Structural Investigation of Resin Glycosides from *Ipomoea lonchophylla*

John K. MacLeod* and Annemarie Ward

Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia

Peter B. Oelrichs

National Research Centre for Environmental Toxicology, Brisbane, Queensland 4108, Australia

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A fraction from *Ipomoea lonchophylla*, which was toxic to mice, contained an inseparable mixture of resin glycosides with differing numbers of C_5 ester groups on the hexasaccharide chain. After alkaline hydrolysis of the esters, the structure of the major component (**1**) was elucidated using high-field NMR spectroscopy, mass spectrometry, chemical studies, and comparison with known resin glycosides. Compound **1** was identified as 3,11-dihydroxytetradecanoic acid 11-*O*-*β*-quinovopyranosyl-(1→2)-*β*-glucopyranosyl-(1→3)-[α-rhamnopyranosyl-(1→4)]-quinovopyranosyl-(1→2)-*β*-glucopyranosyl-(1→2)-*β*-fucopyranoside.

The "dumb lamb syndrome" occurs in the Longreach district of Central Queensland and causes high mortality rates (∼20%) among newly born lambs. It is believed that this disorder occurs during gestation when ewes feed on particular plant species.[1] Two species that have been implicated are *Abelmoschus ficulneus* (L.) Wight (native rosella) and *Ipomoea lonchophylla* J. Black (Convolvulaceae) (cowvine). During a preliminary screening, no toxic extract from *A. ficulneus* was found, but one fraction from *I. lonchophylla* was toxic to mice and is the subject of the present investigation. No tests have so far been carried out to determine whether this toxic extract contributes to "dumb lamb syndrome".

Many species of *Ipomoea* were and often still are used in folk medicine in different parts of the world,[2−5] especially as purgatives.[6] Pharmacological studies on extracts of these plants have reported antimicrobial, analgesic, spasmogenic, spasmolytic, hypotensive, psychotomimetic, and anticancer effects.[7] Chemical investigations have shown that indole alkaloids[8] and resin glycosides[9] are the most common biologically active constituents in the Convolvulaceae. Resin glycosides of the jalapin class are composed of monohydroxy and dihydroxy C_{14} and C_{16} fatty acids glycosidically linked to oligosaccharide chains usually containing four to six sugar units with an ester linkage between the fatty acid and the oligosaccharide chain to form a macrocyclic lactone. The sugars are esterified with varying numbers of lower acid moieties, most commonly 2-methylbutanoic acid and 3-hydroxy-2-methylbutanoic acid.[10]

The dried *I. lonchophylla* plant was extracted using aqueous MeOH. The extract was concentrated, and the residue was taken up in H_2O and re-extracted with *n*-BuOH. Purification of the concentrate from the butanol extract was achieved by size-exclusion chromatography. Using toxicity testing to follow the fractionation, a colorless solid was obtained, which was identified as a mixture of resin glycosides from MS and NMR analyses. The negative FABMS showed that a mixture compounds with of high molecular weight was present with $[M - 1]^-$ ions at *m*/*z* 1651 (minor), 1551 (major), 1451 (major), and 1351 (minor). Fragment ions at *m*/*z* 1533, 1433, and 1333 were due to the loss of H_2O from the corresponding $[M - 1]^-$ ions. In the positive FABMS, corresponding $[M + 2\ Na - H]^+$ ions were present at *m*/*z* 1697, 1597, 1497, and 1397, together with weaker $[M + Na + K - H]^+$ ions 16 mass units higher. A differing number of 3-hydroxy-2-methylbutanoate groups on the sugars would account for the 100 mass units of difference between the molecular ion species in the FABMS.

The mixture of the resin glycosides was subjected to reversed-phase HPLC but could not be effectively separated into individual pure compounds. In order to identify the individual components that made up the glycosidic and aglycon moieties, the resin glycoside mixture was hydrolyzed with 2 M HCl at 90 °C. The Et_2O extract of the hydrolysate was silylated, and GC−MS of the product showed a major component that had a mass spectrum corresponding to that of the *tris*-trimethylsilyl (TMS) derivative of 3,11-dihydroxytetradecanoic acid, containing diagnostic α-cleavage ions at *m*/*z* 145, 233, and 433.[11] A minor component was identified as the TMS derivative of 3,11-dihydroxyhexadecanoic acid. The smaller C_5 acids on the sugars were not observed, as their TMS derivatives coeluted with excess silylating reagent.

The aqueous phase of the acid hydrolysate was lyophilized and silylated. GC−MS analysis of the silylated residue showed the presence of the TMS ethers of glucose (glc) and the 6-deoxyhexoses quinovose (qui), fucose (fuc), and rhamnose (rha) in the approximate ratio of 2:2:1:1. Authentic samples of these four sugars were subjected to the same conditions both individually and as calibrated mixtures to confirm the assignments and ratio. It was assumed that the sugars were present in their natural forms, that is, D-glucose, D-quinovose, D-fucose, and L-rhamnose.

A sample of the resin glycoside mixture was then subjected to alkaline hydrolysis with a 1.6 M NaOH solution, and the resulting mixture was neutralized with 4 M HCl. An aliquot was acidified to pH 1, extracted with Et_2O, and the Et_2O extract methylated with CH_2N_2. By GC−MS, the mass spectra and retention times for the two major peaks corresponded to those of the methyl esters of authentic samples of 3-hydroxy-2-

* To whom correspondence should be addressed. Phone: 61-6-249 3762. FAX: 61-6-249 5995. E-mail: macleod@rsc.anu.edu.au.

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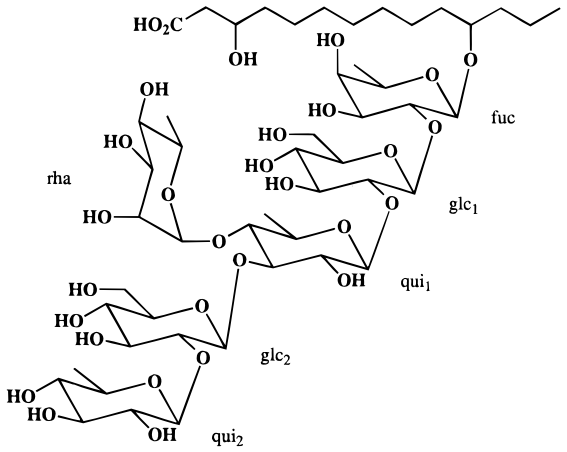
methylbutanoic acid (HMBA) and 2-methylbutanoic acid (MBA). This supported the above proposal that differences of 100 mass units observed for the high mass ions (*m/z* 1651, 1551, 1451, 1351) in the negative FABMS of the original toxic extract could be attributed to the presence of differing numbers of HMBA ester groups.

The neutral aqueous phase from the alkaline hydrolysis was lyophilized, desalted, and purified by reversed-phase HPLC to give a colorless solid (**1**) as the major component. The negative FABMS of **1** showed major ions at *m/z* 1167 $[M - 1]^-$, 1021, 859, 567, 405, and 259, the presence of which enabled the sequence of the oligosaccharide chain to be determined.



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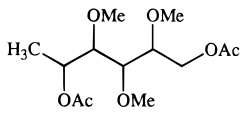
The $[M - 1]^-$ ion for compound **1** at *m/z* 1167 accounted for the presence of one 3,11-dihydroxytetradecanoic acid moiety glycosidically linked to a hexasaccharide consisting of four deoxyhexose (2 × qui, 1 × rha, and 1 × fuc) and two hexose (glc) units, as had been determined by GC−MS. It could also be concluded that the oligosaccharide was bonded to one of the hydroxyl groups and not the carboxylate group of the dihydroxy fatty acid because the linkage had survived alkaline hydrolysis. The fragment ion at *m/z* 1021 represented a mass difference of 146 from the $[M - 1]^-$ ion at *m/z* 1167 and indicated the loss of a terminal deoxyhexose. The major fragment ion at *m/z* 859, 162 mass units lower, represented cleavage at the next sugar in the sequence, a glucose unit. The next most intense ion at *m/z* 567 was 292 mass units lower and represented the loss of two deoxyhexose units. This would be expected if one of these two deoxyhexose sugars was branched. The fragment ion at *m/z* 405 represented a mass difference of 162, equivalent to loss of a glucosyl unit from the ion at *m/z* 567. A further loss of a deoxyhexose sugar gave the *m/z* 259 ion, corresponding to the anion of 3,11-dihydroxytetradecanoic acid, which must be directly attached to this sugar.

The alkaline-hydrolyzed resin glycoside (**1**) was permethylated (dimsyl potassium and MeI), acid hydrolyzed, and the organic extract silylated, in order to determine to which hydroxyl group on the 3,11-dihydroxytetradecanoic acid the oligosaccharide was linked. GC−MS of the silylation mixture showed a major component with intense α-cleavage ions in its mass spectrum at *m/z* 375 and 145, which could be rationalized in terms of a methoxy group at C-3 on the derivatized acid rather than the alternate compound methoxylated at C-11.[11] The glycosidic linkage at C-11 was subsequently confirmed by NMR studies (see below).

An adaptation[12] of the Lindberg procedure was used to determine the number and positions of linkage of each of the two hexose (glc) and four deoxyhexose (qui, fuc, rha) sugars. This involved permethylation of the alkaline-hydrolyzed resin glycoside (**1**) followed by acid hydrolysis, reduction, and acetylation. GC−MS of the product showed the presence of four major components that were identified by comparison with literature data[13] as 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol (**2**); 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methylhexitol (**3**); 1,3,4,5-tetra-*O*-acetyl-6-deoxy-2-*O*-methylhexitol (**4**), and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol (**5**). Taken in conjunction with the earlier negative FABMS sequence analysis, these results permitted the assignment of the positions of linkage between the individual sugar units.



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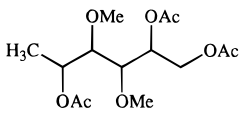

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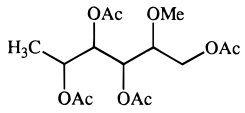

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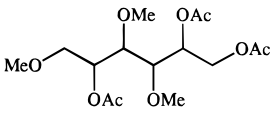

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Only one derivatized hexitol (**5**) was present, from which it could be concluded that both glucose units in the oligosaccharide chain must be 1,2-disubstituted. The relative intensity of the GC signal for this compound was consistent with it representing two glucose units. Because the diacetylated 6-deoxyhexitol (**2**) was the only alditol acetate that could be derived from a terminal sugar unit and because the tetra-*O*-acetyl deoxymethylhexitol (**4**) must be derived from a 1,3,4-trisubstituted deoxyhexose, this implied that there were two terminal deoxyhexose units, as previously suggested by the negative FABMS data. The remaining deoxyhexitol **3** corresponds to a 1,2-disubstituted deoxyhexose and must be the sugar directly linked to the dihydroxy fatty acid. It remained to identify the positions of the individual deoxyhexoses in the chain and the stereochemistry of the sugar linkages. For this, it was necessary to use high-field NMR spectroscopy.

^{1}H- and ^{13}C-NMR assignments for **1** are listed in Table 1. High-field NMR techniques ranging from ^{1}H- at 750 MHz and ^{13}C-NMR experiments to more complex 2D NMR experiments (DQF−COSY, HMQC, HMBC, TOCSY, and ROESY) enabled the assignments to be made. The proton assignments of the aglycon moiety were determined from ^{1}H-NMR and DQF−COSY spectra. The corresponding carbon assignments (Table 1) could then be determined from one-bond HMQC spectra.

Confirmation of the earlier mass spectrometric result that the glycoside was bonded through the hydroxyl group on C-11 and not that on C-3 came from a through-space connectivity observed in the ROESY spectrum (Figure 1) between the proton at 3.81 ppm (H-11 on the fatty acid) and the anomeric proton at 4.84 ppm on the

Table 1. NMR Assignments for the Alkaline Hydrolysis Product (**1**) of the Resin Glycoside Mixture (in pyridine d_5)

sugar	position	δ^1H[a]	δ^{13}C[b]
agl[c]	1		178.1
	2	2.80, br s	44.8
	3	4.40, m	68.9
	4	1.70	34.5
	11	3.81, m	80.2
	12	1.32, m	30.0
	13	1.50	19.0
	14	0.88, t (7.3)	14.5
fuc[c]	1	4.84, d (7.6)	102.2
	2	4.18, dd (7.6, 8.5)	80.3
	3	4.65, t (8.5)	78.1
	4	3.54	76.8
	5	3.82	72.4
	6	1.52, d (6.0)	18.5
glc_1[c]	1	5.76, d (7.6)	102.1
	2	4.27, dd (7.6, 8.2)	75.5[d]
	3	4.13, dd (8.2, 8.9)	79.3
	4	3.96, t (8.9)	72.4
	5	3.74	77.0
	6	4.15	63.2
		4.52, dd (2.0, 10.3)	
qui_1[c]	1	5.86, d (7.8)	102.4
	2	3.95, dd, (7.8, 8.9)	72.2
	3	4.28, dd (8.9, 8.2)	75.5[d]
	4	3.72, dd (8.2, 8.7)	76.8
	5	4.00, dd (8.7, 6.0)	77.15
	6	1.65, d (6.0)	18.7
rha[c]	1	6.41, s	100.0
	2	4.83, d	71.8
	3	5.30, dd (2.1, 9.4)	77.8
	4	4.81, dd (9.4, 9.5)	71.6
	5	5.20, dd (9.5, 6.2)	67.7
	6	1.95, d (6.2)	19.2
glc_2[c]	1	6.21, d (7.5)	100.7
	2	4.03, dd (7.5, 9.2)	72.2
	3	4.48, t (9.2)	76.0
	4	4.24, d (9.2)	77.5[d]
	5	4.07	77.0
	6	4.42	62.8
		4.70	
qui_2[c]	1	5.08, d (8.0)	104.8
	2	4.01, dd (8.0, 8.2)	77.2
	3	3.98, dd (8.2, 9.4)	84.4
	4	3.62, t (9.4)	76.5
	5	3.74	73.5
	6	1.60, d (5.9)	18.7

[a] Spectra recorded at 750 MHz (J values in Hz). [b] ^{13}C chemical shifts from HMQC (500 MHz). [c] Agl = aglycon, fuc = fucose, glc = glucose, qui = quinovose, rha = rhamnose. [d] Assignments may be interchanged.

attached fucosyl moiety. A correlation was also observed between C-11 of the fatty acid at 80.2 ppm and the fucosyl anomeric proton at 4.84 ppm in the HMBC spectrum (Figure 1). The absolute configurations at C-3 and C-11 were not determined, but previous work[6,11,14] has shown that in all resin glycosides investigated to date, both centers possess the *S*-configuration.

The ^{1}H-NMR assignments for the individual monosaccharides were determined with the aid of DQF−COSY and TOCSY spectra, and the corresponding carbon assignments were made with the assistance of the HMQC spectrum. The conformation of the sugars could be deduced from the chemical shifts and coupling constants for the anomeric protons of each of the sugars.[6,15−17] The assignments for each of the sugars were characteristic of β-fucopyranosyl, β-glucopyranosyl, β-quinovopyranosyl, and α-rhamnopyranosyl units by comparison with values reported for corresponding residues in oligosaccharides.[6,11,15−21] It is, however, unusual for the chemical shift of the anomeric proton

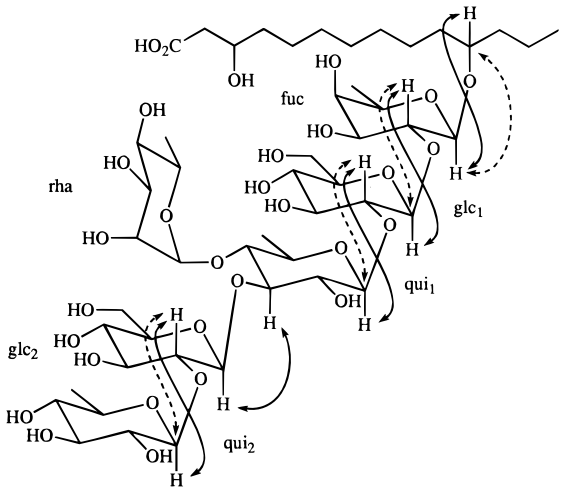

Figure 1. Key ROESY (—) and HMBC (- - -) correlations for establishing sugar linkages for **1**.

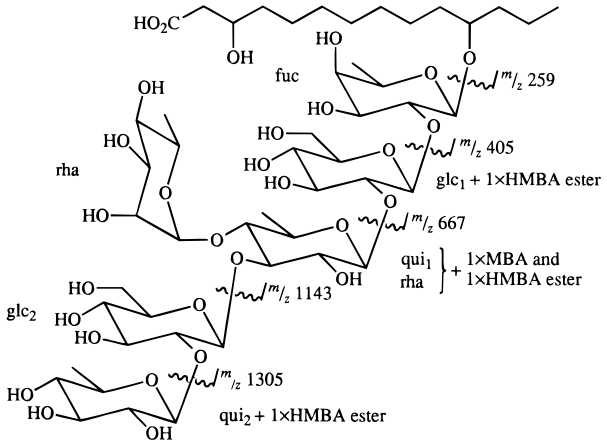

Figure 2. Negative FAB-MS/MS fragmentation pattern and partial ester assignments for the major component (mol wt 1552 Da) in the resin glycoside mixture.

of a 1,2-disubstituted glucose to be as far downfield as 6.21 ppm, as it is normally found between 4.90 and 5.95 ppm.[6,11,16,18] The close proximity of the branching rhamnosyl sugar could be responsible for this downfield shift.

ROESY and HMBC correlations were used to determine the linkages within the hexasaccharide; for example, a connectivity was observed between H-2 on glucose at 4.03 ppm, and the anomeric proton and carbon of the terminal quinovose at 5.08 and 104.8 ppm (Figure 1). In this way, the structure **1** for the ester hydrolysis product of the resin glycoside mixture was established.

Returning to a consideration of the resin glycoside mixture itself, the number and type of ester groups on each sugar in the major component of mol wt 1552 Da were determined by a collision-induced dissociation B/E linked scan of the $[M - 1]^-$ ion at m/z 1551. Fragment ions were present in the B/E scan at m/z 405, 667, 1143, and 1305. Comparison of these m/z values with those in the negative FABMS of the alkaline hydrolysis product **1**, allowed us to locate HMBA and MBA ester groups on the sugars in the hexasaccharide chain as shown in Figure 2. As is also shown, the 1552 Da compound, unlike resin glycosides from other *Ipomoea* species,[17] does not contain a macrocyclic lactone, the mol wt of which would be 18 mass units lower.

Experimental Section

General Experimental Procedures. FABMS and B/E linked scans, using either glycerol, *m*-nitrobenzyl alcohol, or triethanolamine as the matrix, were obtained using a VG ZAB-2SEQ mass spectrometer. GC−MS was carried out on a Hewlett-Packard Model 5970B MSD system using a 12.5 m × 0.20 mm HP-1 column. Optical rotations were measured on a Perkin-Elmer model 241 spectrophotometer. Most ^{1}H- and ^{13}C-NMR experiments were recorded on a Varian 500 MHz NMR spectrometer (DQF−COSY, ROESY, HMQC, and HMBC), while ^{1}H- and TOCSY NMR experiments were performed on a Varian 750 MHz NMR instrument. Chemical shift values are given in δ (ppm). Unless stated otherwise, pyridine-d_5 was used as solvent with C_5H_5N as internal reference (s, singlet; d, doublet; t, triplet; m, multiplet, br, broad). Samples were freeze-dried from D_2O solution several times prior to NMR use to exchange OH protons. Size-exclusion chromatography was carried out using Sephadex LH-20 and LH-60 gel with Pharmacia SR columns. HPLC was performed using a Maxima 820 chromatography workstation with a Waters 510 HPLC pump and either a Waters Lambda-Max 481 AZ LC spectrophotometer or a Waters differential refractometer R401 as detector. A Waters Prep Novapak HR C_{18} column (60A, 6 μm, 7.8 × 300 mm) was used for reversed-phase HPLC.

Plant Material. The aerial parts of *I. lonchophylla* were collected at the Thompson River flood plain, 800 meters SW of Waterloo Homestead, near Longreach in Central Queensland in March 1989, by A. J. Emmott. A voucher specimen (A. Q. 457061) has been deposited at the Queensland Herbarium, Brisbane.

Toxicity Testing. White mice were used as test animals to detect and assay lethal potency. Aliquots were taken from the various fractions, dried, diluted with H_2O, and administered ip. Four animals were used for each fraction to determine the least amount required to kill.[22] The toxic fraction from the LH-20 column showed an LD_{50} value of ca. 100 mg/kg.

Extraction and Isolation. The milled, dried plant material (100 g) was extracted with MeOH−H_2O (1:1, 0.5 L × 3) at approximately 60 °C. The combined extract was concentrated to ca. 200 mL and extracted with *n*-BuOH(100 mL, × 3). The combined *n*-BuOH fraction was taken down to dryness and 0.1 M NH_4OH (100 mL) added to the residue. After centrifugation to remove the resulting precipitate, the supernatant was collected and excess ammonia evaporated off from the aqueous solution. Excess 2 N H_2SO_4 was added and the acidified solution left to stand overnight in a large centrifuge cup. A cloudy precipitate formed, which was spun down. After removal of the supernatant, the precipitate was washed with H_2O until the washings were neutral. The precipitate was taken up in MeOH, concentrated to ca. 20 mL and applied to a Sephadex LH-60 column (3 × 70 cm) made up with MeOH. The toxic fractions (10 mL each) eluted early from the column. The toxic fractions were combined, reduced in volume to about 20 mL, and rechromatographed on a Sephadex LH-20 column. The toxic fraction from the column was taken to dryness giving a colorless powder, identified as a mixture of resin glycosides (205 mg). Reversed-phase HPLC separation of the individual components of the mixture was attempted on a C_{18} column, with either isocratic or gradient elution, a variety of mobile phases, for example, MeOH−H_2O (100:0 → 70:30), and differing solvent flow rates, but was not successful. Negative FABMS *m*/*z* 1651 (9), 1551 (29), 1533 (18), 1451 (19), 1433 (17), 1351 (10), 1333 (11), 1305 (12), 1205 (11), 1143 (15), 1043 (14), 943 (19), 667 (24), 567 (25), 405 (88), 259 (100); positive FABMS *m*/*z* 1697, 1597, 1579, 1513, 1497, 1479, 1413, 1397, 1379.

Acid Hydrolysis. The resin glycoside mixture (2 mg) in 2 M HCl (1 mL) was heated at 90 °C for 2.5 h. The solution was cooled, H_2O (1 mL) was added, and then extracted with Et_2O (4 × 1.5 mL). The combined Et_2O extracts were washed with H_2O (4 × 1 mL), dried (Na_2SO_4), and the solvent was evaporated (0.7 mg). The residue was taken up in dry pyridine (50 μL) and treated with Trisil (1.5 mEq/mL *N*-trimethylsilylimidazole in pyridine) (100 μL) at 70 °C for 30 min. The silylated hydrolysis products were analyzed by GC−MS (50→250 °C, Δ 10 °C/min). A major and minor peak were identified as the TMS derivatives of 3,11-dihydroxytetradecanoic and hexadecanoic acids, respectively, by comparison of their mass spectra with those of other derivatives of the dihydroxy fatty acids.[11] Peak 1: t_R = 18.65 min; EIMS (70 eV) *m*/*z* [M − Me]$^+$ 461 (18), 433 (48), 305 (43), 233 (23), 145 (50), 73 (100). Peak 2: t_R = 20.0 min; *m*/*z* [M − Me]$^+$ 489 (16), 433 (100), 305 (100), 233 (59), 173 (100), 147 (61), 73 (82).

The solution of the combined aqueous phase and washings from the Et_2O extract of the acid hydrolysis was neutralized with 2 M NaOH solution and lyophilized to give a colorless powder. The residue was taken up in dry pyridine (50 μL) and treated with Regisil [10% trimethylchlorosilane and *bis*(trimethylsilyl)-trifluoroacetamide] (100 μL) at 70 °C for 20 min. The silylated hydrolysis products were analyzed by GC−MS (100→250 °C, Δ 10 °C/min) and identified as the following TMS-sugars by comparison with authentic samples. Penta-TMS-glucose: t_R = 12.52 and 13.47 min, area 38%; tetra-TMS-quinovose: t_R = 10.84 and 11.49 min, area 38%; tetra-TMS-rhamnose: t_R = 9.54 and 10.32 min, area 9%; tetra-TMS-fucose: t_R = 10.04 and 10.47 min, area 15%. A mixture of the four sugars (authentic samples) in a molar ratio of 2:2:1:1 was silylated and analyzed by GC−MS. The mass chromatogram showed peak areas of 37%, 40%, 10%, and 13% for glc, qui, rha, and fuc, respectively, confirming the relative proportions of these sugars in the resin glycosides.

Alkaline Hydrolysis. The mixture of resin glycosides (40 mg) was stirred with NaOH solution (5 mL of 0.65 g in 10 mL). After 6 h it was neutralized with 4 M HCl. An aliquot of the aqueous layer was acidified to pH 1 and extracted with Et_2O (4 × 2 mL). The Et_2O extract was washed with H_2O (3 × 1 mL) and dried (Na_2SO_4). To the Et_2O extract was added an aliquot of CH_2N_2 [generated from aqueous NaOH and *N*-methyl-*N*-nitroso-4-toluenesulfonamide (Diazald)] in Et_2O until a yellow color in the reaction mixture persisted. The solution was analyzed by GC−MS (50→250 °C, Δ 10 °C/min). The products were identified as the Me esters of HMBA and MBA by comparison of their retention times and mass spectra with those of authentic samples recorded under the same conditions. HMBA: t_R = 4.45 min; EIMS (70 eV) *m*/*z* [M − Me]$^+$ 117 (9), 101 (19), 88

(100), 57 (77). MBA: t_R = 3.87 min; *m/z* $[M - Me]^+$ 101 (20), 88 (100), 85 (21), 59 (28), 57 (80).

The remaining neutral aqueous phase was freeze-dried, and the solid was chromatographed using LH-20 gel and MeOH to desalt the mixture. One fraction gave a colorless solid (31 mg). Further purification was carried out by using RP-HPLC with a mobile phase of MeOH−H_2O (55:45). The major fraction was a white powder (**1**) (12 mg): mp 162−164 °C; $[\alpha]_D$ −55° (*c* 0.19, MeOH); for ^{1}H- and ^{13}C-NMR spectral data, see Table 1; negative FAB-MS *m/z* $[M - H]^-$ 1167 (100), 1021 (18), 859 (60), 567 (23), 405 (23), 259 (19).

Methylation Analysis of 1. The alkaline-hydrolyzed resin glycoside **1** was subjected to permethylation and acid hydrolysis according to the procedure by Harris *et al.*[12] A portion of the product was dried and dissolved in dry pyridine (100 μL), Regisil (50 μL) was added and the mixture heated at 75 °C for 25 min. GC−MS analysis (100→250 °C, Δ 10 °C/min) gave a peak that was identified from its mass spectrum as the TMS ester of 3-methoxy-11-[(trimethylsilyl)oxy]tetradecanoic acid: t_R = 14.67 min; EIMS (70 eV) *m/z* $[M - Me]^+$ 403 (2), 375 (70), 247 (56), 145 (100), 73 (95).

Modified Lindberg Methylation Analysis of 1. The remaining acid-hydrolyzed methylation product of **1** from above was reduced and acetylated according to the method of Harris *et al.*[12] The resulting sample was analyzed by GC−MS (100→250 °C, Δ 5 °C/min), and four major compounds were identified, by comparison of their mass spectral data with literature values[13]: 1,5-di-*O*-acetyl-6-deoxy-2,3,4-tri-*O*-methylhexitol (**2**): t_R = 12.1 min; 1,2,5-tri-*O*-acetyl-6-deoxy-3,4-di-*O*-methylhexitol (**3**): t_R = 14.2 min; 1,3,4,5-tetra-*O*-acetyl-6-deoxy-2-*O*-methylhexitol (**4**): t_R = 15.6 min; and 1,2,5-tri-*O*-acetyl-3,4,6-tri-*O*-methylhexitol (**5**): t_R = 16.9 min.

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