result from an envelope conformation, with flap at C-8 and a C-8−C-8′ bond of ca 40° (see Scheme 3) out of the ring A plane, as found by X-ray analysis in 2′-bromophyllotoxin.[27] This conformation is also supported by NOEs between 2′/6′-H and 7-H_{ax} (impossible in the half-chair conformation), 8′-H and 9′-H; no NOEs are observed between 7-H_{ax} and 8′-H (expected for half-chair) or between 2′/6′-H and 9-H (expected for half-boat).

Two sets of signals indicative of 1,3,4-trisubstituted aromatic rings appear in the ^{1}H NMR spectrum of **10**, together with β-D-glucopyranosyl signals, two sets of CH_2 signals (each integrating to 4 H) and one CH signal (integrating to 2 H, see Table 2). HMBC correlations are observed between 5-H and C-1/C-3/C-4, 5′-H and C-1′/C-3′/C-4′, 3-OCH_3 and C-3 and, lastly, between 3′-OCH_3 and C-3′. Unfortunately, because of the small quantity of **10** (0.2 mg) and the resulting low signal-to-noise ratio in the HMBC spectrum, no correlation of the anomeric proton with an aromatic carbon signal could be observed. However, as indicated by the chemical shift of the anomeric proton and the observed

NOEs between 3-OMe and 2-H and between 3′-OMe and 2′-H, the glucose has to be attached to C-4. The EIMS exhibits an aglycon peak at $m/z = 362$, revealing an elemental composition of $C_{20}H_{26}O_6$ in the HR mode. This corresponds to eight double bond equivalents, which are already present in the two aromatic rings. With the aid of these data, **10** was assigned as secoisolariciresin-4-yl β-D-glucopyranoside. NMR spectroscopic data fit with values given both for the glucose-containing moiety[28] (assignments not accurate) and for the nonglucosylated moiety.[29] Until now this compound had only been identified in root extracts of *Urtica dioica* (Urticaceae) by GC MS of the trimethylsilyl ether.[30] A trimethylsilylated sample of **10** gave a mass spectrum with a fragmentation pattern comparable to that given in ref.[30]

Conclusions

This paper describes the isolation and structural elucidation of the alkaloidal glucosides (17*RS*)-17-(β-D-glucopyranosyloxy)antidesmone (**2**) and (17*RS*)-8-deoxo-17-(β-D-glucopyranosyloxy)antidesmone (**3**), the megastigmane glucosides blumenyl C β-D-glucopyranoside (**4**), 3-oxo-α-ionyl β-D-glucopyranoside (**5**), blumenyl B β-D-glucopyranoside (**6**) and blumenyl A β-D-glucopyranoside (roseoside, **7**) and the lignan glucosides (+)-lyoniresin-4-yl glucopyranoside (**8**), (+)-4′-methoxylyoniresin-4-yl glucopyranoside (**9**) and secoisolariciresin-4-yl glucopyranoside (**10**).

The presence of the antidesmone glucosides **2** and **3** suggests a biological function for antidesmone, possibly acting in glucosylic storage or as a transportation form. Hydroxylation at C-17, although regiospecific, does not seem to be stereospecific, raising questions concerning the enzymes involved. The obtained (*R*)/(*S*) ratios may have become slightly altered during the isolation process, however, since the diastereomers have different chromatographic properties.

Compound (17*R*)-**3** differs from melochininyl β-D-glucopyranoside, a pyridone alkaloid with a hydroxydodecyl side chain, reported from *Melochia pyramidata* (Sterculiaceae),[31] in the presence of the cycle-forming C-5−C-10 bond. The absolute configuration of the megastigmane glucosides **4**, **5**, **6** and **7** could be determined using CD and NMR spectroscopic data and comparison with literature data, a fast and simple method that avoids cleavage of the glucoside. The lignan glucosides **8** and **9** are described here for the first time. Secoisolariciresin-4-yl glucopyranoside (**10**) has been isolated for the first time and fully characterised by NMR. While a number of 9-*O*- or 9′-*O*-glycosylated lyoniresinols and secoisolariciresinols are known, 4-*O*-glucosylation is seldom observed.[30,32]

Antidesma membranaceum has been shown to be a rich source both of new structures and of known compound types with unusual substitution patterns.

Experimental Section

General Methods: NMR: ^{1}H and 2D Varian Unity 500, 499.87 MHz, ^{13}C Varian Unity 500, 125.7 MHz, chemical shifts were referenced to internal TMS (δ = 0, ^{1}H) and CD_3OD (δ = 49.0, ^{13}C), respectively. − 70 eV EIMS and HR EIMS (resolution ca. 7.500): AMD 402 (AMD Intectra); ESIMS and LC MS/MS: Finnigan TSQ 7000, capillary temperature: 220 °C, ESI positive-ion mode: spray voltage 4.5 kV, collision energy −40 eV, CID pressure 1.8 mT, collision gas: Ar; ESI negative-ion mode: spray voltage 4.0 kV, LC-Tech Ultra Plus pumps, Linear UVIS 200 detector, Sepserve Ultrasep ES RP-18 5μm 1×100 mm column, flow 70μL min^{-1}. − CD and UV: Jasco J-710; $[\alpha]_D$: Jasco DIP-1000. − Gas chromatography/mass spectrometry: Thermo Quest Voyager: EI (70 eV), source temp. 200 °C, column DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), inj. temp. 250 °C, interface temp. 300 °C, carrier gas He, flow rate 1.2 mL min^{-1}, splitless injection; column temp. program: 170 °C for 1 min, then raised to 270 °C at a rate of 25 °C min^{-1} and then elevated to 290°C at a rate of 2 °C min^{-1}. Trimethylsilylated agent: MSTFA (*N*-methyl-*N*-trimethylsilyl-trifluoroacetamide). − Preparative HPLC: Merck−Hitachi L-6250 low-pressure gradient pump, L-4250 UV Detector, Merck Hibar 25×125 mm column, LiChrosorb RP-18, 7μm.

Plant Material: *A. membranaceum* was collected on a Frontier Expedition by the Society for Environmental Exploration, London, in August 1994 in Margrotto Hill, East Usambaras, Murenga District, Tanga Region, Tanzania. It was identified by Mr. Leonard Mwasumbi, Herbarium, Department of Botany, University of Dar es Salaam, Tanzania. The voucher specimen number, Mwasumbi NO 17130, is deposited at the Herbarium, University of Dar es Salaam, Tanzania.

Extraction and Purification: Extraction and isolation was carried out at room temperature (23 °C) in an air-conditioned laboratory. − The dried plant materials − leaves (1391 g) and bark (1541 g) − were extracted separately with 80% MeOH (leaves: 24 L; bark: 30 L; five portions each). The extracts were concentrated in vacuo to the aq. phases and successively extracted with *n*-hexane (2.5 L; 2.3 L), EtOAc (2.5 L; 2.5 L) and 1-butanol (1.9 L; 2.0 L). The organic phases were concentrated in vacuo to yield the dry extracts (1-butanol extract: leaves: 75.04 g; bark: 14.98 g). − A portion of the 1-butanol extract of the leaves (31.4 g) was partitioned using $CHCl_3/MeOH/H_2O$ (43:37:20 v/v/v); the organic layer, after concentration in vacuum, gave 3.306 g of a less polar extract, which was subjected to column chromatography on Sephadex LH-20 (100 g, 5 × 25 cm, MeOH) to yield ten fractions. Fraction I (660 mg) was dissolved in 100 mL of EtOAc and 50 mL of 1-butanol; this solution was extracted successively with 0.2 N, 1 N and 2 N aq. HCl (100 mL each), and the aq. solution was neutralised with K_2CO_3 and extracted with $CHCl_3/MeOH$ (43:37 v/v). Concentration of the organic layer gave 268 mg of residue. Repeated preparative HPLC (flow: 10 mL min^{-1}; gradient: 20% MeOH in 30 min to 50% MeOH, or adapted gradients within this solvent range for final purification; detection: 254 nm) on this fraction and on fractions II, III and IV of the LH-20 column yielded compounds **7** (0.8 mg), **6** (0.8 mg), **5** (2.0 mg), **4** (8.6 mg) and **2** (4.3 mg) (in order of elution from the RP-18 column). − The 1-butanol bark extract was dissolved in 100 mL of EtOAc and 25 mL of 1-butanol and extracted with 0.2 N, 1 N and 2 N aq. HCl. The aq. layer was neutralised with K_2CO_3 and extracted with $CHCl_3/MeOH$ (21.5:18.5 v/v). The residue left from the aq. layer was extracted with MeOH and yielded, after repeated preparative HPLC (15 mL min^{-1}; gradient: 30% MeOH in 25 min to 50% MeOH, then in 25 min to 100% MeOH, or adapted gradients within this solvent range for final purification; detection: 254 nm), compounds **8** (1.7 mg), **10** (0.2 mg), **9** (1.2 mg) and **3** (2.4 mg) (in order of elution).

(17*RS*)-17-(β-D-Glucopyranosyloxy)antidesmone (2): $[\alpha]_D^{23.5}$ = 0.2 (*c* = 0.16, MeOH). − UV: λ_{max}^{MeOH} (log ε) = 248 (4.10), 276 (3.31), 328 nm (3.49). − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 242 (3.41), 285 (0.95), 318 (1.12), 356 nm (−1.71). − ^{1}H, ^{13}C NMR: see Table 1. − ESI MS (pos.-ion mode): *m*/*z* (%) = 498 (20) [M + H]⁺, 336 (100) [aglycon + H]⁺. − ESI TOF MS: *m*/*z* = 498.2654 (calcd. for $C_{25}H_{40}NO_9$ 498.2698), 336.2181 (calcd. for $C_{19}H_{30}NO_4$ 336.2169).

(17*RS*)-8-Deoxo-17-(β-D-glucopyranosyloxy)antidesmone (3): $[\alpha]_D^{23.9}$ = −49.1 (*c* = 0.13, MeOH). − UV: λ_{max}^{MeOH} (log ε) = 203 (4.22), 219 (4.12), 231 (4.11), 275 nm (3.90). − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 225 (−2.48), 259 (−3.31), 283 nm (1.78). − ^{1}H, ^{13}C NMR: see Table 1. − ESI MS (pos.-ion mode): *m*/*z* (%) = 484 (26) [M + H]⁺, 322 (100) [aglycon + H]⁺. − ESI TOF MS: *m*/*z* = 484.2917 (calcd. for $C_{25}H_{42}NO_8$ 484.2905), 322.2381 (calcd. for $C_{19}H_{32}NO_3$ 322.2377).

Blumenyl C β-D-Glucopyranoside (4): $[\alpha]_D^{26.4}$ = 0.2 (*c* = 0.41, MeOH). − UV: λ_{max}^{MeOH} = 238 nm. − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 216 (3.74), 235 (4.28), 333 nm (0.99). − ESI MS (pos.-ion mode, syringe-pump injection): *m*/*z* (%) = 395 (100) [M + Na]⁺, 211 (22) [aglycon + H]⁺. − ^{1}H NMR (CD_3OD, 500 MHz): δ = 5.801 (s, 4-H), 4.324 (d, 8.0, 1′-H), 3.877 (q, 6.0, 9-H), 3.853 (dd, 11.5/2.0, 6′-H_A), 3.642 (dd, 11.5, 5.0, 6′-H_B), 3.348 (dd, 9.0/9.0, 3′-H), 3.284 (dd, 9.0/9.0, 4′-H), 3.250 (m, 5′-H), 3.137 (dd, 9.0/8.0, 2′-H), 2.461 (d, 17.5, 2-H_A), 2.047 (d, 1.0, 13-H), *1.98* (6-H), 1.971 (d, 17.5, 2-H_B), *1.96* (7-H_A), 1.635 (m, 8-H), 1.400 (m, 7-H_B), 1.181 (d, 6.5 10-H), 1.089 (s, 11-H), 1.007 (s, 12-H). − ^{13}C NMR (CD_3OD, derived from 500 MHz HMQC and HMBC): δ = 202.5 (C-3), 170.1 (C-5), 125.4 (C-4), 102.5 (C-1′), 77.9 (C-5′), 78.1 (C-3′), 75.5 (C-9), 75.1 (C-2′), 71.8 (C-4′), 62.9 (C-6′), 52.4 (C-6), 48.0 (C-2), 37.8 (C-8), 37.7 (C-1), 29.0 (C-12), 27.4 (C-11), 26.7 (C-7), 24.9 (C-13), 19.9 (C-10).

3-Oxo-α-ionyl β-D-Glucopyranoside (5): $[\alpha]_D^{26.4}$ = 107.7 (*c* = 0.04, MeOH). − UV: λ_{max}^{MeOH} = 240 nm. − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 203 (−1.77), 243 (14.80), 318 nm (−0.66). − ESI MS (pos.-ion mode, syringe-pump injection): *m*/*z* = 393 [M + Na]⁺. − ^{1}H NMR (CD_3OD, 500 MHz): δ = 5.877 (s, 4-H), 5.774 (dd, 15.5/6.5, 8-H), 5.642 (ddd, 15.5/9.0/1.0, 7-H), 4.397 (ddd, 6.5/6.5/1.0, 9-H), 4.350 (d, 8.0, 1′-H), 3.818 (dd, 12.0/2.5, 6′-H_A), 3.654 (dd, 12.0/5.5, 6′-H_B), 3.338 (dd, 9.0/9.0, 3′-H), 3.284 (dd, 9.0/9.0, 4′-H), 3.209 (ddd, 9.0/5.5/2.5, 5′-H), 3.169 (dd, 9.0/8.0, 2′-H), 2.674 (d, 9.0, 6-H), 2.430 (d, 16.5, 2-H_A), 2.043 (d, 16.5, 2-H_B), 1.934 (d, 1.0, 13-H), 1.287 (s, 10-H), 1.028 (s, 11-H), 1.002 (s, 12-H).

Blumenyl B β-D-Glucopyranoside (6): $[\alpha]_D^{26.4}$ = 0.2 (*c* = 0.04, MeOH). − UV: λ_{max}^{MeOH} = 242 nm. − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 221 (5.65), 252 (−3.11), 328 nm (0.73). − ESI MS (pos.-ion mode, syringe-pump injection): *m*/*z* = 411 [M + Na]⁺. − ^{1}H NMR (CD_3OD, 500 MHz): δ = 5.828 (s, 4-H), 4.380 (m, 9-H), 4.305 (d, 8.0, 1′-H), 3.854 (dd, 12.0/2.0, 6′-H_A), 3.654 (dd, 12.0/5.5, 6′-H_B), 3.341 (dd, 9.0/9.0, 3′-H), 3.278 (dd, 9.0/9.0, 4′-H), 3.244 (dd, 2.0/5.5, 5′-H), 3.129 (dd, 9.0/8.0, 2′-H), 2.610 (d, 18.0, 2-H_A), 2.144 (d, 18.0, 2-H_B), 2.035 (d, 1.0, 13-H), 1.830 (m, 7-H_A), 1.804 (m, 7-H_B), 1.753 (dddd, 11.0/5.0/5.0/2.0, 8-H_A), 1.484 (m, 8-H_B), 1.172 (d, 6.0, 10-H), 1.095 (s, 11-H), 1.014 (s, 12-H).

Blumenyl A β-D-Glucopyranoside (7): $[\alpha]_D^{26.4}$ = 49.1 (*c* = 0.025, MeOH). − UV: λ_{max}^{MeOH} = 235 nm. − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 239 (8.87), 321 nm (−0.46). − ESI MS (pos.-ion mode, syringe-pump injection): *m*/*z* = 409 [M + Na]⁺. − ^{1}H NMR (CD_3OD, 500 MHz): δ = *5.86* (m, 7-H, 8-H, 4-H), 4.425(d, 7.0, 6.5, 9-H), 4.338 (d, 8.0, 1′-H), 3.846 (dd, 12.0/2.0, 6′-H_A), 3.623 (dd, 12.0/6.0, 6′-H_B), 3.346 (dd, 9.0/6.5, 3′-H), *3.23* (4′-H), *3.21* (5′-H), 3.166 (dd, 9.0/8.0, 2′-H), 2.515 (d, 17.0, 2-H_A), 2.147 (d, 17.0, 2-H_B), 1.917 (s, 13-H), 1.286 (d, 6.5, 10-H), 1.036 (s, 11-H), 1.029 (s, 12-H). − ^{1}H NMR (C_6D_6 + CD_3OD, 500 MHz): δ = 6.019 (dd, 16.0/7.0, 8-H), 5.932 (d, 2.0, 4-H), 5.784 (d, 16.0, 7-H)). − ^{13}C NMR (CD_3OD, derived from 500 MHz HMQC and HMBC): δ = 201.3 (C-3), 167.2 (C-5), 134.9 (C-7), 131.4 (C-8), 127.1 (C-4), 102.5 (C-1′), 79.9 (C-6), 77.9 (C-3′), 77.8 (C-5′), 77.0 (C-9), 75.1 (2′), 71.3 (C-4′), 62.3 (C-6′), 50.5 (C-2), 42.2 (C-1), 24.4 (C-13), 23.0 (C-12), 20.8 (C-10), 19.2 (C-11).

(+)-Lyoniresin-4-yl β-D-Glucopyranoside (8): UV: λ_{max}^{MeOH} (log ε) = 205 (4.84), 275 nm (3.50). − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 203 (12.8), 214 (−17.6), 239 (6.9), 271 (2.9), 2.86 nm (−1.1). − ESI MS (neg.-ion mode, syringe-pump injection): *m*/*z* (%) = 581 (44) [M − H]⁻, 419 (100) [aglycon − H]⁻. − HR EI MS: *m*/*z* (%) = 582.2327 (0.93) [$C_{28}H_{38}O_{13}$, calcd. 582.2312], 420.1812 (100) [$C_{22}H_{28}O_8$, calcd. 420.1784], 205 (24), 183 (33), 167 (32). − ^{1}H, ^{13}C NMR: see Table 2.

(+)-4′-*O*-Methyllyoniresin-4-yl β-D-Glucopyranoside (9): UV: λ_{max}^{MeOH} (log ε) = 205 (4.63), 273 nm (3.18). − CD: λ_{max}^{MeOH} (Δε [cm² mmol⁻¹]) = 203 (15.0), 216 (−11.1), 239 (4.8), 268 nm (1.2). − ESI MS (neg.-ion mode, syringe-pump injection): *m*/*z* (%) = 595 (60) [M − H]⁻, 433 (100) [aglycon − H]⁻. − HR EI MS: *m*/*z* (%) = 434.1928 (94) [$C_{23}H_{30}O_8$, calcd. 434.1941], 217 (18), 205 (23), 198 (41), 197 (49), 181 (27). − ^{1}H, ^{13}C NMR: see Table 2.

Secoisolariciresin-4-yl β-D-Glucopyranoside (10): ESI MS (neg.-ion mode, syringe-pump injection): *m*/*z* (%) = 523 (100) [M − H]⁻, 361 (78) [aglycon − H]⁻. − HR EI MS: *m*/*z* (%) = 362.1786 (31) [$C_{20}H_{26}O_6$, calcd. 362.1729], 344 (10), 206 (8), 189 (12), 37 (100). − GC EI MS (trimethylsilylated sample): Kovats retention index = 3984; *m*/*z* (%) = 923 (0.5), 833 (0.8), 743 (1.2), 650 (7), 560 (13), 450 (22), 361 (31), 217 (34), 209 (40), 147 (28), 129 (20), 103 (24), 73 (100). − ^{1}H, ^{13}C NMR: see Table 2.

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