Tobacco Chemistry 64*. A New Sucrose Ester from Greek Tobacco

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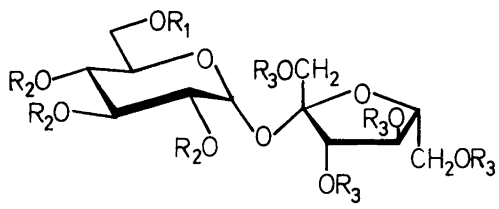
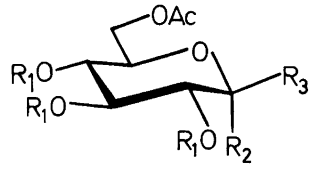
A new sucrose ester has been isolated from Greek tobacco and shown to be 6-*O*-acetyl-2,3,4-tri-*O*-[-3*S*-methylpentanoyl]-sucrose (1) by spectral methods and chemical correlation.

It is well established that tobaccos of the oriental type differ chemically from Virginia and Burley tobaccos in several respects. They have a higher proportion of certain low molecular weight fatty acids such as 3-methylpentanoic acid[2] and contain, in addition to cembranoids, diterpenoids of the labdane class.[3] The genetic differences between these tobacco varieties are apparently also reflected in the content of certain sugars. Thus, 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glycopyranose (*2*)[4] and a few related glucose esters have been isolated from Turkish tobacco.[5] Moreover, analytical studies, employing gas chromatography after conversion to silyl ethers[6,7] or direct chemical ionization mass spectrometry,[8] have shown that oriental tobaccos contain up to 0.2 % of sucrose esters, whereas considerably lower levels are detected in Virginia and Burley tobaccos.

Although available results suggest that these sucrose esters are substituted on the glucose moiety with one unit of acetic acid and three units of C_3–C_8 fatty acids,[6-8] unambigous structural evidence has not, as yet, been presented. We now report the isolation and structure determination of one of these sucrose esters (*1*).



1 R_1 = Ac, R_2 = 3S-methylpentanoyl, R_3 = H
3 R_1 = R_3 = Ac, R_2 = 3S-methylpentanoyl
4 R_1 = R_2 = R_3 = Ac
5 R_1 = R_2 = R_3 = H



R_1 = 3S-methylpentanoyl
2 R_2 = H, R_3 = OH
6 R_2 = OH, R_3 = H
10 R_2 = H, R_3 = OAc
9 R_2 = OAc, R_3 = H

*For part 63, see Ref. 1.

Results

The new compound (*1*) was identified as a disaccharide from the ^{13}C NMR spectrum (Table 1), which contained twelve signals in the region $60 < \delta < 105$. It is a monoacetate (^1H NMR singlet at δ 2.11) converted to a pentaacetate (*3*) on acetylation. In contrast to *1*, *3* gave a ^1H NMR spectrum amenable to detailed analysis. This was carried out by using the results obtained from COSY spectra and spin simulation studies.

A comparison of the ^1H chemical shift values and coupling constants (Table 2) and the ^{13}C NMR data (Table 1) for *3* with the corresponding values for sucrose octaacetate (*4*) strongly suggested that *3* was a sucrose derivative. This assignment was verified by sugar analysis of *1*, which gave glucose and fructose. The ^{13}C NMR spectrum of the new compound (*1*) also exhibited signals at δ 170.7, 171.8, 172.5 and 173.0 consonant with the presence of four ester groups. While one of these was the aforementioned acetate group, the chemical shift values and multiplicities of the remaining signals in the ^{13}C NMR spectrum indicated that the other three ester groups each contained six carbon atoms. Empirical calculations and comparison with data of relevant reference compounds showed that the observed resonances were consistent only with the occurrence of three 3-methylpentanoyl groups. This conclusion was verified and the 3-methylpentanoic component ascribed a 3*S* chirality after isolation of the acidic fraction from an alkaline hydrolysate of *1*.

A comparison of the ^{13}C NMR spectra of *1* and its peracetylated derivative *3* to those of sucrose octaacetate (*4*)[9] and sucrose (*5*)[10] suggested that the most likely positions of esterification were on the pyranose ring, but an exact assignment was not possible by this means. However, as 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glucopyranose (*2*) has been isolated from tobacco,[4] it seemed biogenetically most plausible that the new compound (*1*) was 6-*O*-acetyl-2,3,4-tri-*O*-[-3*S*-methylpentanoyl]-sucrose. This structural assignment was strongly corroborated by the FAB mass spectrum, which contained a peak at *m/z* 701, ascribed to the $[M+Na]^+$ ion, and a base peak at *m/z* 99 due to a 3-methylpentanoyl ion (cf. Scheme 1). A diagnostic ion of mass 499 ($C_{26}H_{43}O_9$ from high resolution EI-MS), which as indicated in Scheme 1, undergoes sequential loss of three 3-methylpentanoyl units and one molecule of acetic acid, probably comprised the tetraesterified pyranose ring. Complementary to this, was the presence of a peak at *m/z* 163, which was attributed to the unsubstituted furanose ring and appropriately shifted to *m/z* 331 in the spectrum of the pentaacetate *3*.

Structural evidence was sought by chemical methods. Thus, acid-catalyzed hydrolysis of *1* using trifluoroacetic acid yielded the α- and β-anomers of a glucose tetraester (*6*, *2*). Although the β-anomer had m.p. and optical rotation in good agreement with those reported[4] for 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glucopyranose (*2*), it was felt that a comparison based solely on these data would not suffice for an unequivocal structural assignment. The chemical correlation shown in Scheme 2 was therefore carried out, whereby 1,6-anhydro-β-D-glycopyranose (*7*) was converted to the 2,3,4-triester *8* by treatment with 3*S*-methylpentanoyl chloride, a reagent obtained via preparative enantiomer separation of (±)-benzyl 3-methylpentanoate on swollen microcrystalline triacetylcellulose. The triester *8* was subjected to acetolysis, which gave a mixture of the α- and β-anomers of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-D-glucopyranose (*9*, *10*). These were identical in all respects to the diacetates obtained from the hydrolysis products *6* and *2*, respectively, hence conclusively assigning a 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-sucrose structure to the new tobacco constituent *1*. A different method for the allocation of the various fatty acid residues in sucrose ester *1* involving long range C–H shift correlation spectroscopy was also applied and will be described elsewhere.[11]

Experimental

The FAB mass spectra were recorded on an LKB 2091 instrument equiped with an Iontech FAB-11NF unit. Xenon atoms of 6 keV energy were used in the bombardment. The sample holder and ion source were kept at room temperature; the samples were dissolved in glycerol. Other instrumental details are given in Ref. 1.

Sugar analysis was carried out by Dr L. Kenne at the Department of Organic Chemistry, Stockholm University using conventional methods. An extract (83 g), obtained by immersing flowers of Greek *Nicotiana tabacum* (Basma Drama) in

chloroform, was initially separated into five fractions, A–E, by flash chromatography (silica gel, hexane/ethyl acetate/methanol as eluents). Part (1.20 g) of the most polar fraction (E, 3.6 g) was further separated by reverse phase HPLC, using columns packed with μ-Bondapak/C18 and Spherisorb 5 ODS, to give 30.3 mg of 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-sucrose (*1*) as a gum, which had $[\alpha]_D$ +51° (*c* 0.26, EtOH) (Found: $[M+Na]^+$ 701. Calc. for $C_{32}H_{54}O_{15}Na$: 701); IR ($CHCl_3$) bands at 3364, 3028, 2966, 2931, 2878, 1744, 1454, 1383, 1267, 1235, 1179, and 1136 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8–1.0 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)$-CH_2–), 1.1–1.5 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.7–2.0 (3H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.0–2.5 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.11 (3H, s, –$OCOCH_3$), 2.8 (1H, broad s, –OH), 3.1 (1H, broad s, –OH), 3.5–4.0 (7H, overlapping signals, H–1′, H–5′, H–6′, and 2×–OH), 4.1–4.5 (5H, overlapping signals, H–5, H–6, H–3′, and H–4′), 4.91 (dd, *J* = 4.0 and 10.3 Hz, H–2), 5.10 (dd, *J* = 9.5 and 10.2 Hz, H–4), 5.53 (dd, *J* = 9.5 and 10.3 Hz, H–3), 5.72 (d, *J* = 4.0 Hz, H–1); MS, FAB [*m/z* (%)]: 701 (M+Na, 3), 499 (4), 457 (0.7), 401 (2), 383 (0.4), 359 (0.3), 341 (0.6), 303 (0.6), 285 (1), 267 (0.6), 261 (3), 243 (2), 205 (3), 169 (8), 163 (5), 145 (8), 127 (14), 115 (26), and 99 (100); MS, EI [*m/z* (%, composition)]: 499 (1, $C_{26}H_{43}O_9$), 401 (2, $C_{20}H_{33}O_8$), 303 (3, $C_{14}H_{23}O_7$), 285 (1, $C_{14}H_{21}O_6$), 261 (5, $C_{12}H_{21}O_6$), 243 (1, $C_{12}H_{19}O_5$), 205 (1, $C_8H_{13}O_6$), 169 (3, $C_8H_9O_4$), 163 (0.4, $C_6H_{11}O_5$), 145 (1, $C_6H_9O_4$), 127 (4, $C_6H_7O_3$), 115 (2, $C_5H_7O_3$ and $C_6H_{11}O_2$), 99 (100, $C_6H_{11}O$ and $C_5H_7O_2$), 71 (51, C_5H_{11} and $C_3H_3O_2$), and 43 (73, C_3H_7 and C_2H_3O).

*Preparation of 1′,3′,4′,6,6′-penta-*O*-acetyl-2,3,4-tri-*O*-[3S-methylpentanoyl]-sucrose* (3). A solution of 5 mg of *1* in 2 ml of Ac_2O-pyridine (1:1) was kept at room temp. overnight. After work-up, 5 mg of *2* was obtained as an oil, which had $[\alpha]_D$ +46° (*c* 0.26, EtOH); IR ($CHCl_3$): 3018, 2965, 2929, 2857, 1745, 1461, 1370, 1232, 1175, 1151, 1093, and 1057 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.80–0.95 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.1–1.4 (6H, overlapping signals 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.7–

Table 1. ^{13}C NMR chemical shifts and assignments for compounds *1* – *6*, *9* and *10*.[a]

Compound	Carbon 1	2	3	4	5	6	1′	2′	3′	4′	5′	6′
1[c]	89.2	70.6	69.2	67.7	68.9	61.6	60.5[b]	104.8	78.8	73.7	82.0	64.8[b]
3[c,d]	89.8	70.2	69.0	67.8	68.7	61.8	63.1	103.8	75.4	74.6	79.0	63.4
4[9]	89.9	70.3	69.6	68.2	68.5	61.8	62.9	104.0	75.7	75.0	79.1	63.6
5[10]	92.9	72.0	73.6	70.2	73.3	61.1	63.3	104.4	77.4	75.0	82.2	63.4
6[c]	90.2	71.2[b]	69.1[b]	67.5	68.1[b]	62.0						
2[c]	95.9	73.3[b]	72.3[b]	68.1	71.3[b]	62.1						
9[c]	89.0	69.9[b]	69.2[b]	67.5	69.1[b]	61.6						
10[c]	91.8	69.9	72.9[b]	67.5	72.1[b]	61.6						

[a] δ Values in $CDCl_3$ relative to TMS; *5* was recorded in D_2O. [b] Assignment may be reversed. [c] $OCOCH_2CH(CH_3)CH_2CH_3$ and $OCOCH_3$; δ 170.7, 171.8, 172.5, and 173.0 for *1*; δ 169.7, 169.9, 170.0, 170.5, 170.7, 171.7, 172.1, and 172.4 for *3*; δ 170.8, 171.9, 172.1, and 172.5 for *6*; δ 170.7, 172.1, and 173.7 for *2*; δ 168.7, 170.6, 171.6, 171.9, and 172.2 for *9*; δ 169.0, 170.6, 171.4, 171.6, and 172.1 for *10*; $OCOCH_2CH(CH_3)CH_2CH_3$: δ 40.9, 41.0 and 41.1 for *1*; δ 40.8, 41.0, and 41.1 for *3*; δ 41.1, and 41.2 for *6*; δ 41.0 and 41.1 for *2*; δ 40.8, 41.0, and 41.1 for *9*; δ 41.0 and 41.1 for *10*; $OCOCH_2CH(CH_3)CH_2CH_3$: δ 31.5 for *1* and *3*; δ 31.5, 31.6, and 31.7 for *6*; δ 31.6 and 31.7 for *2*; δ 31.5 and 31.6 for *9*; δ 31.4, 31.5, and 31.7 for *10*; $OCOCH_2CH(CH_3)CH_2CH_3$: δ 19.2 for *1*, *2*, *3*, *6*, and *9*; δ 19.1 and 19.2 for *10*; $OCOCH_2CH(CH_3)CH_2CH_3$: δ 29.1 and 29.2 for *1*, *9*, and *10*; δ 29.2 for *2*, *3*, and *6*; $OCOCH_2CH(CH_3)CH_2CH_3$: δ 11.2 for *1*, *2*, *3*, *6*, and *10*; δ 11.1 and 11.2 for *9*; $OCOCH_3$: δ 20.7 for *1* and *3*; δ 20.8 for *2*, *6*, and *10*; δ 20.7 and 20.9 for *9*. [d] The spectrum was recorded under WALTZ-16 conditions.[13] Because of the presence of several degenerate peaks and the occurrence of a wide range of $^1J_{C,H}$ values, a two-dimensional heteronuclear *J* method was applied to facilitate the determination of the multiplicities of the lines in the spectrum of *3*.[14]

1.9 (3H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 2.10 (6H, s, 2×$-OCOCH_3$), 2.11 (3H, s, $-OCOCH_3$), 2.12 (3H, s, $-OCOCH_3$), 2.18 (3H, s, $-OCOCH_3$), 2.0–2.4 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 4.1–4.4 (8H, overlapping signals, H–5, H–6, H–1′, H–5′, H–6′), 4.90 (dd, J = 3.7 and 10.4 Hz, H–2), 5.13 (dd, J = 9.6 and 10.1 Hz, H–4), 5.39 (dd, J = 5.9 and 6.0 Hz, H–4′), 5.46 (d, J = 6.0 Hz, H–3′), 5.50 (dd, J = 9.6 and 10.4 Hz, H–3), and 5.70 (d, J = 3.7 Hz, H–1); MS, FAB [m/z (%)]: 869 (M+Na, 0.4), 499 (5), 401 (0.5), 383 (0.4), 331 (30), 303 (0.4), 289 (3), 285 (0.9), 243 (5), 211 (37), 169 (55), 127 (13), 109 (47), and 99 (100).

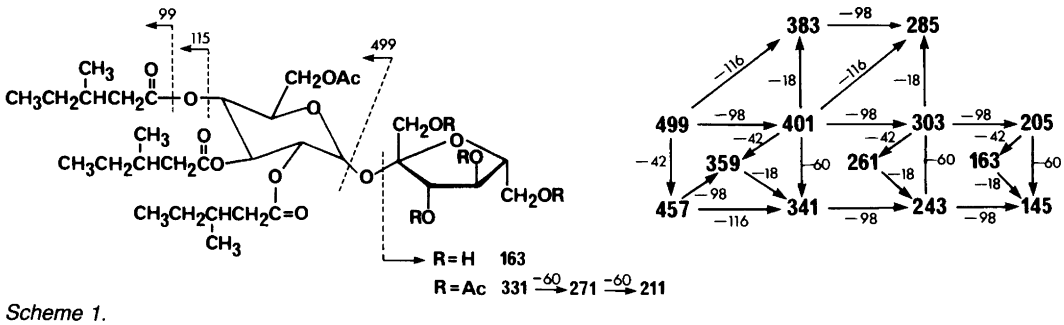
A. Hydrolysis of 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-sucrose (*1*). A solution of 100 mg of *1* in 5 ml of ethanol and O.5 ml of aqueous KOH (45 %) was kept at room temp. for 5h. The reaction mixture was diluted with water, acidified using aqueous H_2SO_4 (10 %) and extracted with Et_2O. The organic phase was dried and the Et_2O carefully removed by distillation. The residue having $[\alpha]_D$ +5° (c 0.5, $CHCl_3$) gave a ^1H NMR spectrum showing the presence of acetic and 3-methylpentanoic acid.

B. Hydrolysis of 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-sucrose (*1*). To 50 mg of *1* was added 2 ml of aqueous trifluoroacetic acid (90 %). The solution was kept at room temperature for 25 min., diluted with toluene and evaporated to give 55 mg of a residue. This was separated by HPLC using a column packed with μ-Bondapak/CN and ethyl acetate/hexane (30:70) as the eluent to give 23 mg of 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-α-D-glucopyranose (*6*) and 7 mg of 6-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glucopyranose (*2*). The former, (*6*), was amorphous and had $[\alpha]_D$ +72° (c 0.2, $CHCl_3$); IR ($CHCl_3$): 3597, 3460, 2967, 2933, 2880, 1746, 1461, and 1383 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8–1.0 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 1.1–1.4 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 1.7–2.0 (3H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 2.0–2.4 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2-$), 2.10 (s, $-OCOCH_3$), 2.90 (broad s, $-OH$), 4.14 (dd, J = 2.6 and −12.3 Hz, H–6a), 4.19 (dd, J = 4.0 and −12.3 Hz, H–6b), 4.26 (ddd, J = 2.6, 4.0, and 10.1 Hz, H–5), 4.89 (dd, J = 3.1 and 10.1 Hz, H–2), 5.13 (t, J = 10.1 Hz, H–4), 5.50 (dd, J = 3.1 and 4 Hz, H–1) and 5.60 (t, J = 10.1 Hz, H–3); MS, EI [m/z (%)]: 499 (M−17, 0.1), 469 (0.1), 442 (0.2), 401 (0.6), 354 (0.5), 285 (0.5), 269 (3), 256 (4), 200 (1), 169 (2), 99 (100), 71 (33), and 43 (31). The latter, (*2*), had m.p. 104–107 °C; $[\alpha]_D$ +30° (c 0.2, $CHCl_3$), reported m.p. 104–106 °C; $[\alpha]_D$ +30.2° (c 4.7, $CHCl_3$);[4] IR

Table 2. Chemical shift values and coupling constants for the protons of the sucrose moieties of *3* and *4*.[a]

	3		*4*[15,16]	
H–1	5.93 d	(3.7 Hz)	5.86 d	(3.7 Hz)
H–2	5.12 dd	(3.7 and 10.5 Hz)	5.03 dd	(3.7 and 10.4 Hz)
H–3	5.96 dd	(9.6 and 10.5 Hz)	5.82 dd	(9.4 and 10.4 Hz)
H–4	5.47 dd	(9.6 and 10.4 Hz)	5.38 dd	(9.4 and 10.3 Hz)
H–5	4.61 ddd	(2.3, 4.5 and 10.4 Hz)	4.51 ddd	(2.6, 4.2 and 10.3 Hz)
H–6a	4.45 dd	(2.3 and −12.4 Hz)	4.36 dd	(2.6 and −12.3 Hz)
H–6b	4.49 dd	(4.5 and −12.4 Hz)	4.41 dd	(4.2 and −12.3 Hz)
H–1′a	4.33 d	(−12.2 Hz)	4.29 d	(−12.4 Hz)
H–1′b	4.40 d	(−12.2 Hz)	4.36 d	(−12.4 Hz)
H–3′	5.77 d	(5.8 Hz)	5.70 d	(5.5 Hz)
H–4′	5.62 dd	(5.8 and 5.9 Hz)	5.54 t	(5.5 Hz)
H–5′	4.22 dt	(4.5 and 5.9 Hz)	4.19 dt	(5.4 and 5.5 Hz)
H–6′a	4.43 dd	(4.5 and −12.0 Hz)	4.40 d	(5.4 Hz)
H–6′b	4.46 dd	(5.9 and −12.0 Hz)		

[a] δ Values in C_6D_6 relative to TMS. Multiplicities are indicated by the usual symbols: d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; t, triplet; dt, doublet of triplets. Figures in parenthesis are coupling constants.



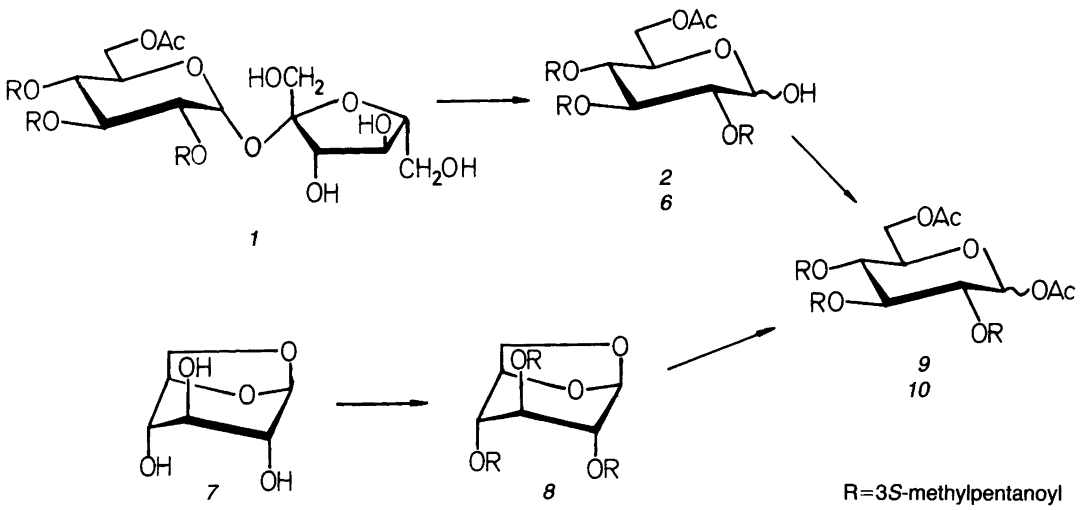
Scheme 1.

($CHCl_3$): 3600, 3465, 2967, 2932, 2878, 1746, 1461, and 1382 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8–1.0 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.1–1.4 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.7–2.0 (3H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.0–2.4 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.10 (s, –OCOCH_3), 3.57 (d, J = 9.2 Hz, –OH), 3.74 (ddd, J = 2.6, 4.6, and 10.0 Hz, H-5), 4.16 (dd, J = 2.6 and –12.3 Hz, H–6a), 4.20 (dd, J = 4.6 and –12.3 Hz, H–6b), 4.72 (dd, J = 8.9 and 9.2 Hz, H–1), 4.89 (dd, J = 8.9 and 9.8 Hz, H–2), 5.13 (dd, J = 9.7 and 10.0 Hz, H–4) and 5.33 (dd, J = 9.7 and 9.8 Hz, H–3); MS, EI [*m/z* (%)]: 499 (M–17, 0.1), 469 (0.2), 442 (0.3), 401 (0.8), 354 (0.6), 285 (0.5), 269 (3), 256 (4), 200 (1), 169 (2), 99 (100), 71 (33), and 43 (31).

MS, FAB of a mixture of the α and β anomers [*m/z* (%)]: 517 (M+H, 0.2), 499 (8), 401 (4), 383 (0.5), 341 (0.3), 303 (0.5), 285 (1), 169 (8), 127 (3), 109 (9), 99 (100), 71 (74), and 43 (53).

*Preparation of 1,6-anhydro-2,3,4-tri-*O*-[3S-methylpentanoyl]-β-*D*-glucopyranose* (8). A solution of 0.11 g of benzyl 3*S*-methylpentanoate in 2 ml of ethanol and 1 ml of KOH (aqueous, 25 %) was stirred at room temperature for 10 min. Dichloromethane was added, and the acid extracted with aqueous NaOH (1M). The aqueous phase was acidified and extracted with Et_2O, the Et_2O phase washed and concentrated to give crude 3*S*-methylpentanoic acid. Without further purification, this acid was dissolved in 0.5 ml of dichloromethane and treated with 50 µl of $SOCl_2$ (1.2 equiv.). After stirring for 1 h, 20 mg of 1,6-anhydro-β-D-glucopyranose (*7*) dissolved in 0.5 ml of pyridine were added. After an additional 30 min, the reaction mixture was applied to the top of a silica gel column and eluted with ethyl acetate/hexane (20:80) to give 39 mg of 1,6-anhydro-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glucopyranose (*8*) as a syrup, $[\alpha]_D$ –28° (*c* 0.7, $CHCl_3$); IR ($CHCl_3$): 2967, 2933, 2879, 1734, 1461, 1382, 1287, 1241, 1176, 1151, 1121, 1097, 1046, and 1025 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.85–1.0 (18 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.2–1.5 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.8–2.0 (3 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.1–2.5 (6H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 3.81 (dd, J = 5.4 and –7.8 Hz, H–6a), 4.10 (dd, J = 1.0 and –7.8 Hz, H–6b), 4.55–4.65 (overlapping signals, H–2, H–4, and H–5), 4.86 (m, H–3), and 5.46 (m, H–1).

*Acetolysis of 1,6-anhydro-2,3,4-tri-*O*-[3S-methylpentanoyl]-β-*D*-glucopyranose* (8). To a solution of 38 mg of *8* in 1 ml of Ac_2O was added 0.1 ml of Ac_2O/H_2SO_4 (1 % H_2SO_4). After 15 min at room temperature, water and dichloromethane were added to the reaction mixture. The organic phase was washed with aqueous $NaHCO_3$ and water and concentrated to yield 38 mg of an oil. This was separated by HPLC (Spherisorb 5 CN, ethyl acetate/hexane 10:90) to give 28 mg of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-α-D-glucopyranose (*9*) and 6 mg of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-β-D-glucopyranose (*10*). The former, (*9*), had m.p. 60–62 °C; $[\alpha]_D$ +80° (*c* 0.3, EtOH); IR (CCl_4): 2966, 2929, 2877, 1755, 1455, 1369, 1224, 1175, 1154, 1120, 1091, 1031, 1011, and 938 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8–1.0 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.1–1.4 (6 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.7–1.9 (3H, overlapping signals,



Scheme 2.

3×$CH_3CH_2CH(CH_3)CH_2$–), 2.0–2.4 (6 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.10 (s, –$OCOCH_3$), 2.17 (s, –$OCOCH_3$), 4.05–4.3 (overlapping multiplets, H–5, H–6a and H–6b), 5.09 (dd, J = 3.7 and 10.3 Hz, H–2), 5.19 (dd, J = 9.6 and 9.7 Hz, H–4), 5.54 (dd, J = 9.6 and 10.3 Hz, H–3), and 6.35 (d, J = 3.7 Hz, H–1); MS, EI [*m/z* (%)]: 515 (M–43, 0.3), 499 (1), 442 (1), 354 (2), 301 (0.7), 285 (0.6), 269 (3), 256 (5), 196 (2), 169 (4), 109 (3), 99 (100), 71 (34), 57 (5), and 43 (38).

The latter, (*10*), had m.p. 80–81 °C; $[\alpha]_D$ +7° (*c* 0.3, EtOH); IR (CCl_4): 2966, 2931, 2878, 1755, 1461, 1382, 1367, 1218, 1178, 1150, 1119, 1079, 1013, and 907 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.8–1.0 (18H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.1–1.4 (6 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 1.7–1.9 (3H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.0–2.4 (6 H, overlapping signals, 3×$CH_3CH_2CH(CH_3)CH_2$–), 2.09 (6 H, s, 2×–$OCOCH_3$), 3.83 (ddd, J = 2.4, 4.5 and 9.6 Hz, H–5), 4.13 (dd, J = 2.4 and –12.5 Hz, H–6a), 4.21 (dd, J = 4.5 and –12.5 Hz, H–6b), 5.17 (dd, J = 9.3 and 9.6 Hz, H–4), 5.17 (dd, J = 7.9 and 9.3 Hz, H–2), 5.30 (t, J = 9.3 Hz, H–3), and 5.72 (d, J = 7.9 Hz, H–1); MS EI [*m/z* (%)]: 515 (M–43, 0.3) 499 (0.2), 442 (0.8), 354 (2), 327 (0.6), 301 (0.6), 284 (1), 269 (2), 256 (6), 196 (2), 169 (3), 109 (2), 99 (100), 71 (36), 57 (5), and 43 (38).

Acetylation of 6-O-acetyl-2,3,4-tri-O-[3S-methylpentanoyl]-D-glucopyranose (*6 and* 2). A solution of 20 mg of a mixture of *6* and *2* in 2 ml of Ac_2O/pyridine (1:1) was kept at room temperature for 1 h. After dilution with toluene and concentration at reduced pressure, the reaction mixture was purified by flash chromatography over silica gel to give 18 mg of mixture. This was separated by HPLC (Spherisorb 5 CN; ethyl acetate/hexane, 10:90) into two diacetates, which were identical in all respects (m.p., mixed m.p., optical rotation, IR, ^1H NMR and MS) to the α- and β-anomers of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-[3*S*-methylpentanoyl]-D-glucopyranose *9* and *10*, respectively.

Preparative enantiomer separation of (±)*-benzyl 3-methylpentanoate.* Methods for the preparation of the triacetylated cellulose and the columns as well as the instrumental details have been described elsewhere.[12] The sample of (±)-benzyl 3-methylpentanoate was dissolved in ethanol/water (95:5), and an injection volume of 4 ml was used for ~100 mg of the ester. Due to the poor separation (separation factor ~1.35), the ester had to be cycled nine times through the columns. After each cycle, the (+) enantiomer was depleted of the (–) enantiomer. The fractions collected were concentrated to give in all ~10 mg of the (+) enantiomer, which was virtually 100% pure as determined by NMR technique ($CDCl_3$; Eu $(hfbc)_3$).

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