Anderson, J. W. Dietary fiber and diabetes. In *Medical Aspects of Dietary Fibre*; Spiller, G. A., Kay, R. M., Eds.; Plenum Medical: New York, 1980.
Batey, I. L. Starch analysis using thermostable α-amylase. *Starch/Staerke* **1982**, *34*, 125–128.
Eastwood, M. A.; Brydon, W. G.; Anderson, D. M. W. The effect of the polysaccharide composition and structure of dietary fibers on cecal fermentation and fecal excretion. *Am. J. Clin. Nutr.* **1986**, *44*, 51–55.
Edwards, C. A.; Blackburn, N. A.; Craigen, L.; Davison, P.; Tomlin, J.; Sugden, K.; Johnson, I. T.; Read, N. W. Viscosity of food gums determined in vitro related to their hypoglycemic actions. *Am. J. Clin. Nutr.* **1987**, *46*, 72–77.
Jenkins, D. J. A. Dietary fiber and carbohydrate metabolism. In *Medical Aspects of Dietary Fibre*; Spiller, G. A., Kay, R. M., Eds.; Plenum Medical: New York, 1980.
Jenkins, D. J. A.; Wolever, T. M. S.; Leeds, A. R.; Gassull, M. A.; Haisman, P.; Dilawari, J.; Goff, D. V.; Metz, G. L.; Alberti, K. G. M.M. Dietary fibres, fiber analogues, and glucose tolerance: importance of viscosity. *Br. Med. J.* **1978**, *1*, 1392–1394.
Jenkins, D. J. A.; Wolever, T. M. S.; Jenkins, A. L.; Josse, R. G.; Wong, G. S. The glycaemic response to carbohydrate foods. *Lancet* **1984**, *II* (8399), 388–391.
Johnson, I. T.; Gee, J. M. Effect of gel forming gums on the intestinal unstirred layer and sugar transport in vitro. *Gut* **1981**, *22*, 398–403.
Johnson, I. T.; Gee, J. M. Influence of viscous incubation media on the resistance to diffusion of the intestinal unstirred water layer in vitro. *Pflügers Arch.* **1982**, *393*, 139–143.
Lund, E. K.; Gee, J. M.; Brown, J. C.; Wood, P. J.; Johnson, I. T. Effect of oat gum on the physicochemical properties of the gastrointestinal contents and on the uptake of D-galactose and cholesterol by rat small intestine in vitro. *Br. J. Nutr.* **1989**, *62*, 91–101.
McCleary, B. V.; Glennie-Holmes, M. Enzymic quantification of (1→3)(1→4)-β-D-glucan in barley and malt. *J. Inst. Brew.* **1985**, *91*, 285–295.
Roehrig, K. The physiological effects of dietary fiber-a review. *Food Hydrocolloids* **1988**, *2*, 1–18.
Wood, P. J. Oat β-glucan; structure, location and properties. In *Oats: Chemistry and Technology*; Webster, F. H., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986.
Wood, P. J.; Siddiqui, I. R.; Paton, D. Extraction of high viscosity gum from oats. *Cereal Chem.* **1978**, *55*, 1038–1049.
Wood, P. J.; Weisz, J.; Fedec, P.; Burrows, V. D. Large scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. *Cereal Chem.* **1989**, *66*, 97–103.
Yiu, S. H.; Wood, P. J.; Weisz, J. Effects of cooking on starch and β-glucan of rolled oats. *Cereal Chem.* **1987**, *64*, 373–379.

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Glycosidic Conjugates of Aliphatic Alcohols from Apple Fruit (*Malus sylvestris* Mill cult. Jonathan)†

Wilfried Schwab and Peter Schreier*

Lehrstuhl für Lebensmittelchemie, Universität Würzburg, Am Hubland, D-8700 Würzburg, West Germany

In an extract obtained from neutralized apple juice, cult. Jonathan, by liquid chromatographic separation on Amberlite XAD resin, 2-methylbutyl, hexyl, and 3-hydroxyoctyl β-D-glucopyranoside as well as 2-methylbutyl and hexyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside were identified after per-O-methylation by capillary gas chromatography (HRGC) and coupled HRGC techniques, i.e., HRGC–mass spectrometry (HRGC–MS) and HRGC–Fourier transform infrared spectroscopy (HRGC–FTIR). The dissaccharide glycosides were additionally characterized by ^1H and ^{13}C NMR spectroscopies after preparative HPLC of the per-O-methylated glycosidic extracts on silica gel.

In the last decade there has been increasing research on precursors and metabolites of aroma components from plants. This led to the identification of different forms of bound aroma substances, i.e., flavorless polyols (Williams et al., 1980; Engel and Tressl, 1983; Winterhalter et al., 1986; Strauss et al., 1988) and glycosidic conjugates (Francis and Allcock, 1969; Banthorpe and Mann, 1971; Croteau and Martinkus, 1979; Williams et al., 1982; 1983; Strauss et al., 1988). Furthermore, the important role of diphosphates as metabolites of aroma components from plant origin has been pointed out (Banthorpe et al., 1977; Croteau, 1987).

Recent research on the aroma precursors of apple has revealed the occurrence of two new glycosides in this fruit, i.e., 3-hydroxyoctyl β-D-glucoside (Schwab et al., 1989) and 4-(1-hydroxy-4-keto-2,6,6-trimethyl-2-cyclohexen-1-yl)but-3-en-2-yl (vomifoliol) 6-*O*-β-D-xylopyranosyl-β-D-glucopyranoside (Schwab and Schreier, 1989). This paper concerns the isolation and characterization of a number of glycosidic conjugates of aliphatic alcohols from apple fruit.

† Dedicated to Prof. Dr. F. Drawert on the occasion of his 65th birthday.

EXPERIMENTAL SECTION

Fruits. Fresh, ripe apple fruits (*Malus sylvestris* Mill cult. Jonathan) were obtained from the local market.

Isolation of Glycosides by the XAD Method (Gunata et al., 1985). After the seeds of 2.0 kg of apples were removed and the apples cut into small pieces, the fruits were submerged in 1 L of 0.2 M phosphate buffer (pH 7.4; containing 0.2 M glucono-δ-lactone as glycosidase inhibitor) and homogenized with a Braun blender for 30 s and centrifuged (4000*g*, 0 °C). The

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Table I. Linear HRGC Retention Data (R_i) as Well as EIMS and Vapor-Phase FTIR Data of Per-O-methylated 2-Methylbutyl (2), Hexyl (3), and 3-Hydroxyoctyl β-D-Glucopyranoside (4) from Apple Fruit

compd	R_i	EIMS: m/z (%)[a]	vapor-phase FTIR, cm^{-1}
2	1733	88 (100), 45 (41), 71 (41), 101 (30), 43 (28), 131 (16), 41 (15), 61 (15)	2969, 2939, 2889, 2840, 1465, 1380, 1108, 992, 937
3	1873	88 (100), 45 (38), 101 (32), 43 (26), 145 (25), 61 (24), 71 (17), 75 (15)	2938, 2840, 1461, 1374, 1109, 988
4	2191	88 (100), 45 (37), 101 (33), 71 (22), 143 (21), 69 (13), 75 (13), 203 (13)	2984, 2938, 2888, 1465, 1374, 1107, 987, 934

[a] The eight most intense peaks are given.

Table II. Linear HRGC Retention Data (R_i) as Well as EIMS and Vapor-Phase FTIR Data of Products 7–9 Obtained after Acidic Hydrolysis (pH 1.0) of Per-O-methylated Glucosides 5 and 6

compd	R_i	EIMS: m/z (%)[a]	vapor-phase FTIR, cm^{-1}
7	1753	88 (100), 71 (31), 101 (31), 43 (29), 45 (23), 41 (16), 131 (13), 73 (12)	3692, 2934, 2835, 1472, 1382, 1096
8	1898	88 (100), 43 (33), 101 (33), 45 (25), 61 (24), 41 (18), 145 (18), 85 (12)	3692, 2940, 2835, 1465, 1375, 1097
9	1329	45 (100), 101 (58), 87 (43), 41 (21), 88 (19), 115 (19), 43 (15), 59 (14)	3650, 3577, 2992, 2938, 2835, 1464, 1374, 1334, 1191, 1109

[a] The eight most intense peaks are given.

supernatant was passed through a conditioned XAD (0.3–1.0 mm; Serva) column. After the column was washed with 1.5 L of distilled water, 0.5 L of pentane, and 0.75 L of ethyl acetate, the glycosidic extract was obtained by eluting with 500 mL of methanol.

Cleanup of the Glycosidic Extract. The methanolic extract was concentrated in vacuo followed by dissolving the residue in 100 mL of 0.2 M phosphate buffer (pH 5.0) and extracting continuously with 100 mL of diethyl ether in a liquid–liquid extractor to separate the volatiles.

Derivatization. Per-O-methylation was performed by the method of Finne et al. (1980).

Fractionation of Per-O-methylated Glycosides. The per-O-methylated glycosidic extract was dissolved in 1.5 mL of chloroform and separated into 60 fractions (each 1 mL) by semipreparative HPLC using eluent I (cf. HPLC section). Fractions 14–17, 18–25, and 26–29 each were combined, concentrated, and analyzed by HRGC, HRGC–MS, and HRGC–FTIR. Combined fractions 26–29 were refractionated with another gradient (II; cf. HPLC section). In this step a fraction containing per-O-methylated glycosides 5 and 6 in a pure (98% by HRGC) 1:1 mixture was obtained.

Acidic Hydrolysis. The 1:1 mixture of 5 and 6 was dissolved in 50 mL of water, acidified to pH 1.0, and refluxed for 2 h. After extraction of the aqueous residue with 3 × 50 mL of diethyl ether, the combined organic layers were concentrated and subjected to HRGC, HRGC–MS, and HRGC–FTIR analyses.

Synthesis of 2-Methylbutyl β-D-Glucopyranoside. *a. 2,3,4,6-Tetra-O-acetyl-β-D-glucoside.* 2-Methylbutyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside was synthesized under the following modified Koenigs–Knorr conditions (Paulsen et al., 1985). To 71.4 mM 2-methylbutanol in 50 mL of dichloromethane were added 7 g of Drierite and 21.5 mM Ag_2O, and the mixture was stirred in the dark at room temperature for 30 min. Then, 19.9 mM α-D-acetobromoglucose in 50 mL of dichloromethane was added within 20 min. After the mixture was stirred in the dark at room temperature for 3 days, it was filtered through Celite, evaporated in vacuo, resolved in 90 mL of pentane–diethyl ether (2:1), and extracted with 90 mL of aqueous methanol (50%). The crude product was purified by LC on silica gel using pentane–ethyl acetate (3:1) as solvent. Yield of purified compound was 30%.

b. Deacetylation. To a solution of 200 mg of 2-methylbutyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside in 20 mL of methanol was added 20 mL of 0.02 M sodium methanolate solution. After 12 h the reaction mixture was neutralized by adding Dowex 50-WX8 (20–50 mesh, H^+ form) and filtered. The crude product was purified by LC on silica gel using ethyl acetate–methanol (9:1) as solvent. Yield of purified compound was 96%.

Synthesis of 2,3,5-Tri-*O*-methyl-α-L-arabinofuranoside. A 50-mg portion of α-L-arabinofuranose was per-O-methylated by the method of Finne et al. (1980). Subsequently, the obtained methyl 2,3,5-tri-*O*-methyl-α-L-arabinofuranoside was dissolved in 100 mL of water, acidified to pH 1.0, and refluxed for 2 h. Extraction with 3 × 100 mL of diethyl ether yielded 2,3,5-tri-*O*-methyl-α-L-arabinofuranoside.

Synthesis of Hexyl 2,3,4,6-Tetra-*O*-methylglucopyranoside. A 50-mg portion of hexyl β-D-glucopyranoside was per-O-methylated by the method of Finne et al. (1980).

Capillary Gas Chromatography (HRGC). A Carlo Erba Fractovap 4100 gas chromatograph with FID equipped with a J&W fused silica DB5-30W capillary column (30 m × 0.25 mm (i.d.), film thickness 0.25 μm) was used. Split injection (1:50) was employed. The temperature program was 60–300 °C at 5 °C/min. Flow rates: carrier gas, 2.0 mL/min He; makeup gas, 30 mL/min N_2; detector gases, 30 mL/min H_2 and 300 mL/min air. The injector temperature was kept at 250 °C and the detector temperature at 300 °C. Volumes of 1.0 μL were injected.

Capillary Gas Chromatography–Mass Spectrometry (HRGC–MS). A Varian Aerograph 1440 gas chromatograph equipped with a Carlo Erba water-cooled on-column injection system was coupled by an open-split interface to a Finnigan MAT 44 mass spectrometer with SS 200 data system. A J&W DB5-30W fused silica capillary column (30 m × 0.25 mm (i.d.), film thickness 0.25 μm) connected to a 2-m uncoated piece of fused silica capillary column as the "retention gap" was used. The conditions were as follows: temperature, from 60 to 300 °C at 5 °C/min; carrier gas flow rate, 2.5 mL/min He; temperature of ion source and all connection parts, 200 °C; electron energy, 70 eV; cathodic current, 0.8 mA; injection volumes, 1.0 μL. Results of qualitative analyses were verified by comparison of HRGC retention (R_i) and mass spectral data with those of authentic reference substances. Quantitative HRGC determinations were carried out on a Hewlett-Packard 3388 A laboratory data system.

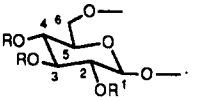
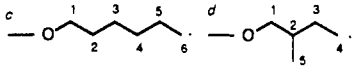
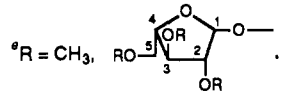
Capillary Gas Chromatography–Fourier Transform Infrared Spectroscopy (HRGC–FTIR). A Dani 6500 gas chromatograph equipped with a J&W DB5-30W fused silica capillary column (30 m × 0.25 mm (i.d.), film thickness 0.25 μm) was interfaced to a Nicolet 20 SXB FT infrared spectrometer. The column was programmed at 5 °C/min from 60 to 300 °C. Carrier gas was 2.5 mL/min He. Light pipe and transfer lines were held at 280 °C; vapor-phase spectra were recorded from 700 to 4000 cm^{-1} with a resolution of 8 cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{13}C and ^1H NMR spectra were recorded at 200 MHz on a Bruker AC-200 instrument, with $CDCl_3$ as solvent and Me_4Si as reference standard.

High-Performance Liquid Chromatography (HPLC). Semipreparative separations (20-μL samples) were carried out

Table III. ^{13}C NMR Data (δ) of Methylated Glucopyranosides (A, B, D), Hexanol (C), and Isolated Disaccharide Glycosides (E, F)[a]

atom	A	B	C	D	E	F
			Glucose Moiety[b]			
C1	98.16	105.00		103.33	103.0	104.2
C2	82.58	84.58		83.73	83.9	83.9
C3	84.28	87.21		86.37	86.4	86.4
C4	80.61	80.48		79.42	80.3	80.3
C5	70.98	75.38		74.54	74.3	74.3
C6	72.41	72.36		71.42	67.0	67.0
			Methoxy Functions of Glucose Moiety			
C1	55.27	56.97				
C2	58.36	60.47		60.14	60.3	60.3
C3	60.69	60.78		60.54	60.8	60.8
C4	60.52	60.57		60.20	60.3	60.3
C6	59.24	59.30		59.18		
			Hexanol Moiety[c]			
C1			62.1	69.83	69.9	
C2			32.9	31.45	31.6	
C3			25.8	25.56	25.8	
C4			31.9	29.50	29.6	
C5			23.0	22.42	22.6	
C6			14.2	13.83	14.0	
			2-Methylbutanol Moiety[d]			
C1						75.2
C2						26.2
C3						34.9
C4						11.3
C5						16.4
			Arabinose Moiety[e]			
C1					106.4	106.4
C2					89.9	89.9
C3					85.8	85.8
C4					84.1	84.1
C5					72.6	72.6
			Methoxy Functions of Arabinose Moiety			
C2					57.9	57.9
C3					59.3	59.3
C5					57.5	57.5

[a] Key: A, methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (Haverkamp et al., 1973); B, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (Haverkamp et al., 1973); C, hexanol (Kalinowsky et al., 1984); D, hexyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside; E, isolated per-O-methylated hexyl disaccharide (**6**); F, isolated per-O-methylated 2-methylbutyl disaccharide (**5**). [b] R = CH_3,  [c]  [d]  [e] R = CH_3,

with a Kontron chromatograph on a silica gel column (250 × 5 mm; 5-μm particle size) and a flow of 1.0 mL/min. A linear gradient (I) of hexane–2-methoxy-2-methylpropane (8:2) (10 min) to hexane–2-methoxy-2-methylpropane (2:8) (10 min) in 30 min was applied. The second linear gradient (II) was achieved with hexane–2-methoxy-2-methylpropane (3:7) (20 min) to hexane–2-methoxy-2-methylpropane (6:4) (5 min) in 15 min. Samples were detected at 254 nm (Kontron 720).

RESULTS AND DISCUSSION

In our recent publication on the simultaneous enzyme catalysis extraction of an extract from apple (*M. sylvestris* Mill cult. Jonathan) fruit, a number of aglycons enzymatically liberated from their glycosidic conjugates have been described (Schwab and Schreier, 1988). Among them, the occurrence of several aliphatic alcohols suggested that these substances, well-known as constituents of apple fruit aroma (Schreier et al., 1978), were also present as glycosides in apple fruit.

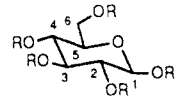
In an extract obtained from neutralized apple juice by liquid chromatographic separation on Amberlite XAD resin (Gunata et al., 1985) using methanol elution, the glycosidic conjugates were studied by HRGC, HRGC–MS, and HRGC–FTIR after per-O-methylation. From the peaks outlined in Figure 1, **1** and **3** were identified as per-O-methylated glucose and hexyl β-D-glucopyranoside, respectively. The identification of **4** (Figure 1) as per-O-methylated 3-hydroxyoctyl β-D-glucopyranoside has been already described elsewhere (Schwab et al., 1989). The chromatographic and spectroscopic data of **2**–**4** are summarized in Table I.

The occurrence of the fragment ion m/z 131 ($CH_3OCH{=}O^+CH_2CH(CH_3)CH_2CH_3$) in the mass spectrum of peak **2** instead of m/z 145 ($CH_3OCH{=}O^+(CH_2)_5CH_3$) in **3** as well as the HRGC retention behavior (cf. Figure 1) suggested for **2** the structure of per-O-methylated 2-methylbutyl β-D-glucopyranoside. Therefore, 2-methylbutyl β-D-glucopyranoside was synthesized by a modified Koenigs–Knorr method (Paulsen et al., 1985). The coincidence of chromatographic and spectroscopic data of the synthesized per-O-methylated glucoside with that of derivatized, unknown compound from the sample revealed the occurrence of 2-methylbutyl β-D-glucopyranoside in apple fruit (cf. Table I).

Table IV. ^{13}C NMR Data (δ) of Methyl Glucopyranosides (G, H) and Methyl Arabinofuranosides (I, J)[a]

atom	G	H	I	J
		Glucose Moiety[b]		
C1	100.0	104.0		
C2	72.2	74.1		
C3	74.1	76.8		
C4	70.6	70.6		
C5	72.5	76.8		
C6	61.6	61.8		
		Arabinose Moiety[c]		
C1			109.3	103.2
C2			81.9	77.5
C3			77.5	75.7
C4			84.9	83.1
C5			62.4	64.2

[a] Key: G, methyl α-D-glucopyranoside (Bock and Pederson, 1983); H, methyl β-D-glucopyranoside (Bock and Pederson, 1983); I, methyl α-L-arabinofuranoside (Gorin and Mazurek, 1975; Bock and Pederson, 1983; Backinowsky et al., 1985; Fujiwara et al., 1985); J, methyl β-L-arabinofuranoside (Gorin and Mazurek, 1975; Bock and Pederson, 1983; Backinowsky et al., 1985; Fujiwara et al., 1985). [b] R = CH_3,



[c] R = CH_3,

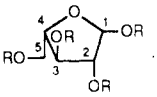
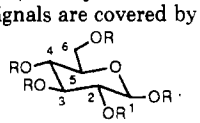
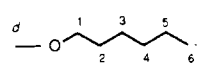


Table V. ^1H NMR Data (δ) of Methylated Glucopyranosides (A–C) and Methyl Arabinofuranoside (D) (Coupling Constants in Hertz; s = Singlet, d = Doublet, t = Triplet, m = Multiplet)[a]

atom	A	B	C	D
		Glucose Moiety[c]		
H1	4.76 (3.5) d	4.12 (7.8) d	4.16 (7.6) d	
H2	3.09 dd	2.83 dd	2.93 dd	
H3	3.29 t	3.09 t	3.08 t	
H4	3.01 t	3.01 t	2.97 t	
H5	3.47 m	3.24 m	3.21 m	
H6	3.48 dd	3.47 dd	*b*	
	3.48 dd	3.54 dd		
		Methoxy Functions of Glucose Moiety		
C1	3.29 s	3.41 s		
C2	3.39 s	3.45 s	3.52 s	
C3	3.49 s	3.51 s	3.57 s	
C4	3.43 s	3.43 s	3.47 s	
C6	3.30 s	3.31 s	3.35 s	
		Hexanol Moiety[d]		
2 H1			1.52 t	
8 H2–5			1.25 m	
3 H6			0.83 t	
		Arabinose Moiety[e]		
H1				4.91
H2				4.04
H3				3.93
H4				4.02
H5				3.80
				3.69

[a] Key: A, methyl 2,3,4,6-tetra-*O*-methyl-α-D-glucopyranoside (Haverkamp et al., 1973); B, methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside (Haverkamp et al., 1973); C, hexyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside; D, methyl α-L-arabinofuranoside (Du Penhoat et al., 1987). [b] Signals are covered by signals for the methoxy functions. [c] R = CH_3,





[e] R = CH_3,

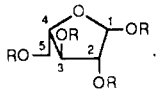
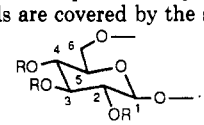


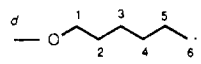
Table VI. ^1H NMR Data (δ) of Partially Methylated Arabinofuranoses (E, F), Arabinan (G), and Isolated Disaccharide Glucosides (H, I) (Coupling Constants in Hertz; s = Singlet, d = Doublet, t = Triplet, m = Multiplet)[a]

atom	E	F	G	H	I
			Glucose Moiety[c]		
H1				4.21 (8) d	4.21 (8) d
H2				2.96 dd	2.96 dd
H3				3.11 t	3.11 t
H4				3.01 t	3.01 t
H5				3.22 m	3.22 m
H6				*b*	*b*
			Methoxy Functions of Glucose Moiety		
C1					
C2				3.50 s	3.50 s
C3				3.58 s	3.58 s
C4				3.45 s	3.45 s
C6					
			Hexanol Moiety[d]		
2 H1				1.61 t	
8 H2–5				1.25 m	
3 H6				0.88 t	
			2-Methylbutanol Moiety[e]		
2 H1					1.61 d
1 H2, 2 H3					1.25 m, 1.25 m
3 H4, 3 H5					0.88 t, 0.92 d
			Arabinose Moiety[f]		
H1	5.23	5.28	5.12 (3)	5.07 s	5.07 s
H2	4.02	4.07	4.12	3.96 t	3.96 t
H3	3.93	4.00	4.04	3.81 t	3.81 t
H4	4.17	3.89	4.24	4.05 m	4.05 m
H5	3.68	3.67	3.91	3.76 dd	3.76 dd
	3.57		3.81	3.67 dd	3.67 dd
			Methoxy Functions of Arabinose Moiety		
C2				3.59 s	3.59 s
C3				3.61 s	3.61 s
C5				3.41 s	3.41 s

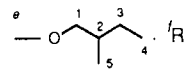
[a] Key: E, 5-*O*-methyl-α-L-arabinofuranose (Snyder and Serianni, 1987); F, 5-*O*-methyl-β-L-arabinofuranose (Snyder and Serianni, 1987); G, arabinan (Du Penhoat et al., 1987); H, isolated per-O-methylated hexyl disaccharide (**6**); I, isolated per-O-methylated 2-methylbutyl disaccharide (**5**). [b] Signals are covered by the signals for the methoxy functions. [c] R = CH_3,



[d]



[e]



[f] R = CH_3 or H,

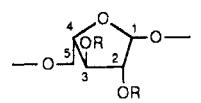


Table VII. ^1H NMR Data of Arabinosides (s = Singlet)

compound	δ(H1)[a] ($J_{H1,H2}$, Hz)	lit. ref
methyl α-L-arabinofuranoside	4.91 (1.8)	Joseleau et al. (1977)
methyl β-L-arabinofuranoside	4.86 (4.5)	Joseleau et al. (1977)
methyl α-L-arabinopyranoside	4.26 (7.5)	Joseleau et al. (1977)
methyl β-L-arabinopyranoside	4.83 (2.8)	Joseleau et al. (1977)
arabinan	5.12 (3)	Du Penhoat et al. (1987)
arabinofuranoside glucoside	5.08 s	Fujiwara et al. (1985)

[a]

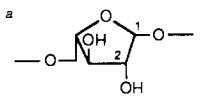
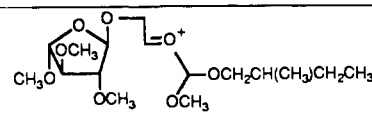
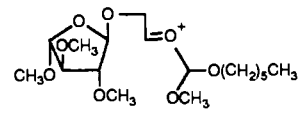
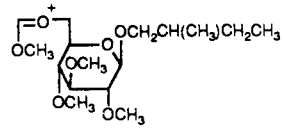
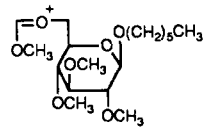
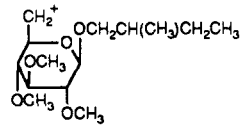
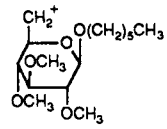
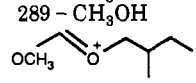
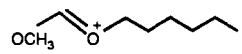
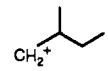
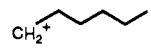


Table VIII. Postulated Fragment Ions in the Mass Spectra (70 eV) of Per-O-methylated 2-Methylbutyl (5) (a) and Hexyl 6-*O*-α-L-Arabinofuranosyl-β-D-glucopyranoside (6) (b) (Cf. Figure 3)

m/z	fragment ion	lit. ref
365 (a)		Kochetkov et al. (1964)
379 (b)		Kochetkov et al. (1964)
335 (a)		Kochetkov et al. (1964)
349 (b)		Heyns et al. (1966) Kochetkov et al. (1964)
275 (a)		Heyns et al. (1966) Kochetkov et al. (1964)
289 (b)		Heyns et al. (1966) Kochetkov et al. (1964)
243 (a)	275 - CH_3OH	Heyns et al. (1966)
257 (b)	289 - CH_3OH	
131 (a)		Heyns et al. (1966)
145 (b)		Heyns et al. (1966)
71 (a)		
85 (b)		
88/101	carbohydrate fragments	Heyns et al. (1966)

Due to the mass spectral fragmentation of peaks **5** and **6** (Figure 1), similar to that of **2** and **3**, and their HRGC retention behavior, **5** and **6** were assumed to be disaccharides of 2-methyl-1-butanol and 1-hexanol, respectively. For their structural elucidation, the per-O-methylated glycosidic extract was fractionated by HPLC on silica gel using eluent I (cf. the Experimental Section) (Figure 2). HRGC and HRGC–MS analyses revealed the occurrence of **2** and **3** in fractions 14–17, **1** and **4** in fractions 18–25, and **5** and **6** in fractions 26–29 (cf. Figure 2). Repeated semipreparative HPLC fractionation using another gradient (II; cf. the Experimental Section) yielded a pure (98% by HRGC) 1:1 mixture of **5** and **6**. After their ^1H and ^{13}C NMR spectra were recorded, **5** and **6** were hydrolyzed at pH 1.0 and the products, isolated from the aqueous residue by solvent extraction, studied by HRGC, HRGC–MS, and HRGC–FTIR. Two of the three products formed, i.e., **7** and **8**, showed mass spectra similar to those of **2** and **3**, but different R_i data (Table II). In addition, the vapor-phase FTIR spectra of **7** and **8** as well as of the third product **9** exhibited OH absorptions that were evaluated as primary and secondary OH valence bands, respectively (Nyquist, 1984) (Table II).

After per-O-methylation of the product mixture **7–9**, HRGC revealed again three peaks, from which two were identified by HRGC–MS and HRGC–FTIR as **2** and **3**; the third one, **10**, appeared as a nonresolved double peak (R_i 1228/1234). From these results it could be concluded that **7** and **8** had to be partially methylated 2-methylbutyl and hexyl β-D-glucopyranosides, respectively, exhibiting an OH group at C6 (cf. FTIR data in Table II; primary OH only at this position). Compound **9** had to be the second sugar moiety bound to the disaccharide structure.

This partially methylated monosaccharide **9**, liberated by acidic hydrolysis from the per-O-methylated disaccharide glycosides **5** and **6**, was identified as 2,3,5-tri-*O*-methyl-α-L-arabinofuranoside (Kochetkov et al., 1963) by comparison of its HRGC, HRGC–MS, and HRGC–FTIR data with those of a synthesized reference compound. Per-O-methylation of synthesized **9** led to its partial degradation forming, inter alia, the above-mentioned double peak **10**.

Further structural elucidation of the carbohydrate moiety of **5** and **6**, suggested to consist of glycopyranose linked at C6 to an arabinofuranose, was performed by NMR

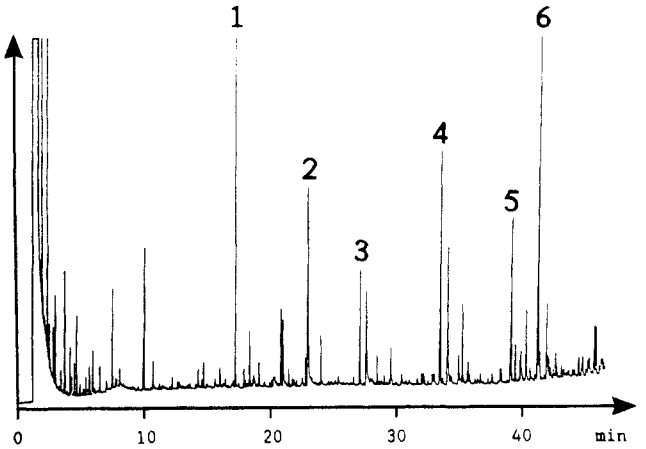


Figure 1. HRGC separation (J&W; 30 m × 0.25 mm (i.d.), fused silica WCOT DB-5 capillary column, df = 0.25 μ) of a per-O-methylated apple extract obtained by XAD adsorption chromatography: 1, per-O-methylated glucose; 2–6, per-O-methylated glycosides. Structures are explained in the text.

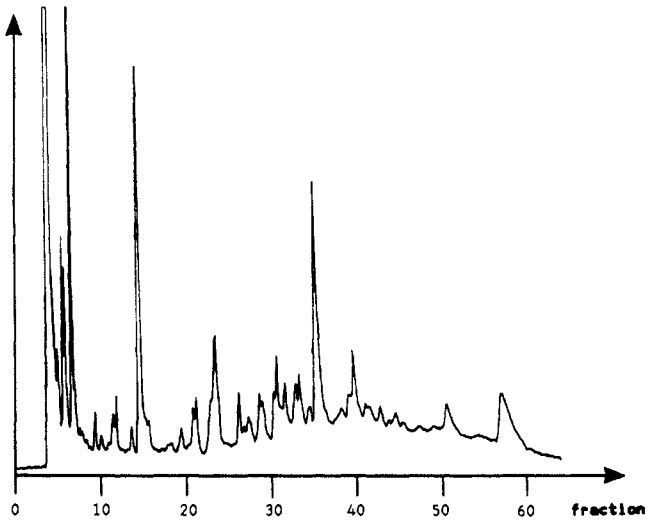


Figure 2. HPLC separation of a per-O-methylated apple extract obtained by XAD adsorption chromatography. For HPLC conditions, see the Experimental Section.

techniques. The ^{13}C NMR data of the disaccharide mixture **5**/**6** and synthesized hexyl tetra-*O*-methyl-β-D-glucopyranoside as well as additional data of per-O-methylated glycosides taken from the literature (Haverkamp et al., 1973; Kalinowski et al., 1984) are outlined in Table III. As shown from Table III, the coincidence of ^{13}C NMR data of per-O-methylated glucose **1** with that of the isolated compounds **5** and **6** was obvious. Due to the high ppm values recorded for the C1 of glucose, β-configuration was concluded. Furthermore, in the ^{13}C NMR spectra of **5** and **6** the signal for the methoxy group at C6 of glucose moiety was lacking, confirming the above-mentioned information obtained by vapor-phase FTIR spectroscopy, i.e., the linkage to the second sugar moiety at C6 of β-glucose.

From the comparisons of signal shifts of per-O-methylated glucopyranoses (Table III) with that of methyl glucopyranoses (Table IV), the following conclusions were made concerning the structure of the unknown arabinose moiety in **5** and **6**. Methylation of an OH group of glucose led to a shift of approximately +9 ppm, as shown for C2, C3, C4, and C6 (but not for C1 and C5) of per-O-methylated (Table III) and methyl glucopyranosides (Table IV). When the ^{13}C NMR data of methyl arabinofuranoses (Table IV) were compared with that of per-O-methylated disaccharide glycosides **5** and **6** (Table III), the shift from OH to CH_3O group was found to be approximately +8 ppm for C2 and C3 and +9 ppm for C5; the position of the signal for C4 was not changed. Due to the carbohydrate instead of the methyl moiety at C1, in comparison to the methyl arabinoside, the signal for C1 of arabinose moiety in **5** and **6** was downfield-shifted. Accordingly, in derivatized arabinans, the signal at C1 of furanoid arabinoses has been found at 106.15 ppm (Backinowsky et al., 1985). Finally, from the comparison of ^{13}C NMR data of methyl arabinoses (Table IV) with that of arabinose moieties of **5** and **6** (Table III) α-configuration was concluded, since coincidence of data for the signal at C4 was given with the α-anomer and the ppm values recorded for C1, C2, and C3 were found to be too low and for C5 too high for the β-anomer. Studying 5-*O*-methylarabinofuranoside, Snyder and Serianni (1987) described 73.5 and 75.1 ppm for the C5 signal of α and β-anomers, respectively. Since all data were related to D-glucopyranoses and L-arabinofuranoses, these configurations can be also considered for the isolated carbohydrate moieties in **5** and **6**.

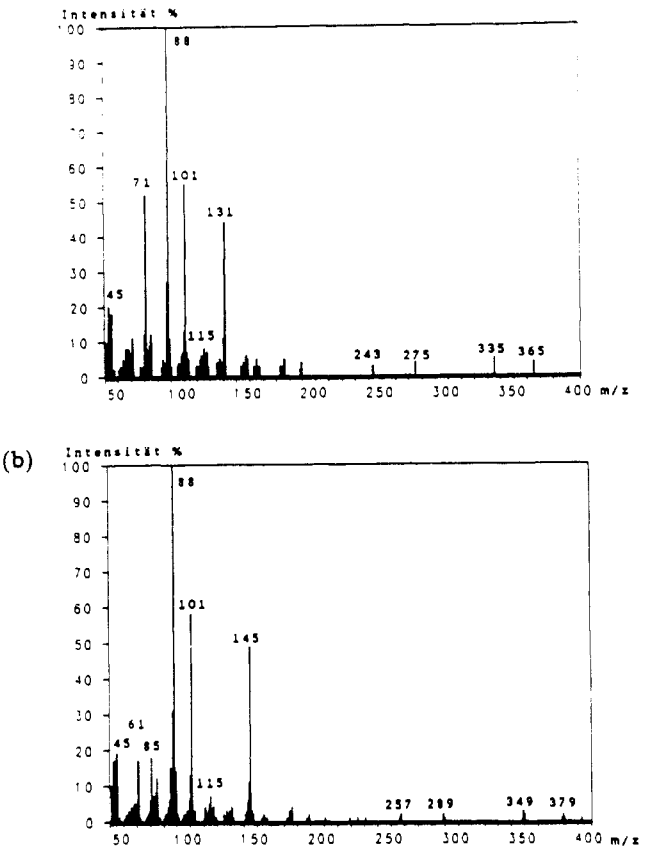


Figure 3. Mass spectra (70 eV) of per-O-methylated 2-methylbutyl (**5**) (a) and hexyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranoside (**6**) (b).

The ^1H NMR data of the disaccharide mixture **5**/**6** are outlined in Table V, which also contains the data of synthesized hexyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside and ^1H NMR information taken from the literature (Haverkamp et al., 1973; Du Penhoat et al., 1987; Snyder and Serriani, 1987). From the data recorded for methyl 2,3,4,6-tetra-*O*-methyl-α-D- and -β-D-glucopyranoside as well as hexyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, β-configuration was proved for the glucopyranoside moiety in **5** and **6**. In addition, the three H atoms of CH_3O at the C6 of glucose were lacking, thus confirming the linkage of the second sugar moiety at this position.

The ^1H NMR signals for the arabinose moieties of **5** and **6** (Table IV) showed a high ppm value for H1 that appeared as singulet. This shift and the low coupling constant $J_{H1,H2}$ indicated the α-furanoid form of arabinose in **5** and **6** (Table VII).

By means of the now clearly defined structures of isolated disaccharides as hexyl and 2-methylbutyl 6-*O*-α-L-arabinofuranosyl-β-D-glucopyranosides, the mass spectral fragmentation of per-O-methylated derivatives **5** and **6** (Figure 3) could be evaluated. Table VIII shows the most important fragment ions and their postulated structures.

While β-D-glucopyranoside glycosides have been often found in nature, 6-α-L-arabinofuranosyl-D-glucopyranosides have been detected only as carbohydrate moieties of monoterpene glycosides (Williams et al., 1982). Additionally known are 6-β-L-arabinopyranosyl-β-D-glucopyranose (vicianose) (Kennedy and White, 1983) and eugenol β-vicianoside (Gein) (Chaudhury and Robertson, 1949).

LITERATURE CITED

Backinowsky, L. V.; Nepogod'ev, S. A.; Kochetkov, N. K. Stereospecific Synthesis of a (1→5)-α-L-Arabinan. *Carbohydr. Res.* **1985**, *137*, C1–C3.

Banthorpe, D. V.; Mann, J. Monoterpene Glucosides from Petals of Tanacetum vulgare. *Phytochemistry* **1971**, *11*, 2589–2591.

Banthorpe, D. V.; Bucknall, G. A.; Gutowski, J. A.; Rowan, M. G. Epoxidation and Hydradation of Prenyl Pyrophosphates by Plant Extracts. *Phytochemistry* **1977**, *16*, 355–358.

Bock, K.; Pederson, C. Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Monosaccharides. *Adv. Carbohydr. Chem. Biochem.* **1983**, *41*, 27–66.

Chaudhury, D. N.; Robertson, A. Syntheses of Glycosides. Part XIV. The Synthesis of Gein and the Hexa-acetyl β-Vicianoside of (−)-Mandelonitrile believed to be the Hexa-acetate of Vicianin. *J. Chem. Soc.* **1949**, 2054–2057.

Croteau, R. Biosynthesis and Catabolism of Monoterpenoids. *Chem. Rev.* **1987**, *87*, 929–954.

Croteau, R.; Martinkus, C. Metabolism of Monoterpenes. Demonstration of (+)-Neomenthyl-β-D-Glucoside as a major Metabolite of (−)-Menthone in Peppermint (*Mentha piperita*). *Plant Physiol.* **1979**, *64*, 169–175.

Du Penhoat, C. H.; Michon, V.; Goldberg, R. Development of Arabinans and Galactans During the Maturation of Hypocotyl Cells of Mung Bean (*Vigna radiata* Wilczek). *Carbohydr. Res.* **1987**, *165*, 31–42.

Engel, K. H.; Tressl, R. Formation of Aroma Components from Nonvolatile Precursors in Passion Fruit. *J. Agric. Food Chem.* **1983**, *31*, 998–1002.

Finne, J.; Krusius, T.; Rauvala, H. Use of Potassium tert-Butoxide in the Methylation of Carbohydrates. *Carbohydr. Res.* **1980**, *80*, 336–339.

Francis, M. J. O.; Allcock, C. Geraniol β-D-Glucoside; Occurrence and Synthesis in Rose Flowers. *Phytochemistry* **1969**, *8*, 1339–1347.

Fujiwara, T.; Takeda, T.; Ogihara, Y. Synthesis of Trisaccharides Related to an Arabinoglucan. *Carbohydr. Res.* **1985**, *141*, 168–171.

Gorin, P. A. J.; Mazurek, M. Further Studies on the Assignment of Signals in ^{13}C Magnetic Resonance Spectra of Aldoses and Derived Methyl Glycosides. *Can. J. Chem.* **1975**, *53*, 1212–1223.

Gunata, Y. Z.; Bayonove, C. L.; Baumes, R. L.; Cordonnier, R. E. The Aroma of Grapes. I. Extraction and Determination of Free and Glycosidically Bound Fraction of Some Grape Aroma Components. *J. Chromatogr.* **1985**, *331*, 83–90.

Haverkamp, J.; van Dongen, J. P. C. M.; Vliegenthart, J. F. G. PMR and CMR Spectroscopy of Methyl 2,3,4,6-Tetra-*O*-Methyl-α- and -β-D-Glucopyranoside. An Application to the Identification of Partially Methylated Glucoses. *Tetrahedron* **1973**, *29*, 3431–3439.

Heyns, K.; Grützmacher, H. F.; Scharmann, H.; Müller, D. Massenspektrometrische Strukturanalysen von Kohlenhydraten. *Fortschr. Chem. Forsch.* **1966**, *5*, 448–490.

Joseleau, J.-P.; Chambat, G.; Vignon, M.; Barnoud, F. Chemical and ^{13}C N.M.R. Studies on Two Arabinans from the Inner Bark of Young Stems of *Rosa glauca*. *Carbohydr. Res.* **1977**, *58*, 165–175.

Kalinowsky, H.-O., Berger, S., Braun, S., Eds. *^{13}C-NMR-Spektroskopie*; Thieme: Stuttgart, 1984.

Kennedy, J. F., White, C. A., Eds. Bioactive Carbohydrates. In *Chemistry, Biochemistry und Biology*; Wiley: Chichester, 1983.

Kochetkov, N. K.; Wulfson, N. S.; Chizhov, O. S.; Zolotarev, B. M. Mass Spectrometry of Carbohydrate Derivatives. *Tetrahedron* **1963**, *19*, 2209–2224.

Kochetkov, N. K.; Chizhov, O. S.; Poliakova, L. A. Mass Spectrometric Study of Hydrocarbons. *Dokl. Akad. Nauk SSSR* **1964**, *158*, 685–688.

Nyquist, R. A., Ed. *The Interaction of Vapor-Phase Infrared Spectra. Group Frequency Data*; Heyden & Son: London, 1984.

Paulsen, H.; Lê-Nguyên, B.; Sinnwell, V.; Heemann, V.; Seehofer, F. Synthese von Glycosiden von Mono-, Sesqui- und Diterpenalkoholen. *Liebigs Ann. Chem.* **1985**, 1513–1536.

Schreier, P.; Drawert, F.; Steiger, G. Anwendung der Maische-Hochkurzzeiterhitzung und deren Einfluss auf die Aromastoffzusammensetzung bei der Herstellung von Apfelbranntwein. *Z. Lebensm. Unters. Forsch.* **1978**, *167*, 16–22.

Schwab, W.; Schreier, P. Simultaneous Enzyme Catalysis Extraction: A Versatile Technique for the Study of Flavor Precursors. *J. Agric. Food Chem.* **1988**, *36*, 1238–1242.

Schwab, W.; Schreier, P. Vomifoliol 1-*O*-β-D-xylopyranosyl-6-*O*-β-D-glucopyranoside: A New Disaccharide Glycoside from Apple Fruit. *Phytochemistry* **1989**, in press.

Schwab, W.; Scheller, G.; Gerlach, D.; Schreier, P. Identification of 3-Hydroxyoctyl β-D-glucoside and Absolute Configuration of Free and Bound Octane-1,3-diol in Apple Fruit. *Phytochemistry* **1989**, *28*, 157–160.

Snyder, J. R.; Serianni, A. S. Synthesis and N.M.R.-Spectral Analysis of Unriched and [1-^{13}C]-Enriched 5-Deoxypentoses and 5-*O*-Methylpentoses. *Carbohydr. Res.* **1987**, *163*, 169–188.

Strauss, C. R.; Wilson, B.; Williams, P. J. Novel Monoterpene Diols and Diol Glycosides in *Vitis vinifera* Grapes. *J. Agric. Food Chem.* **1988**, *36*, 569–573.

Williams, P. J.; Strauss, C. R.; Wilson, B. Hydroxylated Linalool Derivatives as Precursors of Volatile Monoterpenes of Muscat Grapes. *J. Agric. Food Chem.* **1980**, *28*, 766–769.

Williams, P. J.; Strauss, C. R.; Wilson B.; Massy-Westropp, R. A. Novel Monoterpene Disaccharide Glycosides of Vitis *vinifera* grapes and wines. *Phytochemistry* **1982**, *21*, 2013–2020.

Williams, P. J.; Strauss, C. R.; Wilson, B.; Massy-Westropp, R. A. Glycosides of 2-Phenylethanol and Benzyl alcohol in *Vitis vinifera* grapes. *Phytochemistry* **1983**, *22*, 2039–2041.

Winterhalter, P.; Katzenberger, D.; Schreier, P. 6,7-Epoxy-Linalool and Related Oxygenated Terpenoids from *Carica papaya* Fruit. *Phytochemistry* **1986**, *25*, 1347–1350.

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Registry No. 2-Methylbutyl β-D-glucopyranoside, 80202-03-5; hexyl β-D-glucopyranoside, 59080-45-4; 3-hydroxyoctyl β-D-glucopyranoside, 124150-19-2; 2-methylbutyl 6-α-L-arabinofuranosyl-β-D-glycopyranoside, 124069-37-0; hexyl 6-α-L-arabinofuranosyl-β-D-glucopyranoside, 124044-06-0; 2-methylbutanol, 137-32-6; α-D-acetobromoglucose, 572-09-8; 2-methylbutyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside, 124044-07-1; α-L-arabinofuranose, 38029-69-5; 2,3,5-tri-*O*-methyl-α-L-arabinofuranoside, 124150-20-5; hexyl 2,3,4,6-tetra-*O*-methylglucopyranoside, 124044-08-2.