Berchemins A and B: Novel Enzyme-Inhibiting Dimeric Lignan Glycosides from *Berchemia pakistanica*

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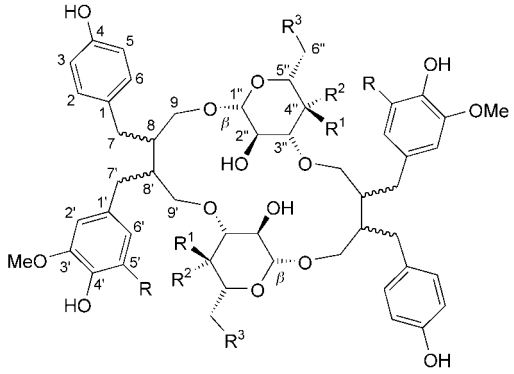
Phytochemical investigation of *Berchemia pakistanica* by extensive bioassay-guided fractionation resulted in the isolation of two novel dimeric lignan glycosides, berchemin A (**1**) and berchemin B (**2**), and of the four known compounds β-sitosterol, β-sitosterol 3-*O*-β-D-glucoside, vavain, and daphnetin 8-β-D-glucoside. The structures of **1** and **2** were elucidated with the help of modern spectroscopic techniques, and the known compounds were identified by comparing their spectral data with those reported in the literature. Berchemins A and B were found to inhibit lipoxygenase and α-glucosidase enzymes in a concentration-dependent fashion.

Introduction. – As part of our ongoing investigations of medicinal plants, we have studied the chemical constituents of the genus *Berchemia* (Rhamnaceae). Many plants of this genus are being used as herbal medicines for cholelithiasis and hepatitis in China and Japan [1]. *Berchemia pakistanica* BROWICZ. is found in hilly regions of southern Pakistan. The plant is used by the people in Quetta valley for the treatment of inflammation, rheumatism, bronchial asthma, and non-insulin-dependent diabetes mellitus. Previously, two bioactive dimeric 4-hydroxybenzoate derivatives [2] have been reported from the aerial parts of this species. However, ^{1}H-NMR analysis of the crude extract and its AcOEt fraction further showed the presence of lignans and other types of compounds. This prompted us to carry out further studies. As a result, two novel bioactive dimeric lignan glycosides, named berchemins A (**1**) and B (**2**), have been isolated and their structures elucidated through spectroscopic studies. Moreover, in the course of this work, the known compounds β-sitosterol [3][4], β-sitosterol 3-*O*-β-D-glucoside [4][5], vavain [6], and daphnetin 8-β-D-glucoside [7] were isolated for the first time from the genus *Berchemia*.

Results and Discussion. – Compounds **1** and **2** were isolated from the MeOH extract of the dried whole plant of *B. pakistanica* by successive column-chromatographic purification. The entire separation strategy was based on bioactivity screening in enzyme-inhibition assays.

Berchemin A (**1**) was isolated as a yellow gummy solid. The UV spectrum exhibited a maximum absorption at λ_{max} 228 and 279 nm, typical for the presence of aromatic rings. The IR spectrum showed strong absorptions at 3380 (O–H) and both 1605 and 1517 cm^{-1} (arom. C=C). The HR-FAB mass spectrum (positive mode) provided a quasi-molecular ion peak at *m/z* 1013.4375 ($[M+H]^+$), suggesting a molecular formula of



1 R = MeO, R^1 = H, R^2 = R^3 = OH
2 R = H, R^1 = OH, R^2 = R^3 = H

$C_{52}H_{68}O_{20}$, implying 19 degrees of unsaturation; this formula was further confirmed by elemental analysis (see the *Exper. Part*). The ^{13}C-NMR spectrum (BB, DEPT; *Table 1*) of **1** showed signals due to 24 aromatic C-atoms, four MeO, ten CH_2, and 14 CH groups. The ^{13}C-NMR and MS data indicated that **1** was a dimeric

Table 1. *^{1}H- and ^{13}C-NMR Data for Berchemin A* (**1**). In CD_3OD at 400 (^{1}H) and 100 MHz (^{13}C), resp.; δ in ppm, J in Hz. Arbitrary atom numbering (see chemical formula).

Position	δ(H)	COSY-45°	δ(C)	HMBC[a])
1			133.9	
2, 6	7.25 (*d*, *J* = 8.0)	3, 5	122.7	4, 7
3, 5	6.85 (*d*, *J* = 8.0)	2, 6	115.7	1
4			152.7	
7	2.49 (*m*)	8	36.0	2, 6, 9, 8′
8	1.91 (*m*)	8′, 9, 7	44.0	1, 7′, 9′
9a	3.84 (*dd*, *J* = 12.2, 3.9)	8, 9b	68.6	7, 8′, 1″
9b	3.51 (*dd*, *J* = 12.2, 6.1)	8, 9a		
1′			134.2	
2′, 6′	6.59 (*s*)		106.6	4′, 7′
3′, 5′			148.8	
4′			137.1	
7′	2.44 (*m*)	8′	35.1	2′, 6′, 9′, 8
8′	2.13 (*m*)	8, 7′, 9′	44.3	1′, 7, 9
9′a	3.90 (*dd*, *J* = 13.0, 4.1)	8′, 9′b	69.9	8, 7′, 3″
9′b	3.72 (*dd*, *J* = 13.0, 6.4)	8′, 9′a		
1″	5.09 (*d*, *J* = 7.6)	2″	104.1	3″, 5″, 9
2″	3.58 (*m*)	1″, 3″	74.5	4″
3″	4.21 (*m*)	2″, 4″	81.0	5″, 1″
4″	3.54 (*m*)	5″, 3″	78.2	2″, 6″
5″	3.60 (*m*)	6″, 4″	78.9	3″, 1″
6″a	3.68 (*dd*, *J* = 11.8, 2.4)	5″, 6″b	62.6	4″
6″b	3.45 (*dd*, *J* = 11.8, 5.3)	5″, 6″a		
MeO	3.81 (*s*)		56.8	3′, 5′
Ar–O*H*	8.9 (*s*)			

[a]) Key (H → C) correlations only.

diphenylbutane-type lignan glucoside. The hexose moieties could be identified by spectral comparison of ^{13}C-NMR data with literature values [8]. Ether cleavage of **1** with BCl_3 (see the *Exper. Part*) yielded, besides a host of other products, D-glucose (Glc), identified through comparison with an authentic sample and by optical rotation.

The ^{1}H-NMR spectrum of **1** showed the presence of three different types of aromatic H-atoms at δ(H) 7.25 (*d*, *J* = 8.0, 4 H), 6.85 (*d*, *J* = 8.0, 4 H), and 6.59 (*s*, 4 H); two CH_2 groups at δ(H) 2.49 (*m*, 4 H) and 2.44 (*m*, 4 H); two oxygenated CH_2 groups at δ(H) 3.90 (*dd*, *J* = 13.0, 4.1, 3 H), 3.72 (*dd*, *J* = 13.0, 6.4, 2 H), 3.84 (*dd*, *J* = 12.2, 3.9, 2 H), and 3.51 (*dd*, *J* = 12.2, 6.1, 2 H); two CH groups at δ(H) 2.13 (*m*, 2 H) and 1.91 (*m*, 2 H), and a MeO resonance at δ(H) 3.81 (*s*, 12 H). The *doublet* for the anomeric H-atoms at δ(H) 5.09 (*d*, *J* = 7.6) permitted assignment of the β-configuration to the Glc moiety. The spectrum further showed a downfield-shifted H–C(3″) resonance of Glc at δ(H) 4.21 (*m*, 2 H), indicating attachment of the lignan moieties at the 3″-O-atoms[1]). Further, O–CH and O–CH_2 resonances at δ(H) 3.54–3.60 (*m*, 6 H) and 3.68, 3.45 (2*dd*, *J* = 11.8, 2.4 and 11.8, 5.3, resp., 2 × 2 H) were observed, respectively.

Based on the above spectral data and by comparison with previously reported lignans [9–11], the structure of berchemin A (**1**) was assigned as 4,4′-{[(1*S*,8*R*,10*R*,11*S*,12*S*,19*R*,21*R*,22*S*,23*R*,24*R*)-11,22,23,24-tetrahydroxy-5,16-bis(4-hydroxybenzyl)-10,21-bis(hydroxymethyl)-2,7,9,13,18,20-hexaoxatricyclo[17.3.1.1^{8,12}]tetracosane-4,15-diyl]bis(methylene)}bis(2,6-di methoxyphenol). This was further supported through HMQC, HMBC, and COSY-45° experiments (*Table 1*). The ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those previously reported for a diphenylbutane-type lignan glucoside [9]. The C(9) and C(9′) resonances could be assigned through the differential downfield chemical shifts of C(9′) due to the attachment of sugar moieties. Since no NOE was observed between H–C(8) and H–C(8′), a relative *trans*-configuration was assumed, as found earlier in such monomeric lignans [11][12]; however, this assumption cannot be regarded as conclusive due to the flexible nature of the molecule.

Berchemin B (**2**), isolated as a yellow gummy solid, had the molecular formula $C_{50}H_{64}O_{16}$, requiring 19 degrees of unsaturation, as established by HR-FAB-MS. It differed from compound **1** mainly by the absence of two MeO resonances and the presence of a D-fucose (Fuc) instead of a D-glucose (Glc) moiety.

The UV and IR spectra of **2** were similar to those of **1**. The ^{1}H-NMR data of **2**, together with data from a standard series of COSY-45° and HMBC experiments (*Table 2*), indicated the presence of two aromatic CH groups instead of two quaternary C-atoms, as well as the absence of the two $HOCH_2$ signals. Another notable difference in the ^{1}H-NMR spectrum of **2** relative to that of **1** was the absence of the previously observed aromatic *singlet* due to nonsymmetric phenyl rings. This resonance was replaced by signals at δ(H) 7.10 (*d*, *J* = 1.9, 2 H), 6.96 (*d*, *J* = 8.1, 2 H), and 6.88 (*dd*, *J* = 8.1, 1.9, 2 H). In addition, a new signal at δ(H) 1.17 (*d*, *J* = 6.2, 6 H) due to two additional Me groups was observed in the ^{1}H-NMR spectrum; and the C-atom of this Me group showed an HMBC correlation with H–C(4″) at δ(H) 4.03 (*m*), indicating the attachment of the Me group at C(5″) of the two symmetry-related phenyl rings. The presence of the Fuc moiety in **2** was confirmed by comparison of the ^{13}C-NMR spectrum with standard reference data [13] [14], and also by ether cleavage of **2** with BCl_3, giving rise to D-fucose, as identified by comparison with an authentic sample and on the basis of optical rotation.

The above data allowed us to assign the structure of berchemin B (**2**) as 4,4′-{[(1*S*,8*R*,10*R*,11*S*,12*S*,19*R*,21*R*,22*S*,23*R*,24*R*)-11,22,23,24-tetrahydroxy-5,16-bis(4-hydro-

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[1]) Arbitrary atom numbering (see chemical formula).

Table 2. *^{1}H- and ^{13}C-NMR Data for Berchemin B* (**2**). In CD_3OD at 500 (^{1}H) and 125 MHz (^{13}C), resp.; δ in ppm, J in Hz. Arbitrary atom numbering (see chemical formula).

Position	δ(H)	COSY-45°	δ(C)	HMBC [a])
1			132.1	
2, 6	7.20 (*d*, *J* = 7.9)	3, 5	122.1	4, 7
3, 5	6.82 (*d*, *J* = 7.9)	2, 6	117.5	1
4			153.6	
7	2.45 (*m*)	8	35.5	2, 6, 9, 8′
8	2.0 (*m*)	8′, 7, 9	41.9	1, 7′, 9′
9a	3.60 (*dd*, *J* = 11.8, 2.3)	8, 9b	69.1	7, 8′, 1″
9b	3.21 (*dd*, *J* = 11.8, 5.4)	8, 9a		
1′			133.4	
2′	7.10 (*d*, *J* = 1.9)		111.7	4′, 6′, 7′
3′			146.6	
4′			144.0	
5′	6.96 (*d*, *J* = 8.1)	6′	114.3	3′, 1′
6′	6.88 (*dd*, *J* = 8.1, 1.9)	5′	121.5	2′, 4′, 7′
7′	2.41 (*m*)	8′	33.2	8, 2′, 6′, 9′
8′	2.15 (*m*)	8, 7′, 9′	41.6	7, 9, 1′
9′a	4.08 (*dd*, *J* = 12.4, 2.6)	8′, 9′b	70.0	8, 7′, 3″
9′b	3.92 (*dd*, *J* = 12.4, 4.3)	8′, 9′a		
1″	5.1 (*d*, 7.9)	2″	105.0	9, 3″, 5″
2″	4.12 (*m*)	1″, 3″	72.0	4″
3″	4.25 (*dd*, *J* = 9.8, 3.7)	2″, 4″	84.9	1″, 5″, 9′
4″	4.03 (*m*)	3″, 5″	72.3	2″, 6″
5″	3.89 (*m*)	4″, 6″	71.7	1″, 3″
6″	1.17 (*d*, *J* = 6.2)	5″	17.8	4″
3′-OMe	3.88 (*s*)		56.0	3′
Ar–O*H*	8.6 (*s*)			

[a]) Key (H → C) correlations only.

xybenzyl)-10,21-dimethyl-2,7,9,13,18,20-hexaoxatricyclo[17.3.1.1^{8,12}]tetracosane-4,15-diyl]bis(methylene)}bis(2-methoxyphenol).

The known compounds, β-sitosterol, β-sitosterol 3-*O*-β-D-glucoside, vavain, and daphnetin 8-β-D-glucoside, were identified by comparison of their spectral data with those reported previously [3–7].

The inhibitory activities of **1** and **2** against lipoxygenase [2] were tested as a function of concentration (*Fig. 1*). The calculated IC_{50} values given in *Table 3* indicate that both berchemin A (**1**) and berchemin B (**2**) are promising lipoxygenase inhibitors.

The new compounds **1** and **2** were also also tested for α-glucosidase inhibition [2] (*Fig. 2*). However, the IC_{50} value of **2** was modest, and that of **1** was significantly higher than that of deoxynojirimycin used as standard inhibitor. Note that the IC_{50} value of this standard has been only partly confirmed by different research groups. Although lying in the same range, our value reported by *Ali et al.* [15] is somewhat higher than that given by *Takahashi et al.* [16]. This might be due to a slight modification adopted in the present work.

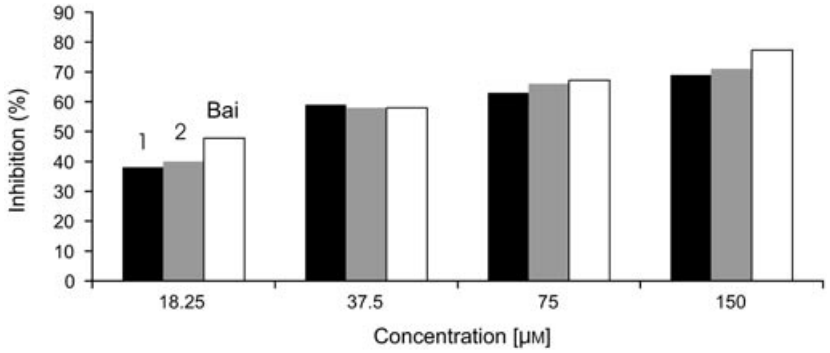

Fig. 1. *Concentration-dependent inhibition of lipoxygenase by compounds* **1** (black) and **2** (grey) vs. *the reference inhibitor baicalein* (Bai; white)

Table 3. *Inhibitory Activities of Compounds* **1** *and* **2** *towards α-Glucosidase and Lipoxygenase Relative to Deoxynojirimycin and Baicalein as Reference Inhibitors, Respectively.* S.E.M. = standard error of the mean of three to five independent assays.

Inhibitor	$IC_{50} \pm$ S.E.M. [μM]	
	α-Glucosidase	Lipoxygenase
1	> 1000	32.0 ± 0.30
2	352 ± 6.90	37.1 ± 0.15
Deoxynojirimycin	425 ± 8.14	–
Baicalein	–	22.5 ± 0.50

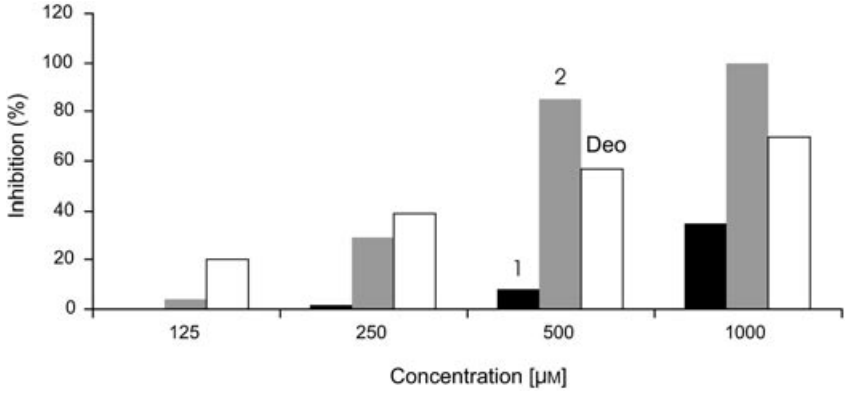

Fig. 2. *Concentration-dependent inhibition of α-glucosidase by compounds* **1** (black) and **2** (grey) vs. *the reference inhibitor deoxynojirimycin* (Deo; white)

Experimental Part

General. Column chromatography (CC): Silica gel (70–230 mesh). Flash-column chromatography (FC): Silica gel (220–440 mesh). TLC: precoated silica-gel *G-25-UV*$_{254}$ aluminium plates; detection at 254 and 366 nm, and by spraying with ceric sulfate reagent, followed by heating. Optical rotation: *Jasco DIP-360* digital polarimeter. UV Spectra: *Hitachi U*-3200 spectrophotometer; λ_{max} in nm, log ε. IR Spectra: *Jasco A-302*

spectrophotometer; in cm^{-1}. NMR: *Bruker* apparatus, at 400/100 or 500/125 MHz, resp., for ^1H and ^{13}C; δ in ppm rel. to $SiMe_4$ (=0 ppm) as internal standard. EI-FAB-MS and HR-EI-MS: *JMS-HX-110* and *JMS-DA-500* mass spectrometers; in *m/z* (rel. %).

Plant Material. The whole plant of *B. pakistanica* was collected in September 2001 from Baluchistan, and identified by Prof. *R. B. Tareen*, Department of Botany, University of Baluchistan, Pakistan. A voucher specimen (No. BBU-100) was deposited in the herbarium at the University of Baluchistan.

Extraction and Isolation. Air-dried *B. pakistanica* (27 kg) was extracted with MeOH at r.t. for two weeks. The MeOH extract was evaporated to a dark greenish residue (580 g), which was dissolved in H_2O to form a suspension, and then partitioned between hexane/H_2O, AcOEt/H_2O, and BuOH/H_2O. The AcOEt-soluble fraction (162 g) was purified by CC (SiO_2; hexane/AcOEt/MeOH gradient): four fractions (*Fr.1–4*). *Fr.2* (51 g) was further purified by CC (SiO_2; hexane/AcOEt 8:2 → 0:10) to afford eight secondary fractions (*Fr.2a–h*). *Fr.2a* (12 g) was further chromatographed (SiO_2; hexane/AcOEt 7.5:2.5, then 1:1) to yield β-sitosterol (30 mg) and β-sitosterol 3-*O*-β-D-glucoside (32 mg), resp. *Fr.2c* (14 g) was purified by CC (SiO_2; hexane/AcOEt 3:7 and 2:8) to afford vavain (28 mg) and daphnetin 8-β-D-glucoside (26 mg), resp. *Fr.2d* (16 g) was purified by CC (SiO_2; AcOEt and AcOEt/MeOH 9:1) to afford **2** (32 mg) and **1** (28 mg), respectively.

Enzyme-Inhibition Assays. a) *Lipoxygenase Assay.* Activity against lipoxygenase was conveniently measured by slightly modifying the spectrometric method developed by *Tappel* [17]. Lipoxygenase type I-B and linoleic acid were purchased from *Sigma* (St. Loius, MO, USA). All other chemicals were of anal. grade. In the assay protocol, 160 µl of 100 mM sodium phosphate buffer (pH 8.0), 10 µl of test-compound soln., and 20 µl of lipoxygenase soln. were mixed and incubated for 10 min at 25°. The reaction was then initiated by the addition of 10 µl of a soln. of linoleic acid (substrate), resulting in the formation of (9*Z*,11*E*,13*S*)-13-(hydroperoxy)octadeca-9,11-dienoate. The change in absorbance at 234 nm was followed for 8 min. Test compounds and the control were dissolved in MeOH. Baicalein (*Aldrich*) was used as positive control. All reactions were performed at least in triplicate on a 96-well micro-plate in *SpectraMax 340* (*Molecular Devices*, USA). The IC_{50} values were calculated using the 'EZ-Fit Enzyme' kinetics program (*Perrella Scientific Inc.*, Amherst, MA, USA). The percent inhibition was calculated as $100 \times (E-S)/E$, where E is the activity of the enzyme without test compound, and S is the activity of the enzyme in the presence of test compound.

b) *α-Glucosidase Assay.* The inhibitory activity of the compounds against α-glucosidase from *Saccharomyces* sp. (*Wako pure Chemical Industries Ltd.*; Wako 076-02841) was measured spectrophotometrically at pH 6.9 at 37°, using 1 mM 4-nitrophenyl α-D-glucopyranoside (PNP-G) as the substrate and 0.69 units/ml enzyme, in 50 mM sodium phosphate buffer, containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) was used as a positive control. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with a UV spectrophotometer (*Molecular Devices*, USA) [15].

4,4′-{[(1S,8R,10R,11S,12S,19R,21R,22S,23R,24R)-11,22,23,24-Tetrahydroxy-5,16-bis(4-hydroxybenzyl)-10,21-bis(hydroxymethyl)-2,7,9,13,18,20-hexaoxatricyclo[17.3.1.1^{8,12}]tetracosane-4,15-diyl]bis(methylene)}-bis(2,6-dimethoxyphenol) (= *Berchemin A*; **1**). Yellow gummy solid. Yield: 28 mg (1.04 ppm). $[\alpha]_D^{27} = +15.4$ ($c = 0.070$, MeOH). UV (MeOH): 228 (4.05), 279 (3.82). IR (KBr): 3380, 1605, 1517. ^1H- and ^{13}C-NMR: see *Table 1*. FAB-MS: 1013 ($[M+H]^+$), 507, 489, 459, 411, 345, 329, 273, 168. HR-FAB-MS: 1013.4375 ($[M+H]^+$, $C_{52}H_{69}O_{20}^+$; calc. 1013.4382), 977.4162 ($[M+H-2H_2O]^+$, $C_{52}H_{65}O_{18}^+$; calc. 977.4170), 507.2224 ($[1/2M+H]^+$, $C_{26}H_{35}O_{10}^+$; calc. 507.2230), 489.2118 ($[1/2M+H-H_2O]^+$, $C_{26}H_{33}O_9^+$, calc. 489.2124), 345.1695 ($[1/2M+H-\text{sugar}]^+$, $C_{20}H_{25}O_5^+$; calc. 345.1702). EI-MS: 345 (36, $[1/2M+H-\text{sugar}]^+$), 328 (16), 327 (25), 272 (32), 254 (18), 168 (83), 167 (41),153 (67), 138 (21), 137 (49), 124 (9), 122 (10), 109 (16), 108 (20), 94 (38), 92 (45), 77 (59). Anal. calc for $C_{52}H_{68}O_{20}$: C 61.65, H 6.77; found: C 61.77, H 6.63.

4,4′-{[(1S,8R,10R,11S,12S,19R,21R,22S,23R,24R)-11,22,23,24-Tetrahydroxy-5,16-bis(4-hydroxybenzyl)-10,21-dimethyl-2,7,9,13,18,20-hexaoxatricyclo[17.3.1.1^{8,12}]tetracosane-4,15-diyl]bis(methylene)}bis(2-methoxyphenol) (= *Berchemin B*; **2**). Yellow gummy solid. Yield: 32 mg (1.19 ppm). $[\alpha]_D^{27} = -9.3$ ($c = 0.150$, MeOH). UV (MeOH): 230 (3.91), 280 (3.79). IR (KBr): 3378, 1615, 1510. ^1H- and ^{13}C-NMR: see *Table 2*. FAB-MS: 921 ($[M+H]^+$), 461, 443, 314, 298. HR-FAB-MS: 921.4266 ($[M+H]^+$, $C_{50}H_{65}O_{16}^+$; calc. 921.4272), 461.2168 ($[1/2M+H]^+$, $C_{25}H_{33}O_8^+$; calc. 461.2175), 443.2061 ($[1/2M+H-H_2O]^+$, $C_{25}H_{31}O_7^+$; calc. 443.2069), 299.1638 ($[1/2M+H-\text{sugar}]^+$, $C_{19}H_{23}O_3^+$; calc. 99.1647), 283.1692 ($[1/2M+H-\text{sugar}]^+$, $C_{19}H_{23}O_2^+$; calc. 283.1698). EI-MS: 299 (49, $[1/2M+H-\text{sugar}]^+$), 283 (22), 272 (30), 267 (19), 254 (11), 168 (45), 150 (36), 138 (40), 137 (100), 124 (80), 108 (18), 94 (36), 77 (64). Anal. calc. for $C_{50}H_{64}O_{16}$: C 65.20, H 7.00; found: C 65.05, H 6.91.

Ether Cleavage of **1** *and* **2**. Compound **1** or **2** (14 mg) was dissolved in anh. CH_2Cl_2 (10 ml) and cooled in a dry ice/acetone bath. BCl_3 (3 g), cooled to −80°, was added. The mixture was kept at this temp. for 30 min, then allow to warm to r.t., and kept for 24 h under anh. conditions. The solvent and the remaining BCl_3 were removed under reduced pressure, and the residue was treated with MeOH (3 × 12 ml), evaporating to dryness under

reduced pressure after each addition. The residue was found to be a mixture of products among which the sugar (D-glucose (Glc) in the case of **1**, D-fucose (Fuc) in the case of **2**) could be identified by paper chromatography (*Whatman No.1*; BuOH/EtOH/H_2O 4:1:5). The mixture was subjected to prep. TLC (SiO_2; C_6H_6/EtOH 2:1) to obtain the free sugar, which was identified as Glc or Fuc, respectively, by the corresponding optical rotations ($[\alpha]_D^{27} = +52.5$ and $+76$, resp.).

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