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SYNTHESIS AND ^{13}C-N.M.R. SPECTROSCOPIC INVESTIGATION OF THREE METHYL RHAMNOTRIOSIDES

ANDRÁS LIPTÁK, JÁNOS HARANGI,
Institute of Biochemistry, L. Kossuth University, H-4010 Debrecen (Hungary)

GYULA BATTA,
Institute of Organic Chemistry, L. Kossuth University, H-4010 Debrecen (Hungary)

OTTO SELIGMANN, AND HILDEBERT WAGNER
Institut für Pharmazeutische Biologie der Universität München, D-8000 München 2 (West Germany)
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ABSTRACT

Methyl 2,3-*O*-isopropylidene-4-*O*-(2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside, methyl 2,4-di-*O*-benzyl-3-*O*-(2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside, and methyl 3,4-di-*O*-benzyl-2-*O*-(2,3-*O*-isopropylidene-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside, obtained by isopropylidenation of the respective methyl rhamnobiosides, were glycosylated with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide. Removal of the protecting groups from the products gave the methyl glycosides of α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→4)-α-L-Rha*p*, α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, and α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→2)-α-L-Rha*p*. These trisaccharide glycosides and their hepta-acetates have been studied by ^{13}C-n.m.r. spectroscopy.

INTRODUCTION

Oligosaccharides containing L-rhamnose are the components of a number of naturally occurring plant glycosides[1–3], glycolipids[4–8], and bacterial cell-wall polysaccharides[9]. The importance of these different classes of compounds explains the efforts which have been made to synthesise rhamnose-containing oligosaccharides[10–20] having different bond-types and anomeric configurations.

We now describe the synthesis of the methyl α-glycosides (**13–15**) of the trisaccharides α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→4)-α-L-Rha*p*, α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→3)-α-L-Rha*p*, and α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→2)-α-L-Rha*p*, of which the last has been reported[21] as the linear part of the repeating unit of the lipopolysaccharide isolated from *Pseudomonas maltophilia* N.C.T.C. 10257. The tetrasaccharide repeating-unit has the structure depicted.

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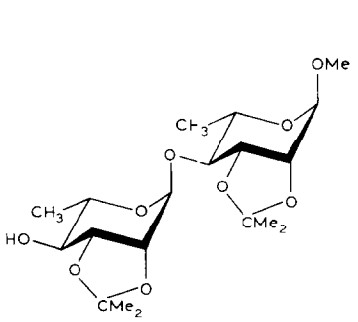
3-*O*-Me-β-L-Xyl*p*
1
↓
4
→3)-α-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→

The other two trisaccharides were synthesised for ^{13}C-n.m.r. spectroscopic investigations.

RESULTS AND DISCUSSION

Methyl 2,3-*O*-isopropylidene-4-*O*-[10] (**1**), methyl 2,4-di-*O*-benzyl-3-*O*-[14] (**2**), and methyl 3,4-di-*O*-benzyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside[14] (**3**) were severally saponified and the products, without isolation, were treated with 2,2-dimethoxypropane in the presence of toluene-*p*-sulphonic acid. Isopropylidenation was complete within 15 min to give the respective partially protected disaccharide derivatives (**4–6**) having HO-4′ unsubstituted. Compounds **4–6** were severally rhamnosylated, according to the Helferich procedure, to give the fully substituted trisaccharide derivatives **7–9**. Hydrolysis of **7** with trifluoroacetic acid removed the isopropylidene groups and acetylation of the product gave the crystalline methyl trisaccharide-glycoside hepta-acetate **10**. Likewise, hydrogenolysis of **8** and **9** cleaved the benzyl groups, and subsequent hydrolysis



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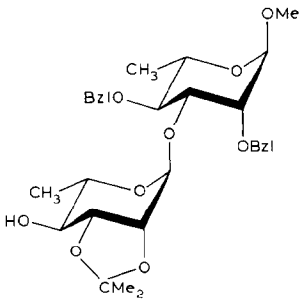

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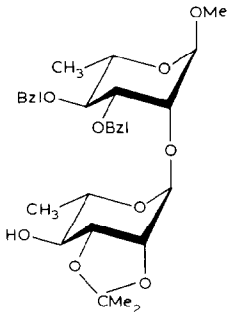

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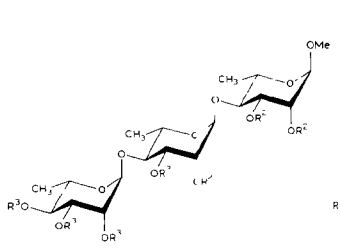
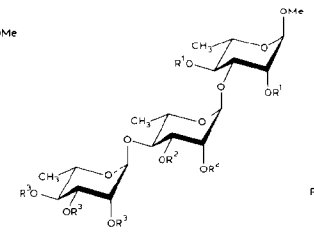
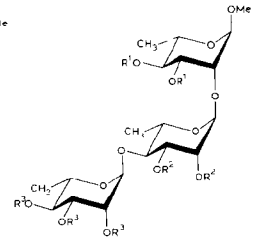


7
10
13



8 R^1 = Bzl, R^2, R^3 = CMe_2, R^4 = Ac
11 R^1 = R^2 = R^3 = Ac
14 R^1 = R^2 = R^3 = H



9
12
15

with trifluoroacetic acid removed the isopropylidene group, and gave **11** and **12**, respectively. Zemplén deacetylation of **10–12** yielded the crystalline methyl glycosides **13–15**.

Since the configurations of the interglycosidic linkages of the starting disaccharides **1–3** were known, only those of the newly formed linkage needed verification. The chemical shift of the signal of the anomeric carbon of α- and β-L-rhamnopyranosides[22–24] is markedly dependent on the structure and chirality of the aglycons, and the value of $J_{C\text{-}1,H\text{-}1}$ and the chemical shifts of the signals of C-3 and C-5 are also diagnostic[23,24].

The ^{13}C-n.m.r. data for **10–15** are listed in Table I. The most characteristic chemical shifts are underlined. From the data, it can be seen that the non-reducing end-group is α for each trisaccharide derivative. The chemical shift of the C-1 signal for **12** is higher than that for **10** or **11**, whereas glycosylation at position 2 results in a negative β-shift at C-1. However, acetylation causes a greater β-shift, and, hence, the C-1 atoms of **10** and **11** resonate at higher field than C-1 of **12**. In the spectra of the deacetylated compounds **13–15**, there are some characteristic lines that are valuable for the determination of bond types. When a (1→2) linkage is present, there is a strong negative β-shift at C-1 (**15**), and the (1→4) linkage causes a shift of +0.5–0.6 p.p.m. at C-6. The assignment of the most characteristic lines of the spectra of **13–15** was straightforward, and valuable information concerning

TABLE I

^{13}C-N M.R. CHEMICAL SHIFTS OF METHYL RHAMNOTRIOSIDES AND THEIR PERACETYLATED DERIVATIVES

Atom	**10**	**11**	**12**	**13**		**14**		**15**	
C-1	98.46	98.40	99.57	101.48		101.49		100.20	
C-2	70.39	71.00	76.30	71.79		70.60	(71.13)[a]	76.13	
C-3	71.80	75.48	70.88	71.62		78.71		70.65	(70.82)
C-4	79.14	72.35	71.46	80.93		72.17		72.80	
C-5	67.45	67.31	66.37	67.55		69.31		69.20	
C-6	18.17	17.49	17.54	18.07		17.43		17.37	
C-1′	99.33	98.81	99.45	102.35		102.75		102.64	
C-2′	70.26	70.41	70.22	71.62		71.41		71.22	
C-3′	71.16	71.19	70.17	71.62	(71.09)	71.56	(70.60)	71.71	(70.65)
C-4′	78.88	78.97	79.02	80.35		80.50		80.47	
C-5′	67.90	67.75	67.75	68.57		68.24		68.18	
C-6′	17.95	17.98	18.05	18.07		18.08		18.01	
C-1″	99.33	99.32	99.06	102.35		102.29		102.24	
C-2″	70.12	70.20	70.22	71.09	(71.62)	71.13	(71.56)	70.98	(71.71)
C-3″	68.82	68.77	68.70	70.94		71.00		70.82	(70.98)
C-4″	71.28	71.10	71.04	72.54		72.63		72.48	
C-5″	66.84	66.56	66.37	70.12		70.08		70.00	
C-6″	17.27	17.27	17.27	17.25		17.26		17.15	
OCH_3	55.10	55.03	54.90	55.30		55.23		55.57	

[a]Data given in brackets were assigned on the basis of comparison with model compounds.

the bond types and anomeric configurations could be drawn from the values of the α- and β-shifts. However, the exact assignments of some lines were questionable (the assignments based on comparison with model compounds are given in brackets); hence. for the trisaccharide derivatives **13–15**, unambiguous ^{13}C assignments were obtained by using two-dimensional n.m.r. methods. After having assigned the ^{1}H-n.m.r. (200 MHz) spectra of these compounds[25] with the aid of homonuclear shift-correlation (COSY) spectroscopy[26], various types of 2D heteronuclear shift-correlation experiments[27,28] and heteronuclear relayed coherence transfer spectroscopy[29] were used to establish the assignments given in Table I. Details of the two-dimensional experiments will be published elsewhere.

EXPERIMENTAL

General methods. — Melting points were determined with a Kofler apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at 23 ±1°. ^{13}C-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) or D_2O (internal 1,4-dioxane, δ 67.5) with a Bruker WP-80 instrument at ambient temperature. A 1-μs pulse width and 4000-Hz spectral width were used, and an average scan number of 60,000. The 2D measurements were obtained with a Bruker WP-200 SY instrument. For sensitivity purposes in heteronuclear shift-correlated experiments at 50.3 MHz, a solution of the compound (**13–15**, 100–150 mg) in D_2O (435 μL) in a cylindrical sample-bulb (Wilmad 529-E-10) was used. Other technical details of the 2D experiments will be described elsewhere. T.l.c. was performed on Kieselgel G (Merck).

Methyl 2,3-O-isopropylidene-4-O-(2,3-O-isopropylidene-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**4**). — A solution of **1** (2.45 g) in methanol (50 mL) containing a catalytic amount of sodium methoxide was left overnight at room temperature. Sodium ions were removed with Amberlite IR-120 (H^+) resin, the solution was concentrated, and the residue was treated with 2,2-dimethoxypropane (6 mL) in the presence of toluene-*p*-sulphonic acid (0.05 g) for 15 min at room temperature. The mixture was diluted with dichloromethane (100 mL), washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and water (3 × 50 mL), dried, and concentrated, to give syrupy **4** (1.86 g, 92%), $[\alpha]_D$ −46° (*c* 1.5, chloroform), R_F 0.47 (dichloromethane–acetone, 9:1). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.52 (s, 1 H, H-1′), 4.81 (s, 1 H, H-1), 3.31 (s, 3 H, OMe), 2.84 (b, 1 H, OH), and 1.60–1.18 (m, 18 H, 6 CMe).

Anal. Calc. for $C_{19}H_{32}O_9$: C, 56.42; H, 7.97. Found: C, 56.65; H, 8.03.

Methyl 2,4-di-O-benzyl-3-O-(2,3-O-isopropylidene-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**5**). — Compound **2** (2.8 g) was treated as described above for **1**, to give syrupy **5** (2.15 g, 89%), $[\alpha]_D$ −12° (*c* 1.15, chloroform), R_F 0.45 (light petroleum–ethyl acetate, 2:1).

Anal. Calc. for $C_{30}H_{40}O_9$: C, 66.15; H, 7.40. Found: C, 66.42; H, 7.56.

Methyl 3,4-di-O-benzyl-2-O-(2,3-O-isopropylidene-α-L-rhamnopyranosyl)-α-

L-*rhamnopyranoside* (**6**). — Compound **3** (2.50 g) was treated as described above for **1**, and the product was crystallised from cyclohexane–hexane (20 mL, 1:1) to give **6** (2.06 g, 94%), m.p. 100°, $[\alpha]_D$ −13.5° (*c* 0.95 chloroform), R_F 0.48 (light petroleum–ethyl acetate, 2:1).

Anal. Calc. for $C_{30}H_{40}O_9$: C, 66.15; H, 7.40. Found: C, 66.60; H, 7.59.

Methyl 2,3-O-isopropylidene-4-O-[2,3-O-isopropylidene-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**7**). — A solution of **4** (1.21 g) in dry acetonitrile (8 mL) was stirred with $Hg(CN)_2$ (1.13 g) and 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (1.59 g) for 4 h at room temperature. The mixture was diluted with dichloromethane (50 mL), filtered, washed with aqueous 5% KI (3 × 30 mL) and water (3 × 30 mL), dried (Na_2SO_4), and concentrated. The crude product (2.1 g) was purified on Kieselgel G (100 g) by elution with dichloromethane–ethyl acetate (85:15), to give **7** (1.48 g, 73.3%), m.p. 76–81° (from ethanol–water), $[\alpha]_D$ −72° (*c* 0.66 chloroform), R_F 0.71 (dichloromethane–ethyl acetate, 85:15).

Anal. Calc. for $C_{31}H_{44}O_{16}$: C, 55.34; H, 6.59. Found: C, 55.80; H, 6.71.

Methyl 2,4-di-O-benzyl-3-O-[2,3-O-isopropylidene-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**8**). — Compound **5** (1.85 g) was glycosylated as described above for **7**. The crude product was purified on Kieselgel G (120 g) by elution with dichloromethane–ethyl acetate (9:1), to give syrupy **8** (1.58 g, 57%), $[\alpha]_D$ −47° (*c* 1.1 chloroform), R_F 0.85 (dichloromethane–ethyl acetate, 9:1).

Anal. Calc. for $C_{42}H_{56}O_{16}$: C, 61.75; H, 6.91. Found: C, 61.35; H, 7.06.

Methyl 3,4-di-O-benzyl-2-O-[2,3-O-isopropylidene-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**9**). — Compound **6** (1.6 g) was glycosylated as described above for **7**. The crude syrupy product was purified on Kieselgel G (75 g) by elution with dichloromethane–ethyl acetate (9:1), to give **9** (1.26 g, 53%), $[\alpha]_D$ −48° (*c* 1.5 chloroform), R_F 0.82 (dichloromethane–ethyl acetate, 9:1).

Anal. Calc. for $C_{42}H_{56}O_{16}$: C, 61.75; H, 6.91. Found: C, 62.00; H, 7.03.

Methyl 2,3-di-O-acetyl-4-O-[2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**10**). — A solution of **7** (1.2 g) in dichloromethane (72 mL) was treated with trifluoroacetic acid (9 mL) containing water (0.09 mL) at room temperature. The reaction was monitored by t.l.c. (dichloromethane–acetone, 7:3); after 1 h, both isopropylidene groups had been hydrolysed. The solution was concentrated *in vacuo*, and toluene (10 mL) was distilled from the residue, which was then treated with acetic anhydride (10 mL) and pyridine (10 mL) for 4 h. The reaction mixture was concentrated, the syrupy residue was treated with ice–water, and the crude product was collected and crystallised from ethanol (20 mL) to give **10** (1.05 g, 77%), m.p. 228–230°, $[\alpha]_D$ −47° (*c* 0.66, chloroform), R_F 0.69 (dichloromethane–acetone, 9:1).

Anal. Calc. for $C_{33}H_{48}O_{20}$: C, 51.82; H, 6.32. Found: C, 51.92; H, 6.40.

Methyl 2,4-di-O-acetyl-3-O-[2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-

rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**11**). — Compound **8** (1.45 g) was treated with trifluoroacetic acid (6 mL) in dichloromethane (50 mL). When hydrolysis of the isopropylidene group was complete (t.l.c., 1 h), the mixture was concentrated, and the residue was dried, and hydrogenolysed over 10% Pd/C (0.3 g) in 1:1 ethanol–ethyl acetate (50 mL) for 12 h. The usual work-up gave a syrupy product that was treated with acetic anhydride (5 mL) in pyridine (5 mL). The crude product was crystallised from ethanol (4 mL) to give **11** (1.1 g, 81%), m.p. 162–164°, $[\alpha]_D$ −40° (*c* 0.76, chloroform), R_F 0.68 (dichloromethane–acetone, 9:1).

Anal. Calc. for $C_{33}H_{48}O_{20}$: C, 51.82; H, 6.32. Found: C, 51.67; H, 6.42.

Methyl 3,4-di-O-acetyl-2-O-[2,3-di-O-acetyl-4-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (**12**). — Compound **9** (0.89 g) was treated as described above for **8**, to give **12** (0.65 g, 78%), m.p. 95–98° (from ethanol–water), $[\alpha]_D$ −46° (*c* 1, chloroform), R_F 0.74 (dichloromethane–acetone, 9:1).

Anal. Calc. for $C_{33}H_{48}O_{20}$: C, 51.82; H, 6.32. Found: C, 51.99; H, 6.39.

Methyl O-α-L-rhamnopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside (**13**). — Compound **10** (0.80 g) was saponified with NaOMe (10 mg) in dry methanol (20 mL) for 8 h. The solution was neutralised with Amberlite IR-120 (H^+) resin, filtered, and concentrated. The syrupy residue crystallised spontaneously to give **13** (0.46 g, 93.5%), m.p. 148–151°, $[\alpha]_D$ −113° (*c* 0.8, water).

Anal. Calc. for $C_{19}H_{34}O_{13}$: C, 48.50; H, 7.28. Found: C, 48.67; H, 7.40.

Methyl O-α-L-rhamnopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→3)-α-L-rhamnopyranoside (**14**). — Compound **11** (0.57 g) was saponified, as described for **10**, to give **14** (0.30 g, 85%), m.p. 92–96°, $[\alpha]_D$ −86° (*c* 0.6, water), R_F 0.69 (1-butanol–methanol–water, 2:1:1).

Anal. Calc. for $C_{19}H_{34}O_{13}$: C, 48.50; H, 7.28. Found: C, 48.71; H, 7.35.

Methyl O-α-L-rhamnopyranosyl-(1→4)-O-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranoside (**15**). — Compound **12** (0.30 g) was saponified, as described for **10**, to give **15** (0.169 g, 91.5%), m.p. 88–92°, $[\alpha]_D$ −70° (*c* 0.7, water), R_F 0.66 (1-butanol–methanol–water, 2:1:1).

Anal. Calc. for $C_{19}H_{34}O_{13}$: C, 48.50; H, 7.28. Found: C, 48.47; H, 7.37.

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