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Note

Synthesis of some oligosaccharide methyl glycosides

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The simplicity of the methanolic HCl method of glycoside formation has led to its wide use in the monosaccharide series. Conditions may be adjusted to permit selected glycosides to be formed in maximum yield, and isolation by a variety of chromatographic methods has been applied.

We have shown[1] that reversed-phase l.c. may be used to resolve and isolate many monosaccharide methyl glycosides in a short time. In addition, we found[2] that the glycosidic linkage of maltose is sufficiently resistant to methanolysis (1% methanolic HCl, at r.t.) to allow the ready isolation of methyl α- and β-maltoside. As chemical and enzymic methods for the formation of oligosaccharide glycosides are somewhat tedious, we report here an extension to trisaccharides of our procedure[2]. There appears to be no reason why the glycosides of larger oligosaccharides could not be prepared if necessary, although a limiting factor would be the availability of the starting oligomers.

The methyl glycosides were readily prepared, as the rate of scission of the inter-residue glycosidic bonds was lower than that of glycosidation.

Under the conditions used, the principal isomaltose and isomaltotriose glycosides isolated had the original reducing-end residues converted into the previously unknown furanoside forms. Small proportions of the pyranosides were also formed.

Scott and Senti[3] prepared a homologous series of methyl glycosides by methanolysis of dextrans. Their compounds were assigned the pyranoid form, on the basis of optical rotation, and stability to mild hydrolysis with acid. Jeanes *et al.*[4] prepared an isomaltose series terminated by a methyl α-D-glucopyranoside residue, which was the acceptor for D-glucosyl groups transferred from sucrose by a dextransucrase.

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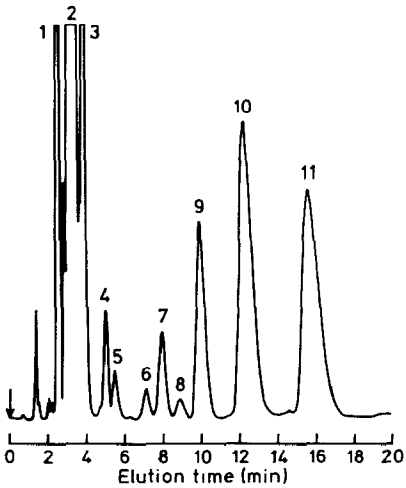

Fig. 1. L.c. profile of the products formed by treatment of maltotriose with methanolic HCl for 89 h. Column, Dextropak; solvent, water at 2 mL/min; pressure, 10 MPa. Peak 1, methanol; 2, maltotriose; 3, methyl α- & β-D-glucopyranoside; 4, methyl α-D-glucofuranoside; 5, methyl β-D-glucofuranoside; 6 & 8, unidentified; 7, methyl α-maltoside; 9, methyl β-maltoside; 10, methyl α-maltotrioside; and 11, methyl β-maltotrioside.

RESULTS AND DISCUSSION

Maltotriosides

Fig. 1 shows the elution profile, in l.c., of the products from the treatment of maltotriose with methanolic hydrogen chloride. As would be expected, methyl D-glucosides and methyl maltosides are present. Two small peaks, 6 and 8, remain unidentified, and possibly arise from impurities in the maltotriose. They are in the region of disaccharide glycosides, but furanosides cannot be formed in the (1→4)-linked maltose series. It is considered unlikely that they represent non-(1→4)-linked reversion products, as no evidence for the formation of such compounds has been obtained in previous experiments involving monosaccharide[1] or disaccharide[2] glycosidations. Likewise, no evidence for the formation of dimethyl acetals has been noted in previous experiments.

During a time study of the maltotriose glycoside formation it was noted that, even after 96 h, a substantial proportion of unreacted maltotriose remained. The pattern in Fig. 1 was achieved in ~80 h, and remained similar as long as maltotriose was still present. ^{1}H-N.m.r. spectroscopy readily identified peaks 7 and 9 as those for methyl α- and β-maltoside, respectively, and peaks 10 and 11 as methyl α- and β-maltotrioside, respectively. The ^{13}C-n.m.r. spectrum of the methyl β-trioside showed 18 peaks (see Table I), the only two coincident peaks being those for C-6′ and C-6″. The α-trioside showed 17 peaks, all three C-6 resonances being coincident. The ^{13}C-n.m.r. spectrum of maltotriose exhibited 26 peaks, the complexity arising due to the two anomeric forms of the reducing group. The influence

TABLE I

^{13}C-N.M.R CHEMICAL SHIFTS (p.p.m.) FOR METHYL α- AND β-MALTOTRIOSIDE [a] AT 75 MHz

Glycoside	*Unit*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*
Methyl α-maltotrioside	"reducing" end	99.13	71.08	73.52	76.96	70.13	60.56
	middle	99.81	71.59	72.75	77.21	71.25	60.56
	nonreducing	99.55	72.92	73.35	69.40	71.79	60.56
Methyl β-maltotrioside	"reducing" end	103.12	72.98	76.21	77.14	74.58	60.75
	middle	99.82	71.51	72.76	76.96	71.27	60.56
	nonreducing	99.51	72.94	73.33	69.40	71.80	60.56

[a]Reference standard: external Me_4Si.

of the free α- and β-anomeric groups extended at least to C-4 of the middle D-glucosyl residue, there being 4 peaks (at δ 77.35, 77.13, 77.01, and 76.98) in the linked C-4 region. Careful comparison of the spectra showed that there were five groups of resonances that were within 0.05 p.p.m. across the three compounds maltotriose, methyl α-maltotrioside, and methyl β-maltotrioside. These, together with the values of Usui *et al.*[5] and Colson *et al.*[6], permitted the assignments in Table I to be made, but some of these must be regarded as tentative, as no other n.m.r. experiments were performed in order confirm them. Attempts to synthesize glycosides of the maltose series by methanolysis of amylose[7] were only partly successful. The products were mainly the methyl D-glucopyranosides, with a small proportion of the maltose pyranosides. Much of the amylose remained unreacted and insoluble in the methanolysis mixture.

Methyl isomaltosides

Fig. 2a shows the 10-MPa liquid chromatogram of the glycosidation products from isomaltose after glycosidation for 18 h. In contrast to maltotriose (and maltose[2]), most of the starting material had reacted by then. This is attributed to the fact that, in the (1→6)-linked series, furanosides form rapidly. Peaks 7 and 8 are those of the β- and α-furanosides, respectively. Even after 95 h (see Fig. 2b), substantial proportions of the furanosides are still present, although a higher proportion of a pyranoside (peak 6) is present. The other pyranoside peak is, presumably, under one of the franoside peaks; its concentration was too small to permit it to be detected in the n.m.r. spectra of the furanosides isolated. Also, the stability of the α-D-(1→6) linkage to methanolysis is greater than that of the α-D-(1→4) linkage, as relatively little of the glucosides appear within 95 h (see Fig. 2b) compared with those of maltose[2] and maltotriose (see Fig. 1) prepared under similar conditions; this is in keeping with observations concerning the relative resistance of these linkages to hydrolysis with aqueous acid[8].

Peaks 1–5 of Fig. 2 were identified by comparisons of their retention times with those of the appropriate standards.

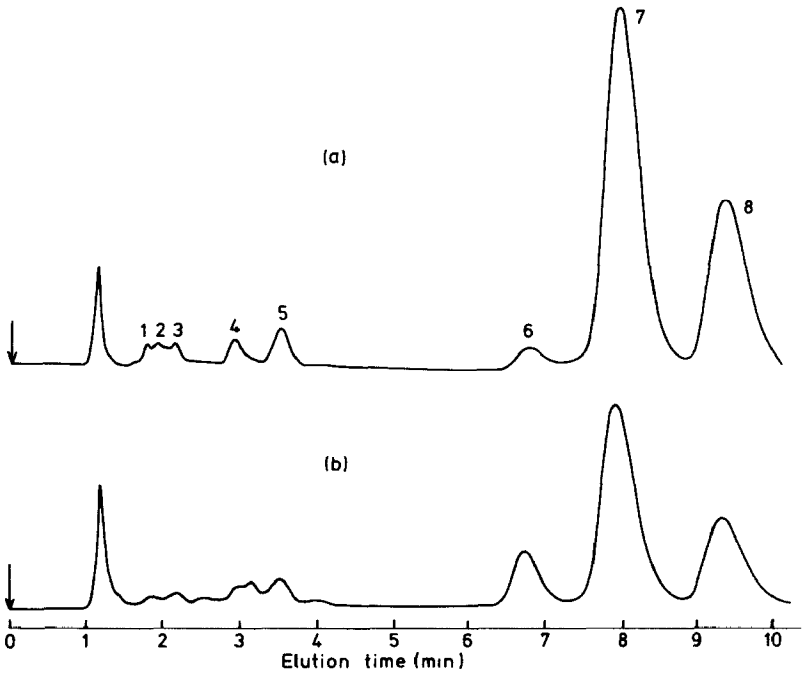

Fig. 2. L.c. profile of the products formed by treatment of isomaltose with methanolic HCl. Column, Dextropak; solvent, water at 2 mL/min; pressure, 10 MPa. (a) After 18 h; (b) after 95 h at r.t. Peak 1, isomaltose; 2–5, methyl D-glucosides; 6, methyl 6-*O*-β-D-glucopyranosyl-α-D-glucopyranoside (tentative); 7, methyl 6-*O*-α-D-glucopyranosyl-β-D-glucofuranoside; and 8, methyl 6-*O*-α-D-glucopyranosyl-α-D-glucofuranoside.

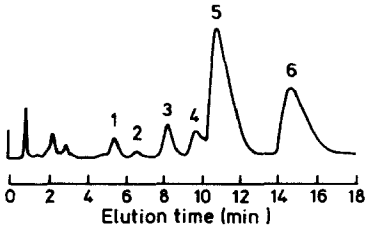

Fig. 3. L.c. profile of the products formed by treatment of isomaltotriose with methanolic HCl for 42 h. Column, Dextropak; solvent, water at 2 mL/min; pressure, 10 MPa. Peaks 1 and 2, unidentified; 3, methyl 6-*O*-α-isomaltosyl-α-D-glucopyranoside; 4, methyl 6-*O*-α-isomaltosyl-β-D-glucopyranoside; 5, methyl 6-*O*-α-isomaltosyl-β-D-glucofuranoside; and 6, methyl 6-*O*-α-isomaltosyl-α-D-glucofuranoside.

Isomaltotriosides

Fig. 3 shows the pattern of glycosidation of isomaltotriose (IM_3) after 42 h. Again, the stability of the (1→6) linkage is evident, as 90% of the products consist of the pyranosides (peaks 3 and 4) and furanosides (peaks 5 and 6) of IM_3. The pyranosides and furanosides were readily identified by ^1H- and ^{13}C-n.m.r. spectroscopy.

It is believed that this is the first reported synthesis of the furanosides glycosides in the isomaltose series. The use of high-field ^1H- and ^{13}C-n.m.r. spectroscopy[9] was invaluable in assigning structures to the various glycosides in this study. Thus, in the proton spectra of the furanoside moieties, the presence of the furanoid ring was indicated by a multiplet between 4.0 and 4.5 p.p.m.

The anomeric protons of the β anomers gave singlets at ~4.75 p.p.m., and the low-field position observed for the β-furanosides, namely, doublets ($J_{1,2}$ ~4 Hz) at 5.05 p.p.m., is also consistent with furanosides. ^{13}C-N.m.r. spectra also showed the downfield shifts of furanosides relative to pyranosides[10,11]. Typically, C-1 of the α-furanoside resonated at ~103 p.p.m., and of the β, at ~110 p.p.m.

Methanolysis of dextran B512(F) in the form of Dextran T_{10} (Pharmacia) would be expected[3] to yield glycosides of the isomaltose series in the pyranose forms. On using the conditions of Scott and Senti[3], *viz.*, refluxing (6 h) in methanolic HCl (M) with added water (0.4%), it was found that the principal products were the methyl glucopyranosides and the pyranose forms of methyl isomaltosides. Traces of the pyranoside isomaltotriosides were detected. Thus, by optimizing the conditions, it should be possible to isolate the pyranose forms up to at least the isomaltotriosides.

EXPERIMENTAL

The l.c. system comprised a Waters M6000 pump, a U6K injector, an RCM100 radial-compression module, and a Waters Dextropak cartridge (10 × 0.8 cm) which, for use, was compressed in the RCM100. Detection was achieved by refractive index (ERMA ERC-7510, ERMA Optical Co, Tokyo). The solvent for l.c. separations was water purified by a Milli-Q system (Millipore Corp.). Maltotriose was obtained from Sigma Chemicals. Isomaltose and isomaltriose were gifts from Dr. G. J. Walker. Optical rotations were determined with a Bendix NPL Automatic Polarimeter 143C. N.m.r. spectra (^{13}C and ^1H) were recorded at 75 and 300 MHz, respectively, for solutions in deuterium oxide at 27°, in 5-mm or 10-mm tubes, with a Bruker CXP-300 spectrometer operated in the Fourier-transform mode, the reference for ^1H being internal acetone (1.81 p.p.m.), and, for ^{13}C, Me_4Si (external, concentric tube). Methanolic hydrogen chloride, 1% (v/v), was prepared by dissolving acetyl chloride in dry methanol.

Typically, glycosides were formed by shaking the dried oligosaccharide with methanolic hydrogen chloride (5 mg/mL). Samples (1 mL) were withdrawn at intervals, made neutral with Amberlite IRA-400 (HCO_3^-) resin, and evaporated at 40°. The product was mixed with water, the suspension filtered, and the filtrate subjected to l.c. in water, in a Dextropak, at a flow-rate of 2 mL/min. When reaction times for optimal formation of glycosides had been determined in this way, batches commencing with 50–100 mg of carbohydrate were prepared, and the material in each peak of interest was isolated by repeated injections, and collections from the exit tube of the detector. Chromatographic purity was checked by re-injection of collected samples.

Glycosides of maltotriose. — Treatment of maltotriose (500 mg) for 89 h under the conditions described yielded two major products (peaks 10 and 11 in Fig. 1). The first of these was characterized as *methyl 4-O-α-maltosyl-α-D-glucopyranoside,* yield 19.5 mg, $[\alpha]_D$ +180° (*c* 0.97, water) {lit.[7] $[\alpha]_D$ +200°, water}; ^{1}H-n.m.r.: δ 5.29 (d, 2 H, $J_{1,2}$ 3.7 Hz, H-1′,1″); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.56. Found: C, 43.9; H, 6.61.

The second product was *methyl 4-O-α-maltosyl-β-D-glucopyranoside,* $[\alpha]_D$ +114° (*c* 1.01, water); ^{1}H-n.m.r.: δ 4.29 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1) and 5.28 (d, 2 H, $J_{1,2}$ 3.8 Hz, H-1′,1″); for ^{13}C-n.m.r., see Table I.

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.56. Found: C, 43.8; H, 6.63.

Glycosides of isomaltose. — Treatment of isomaltose (40 mg) for 42 h under the conditions described yielded, as major products, peaks 7 and 8 in Fig. 2. The first was characterized as *methyl 6-O-α-D-glucopyranosyl-α-D-glucofuranoside,* yield 1.7 mg; $[\alpha]_D$ +112.8° (*c* 0.14, water); insufficient for analysis; ^{1}H-n.m.r.: δ 4.86 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1′) and 5.06 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1).

The second product was *methyl 6-O-α-D-glucopyranosyl-β-D-glucofuranoside,* yield 2.5 mg; $[\alpha]_D$ +64.9° (*c* 0.21, water); insufficient for analysis; ^{1}H-n.m.r.: δ 4.70 (s, 1 H, $J_{1,2}$ ~0 Hz, H-1) and 4.86 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1′); ^{13}C-n.m.r.: δ 108.8 (C-1) and 102.7 (C-1′).

Glycosides of isomaltriose. — Treatment of isomaltotriose (50 mg) for 42 h under the standard conditions yielded four glycosides (peaks 3–6 in Fig. 3) which were respectively identified as follows. *Methyl 6-O-α-isomaltosyl-α-D-glucopyranoside,* yield 3 mg; $[\alpha]_D$ +43.9° (*c* 0.18, water); insufficient for analysis; ^{1}H-n.m.r.: δ 4.73 (t, 2 H, H-1′,1″) and 4.60 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1).

Methyl 6-O-α-isomaltosyl-β-D-glucopyranoside, yield 3.5 mg; $[\alpha]_D$ +9.3° (*c* 0.16, water); insufficient for analysis; ^{1}H-n.m.r.: δ 4.73 (t, 2 H, H-1′,1″) and 4.18 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1).

Methyl 6-O-α-isomaltosyl-α-D-glucofuranoside, yield 6.5 mg; $[\alpha]_D$ +94.8° (*c* 0.25, water); ^{1}H-n.m.r.: δ 5.05 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1) and 4.86 (t, 2 H, $J_{1,2}$ 3.4 Hz, H-1′,1″); ^{13}C-n.m.r.: 103.1 (C-1) and 98.0 (C-1′,C-1″).

Anal. Calc. for $C_{19}H_{34}O_{16}$: C, 44.01; H, 6.36. Found: C, 43.8; H, 6.69.

Methyl 6-O-α-isomaltosyl-β-D-glucofuranoside, yield 11.3 mg; $[\alpha]_D$ +54.6° (*c* 0.47, water); ^{1}H-n.m.r.: δ 4.91 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1′); 4.88 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1″), and 4.80 (s, 1 H, $J_{1,2}$ ~0 Hz, H-1); ^{13}C-n.m.r.: δ 110.0 (C-1), 100.0 (C-1′), and 99.7 (C-1″).

Anal. Calc. for $C_{19}H_{34}O_{15}$: C, 44.01; H, 6.56. Found: C, 43.7; H, 5.74.

ACKNOWLEDGMENTS

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