SECTION C
Organic Chemistry

Chemical Modification of Trehalose. Part II.[1] Synthesis of the *galacto*-Analogue of Trehalose

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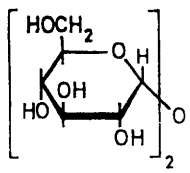
4,6:4′6′-Di-*O*-ethylidene- and -di-*O*-benzylidene derivatives of trehalose were benzoylated, and the acetal groups were removed. The resulting 2,2′,3,3′-tetra-*O*-benzoyltrehalose was then converted into either the 4,4′,6,6′-tetra-*O*-methanesulphonate by methylsulphonylation or into the 4,4′-di-*O*-methylsulphonyl-hexabenzoate by sequential selective di-*O*-benzoylation and methylsulphonylation. Treatment of either of these with sodium benzoate in hexamethylphosphoric triamide gave, with inversion of configuration at the 4- and 4′-positions, the *galacto*-analogue of trehalose as the octa-*O*-benzoate. De-*O*-benzoylation gave the crystalline disaccharide α-D-galacto-pyranosyl α-D-galacto-pyranoside (*galacto*-trehalose).

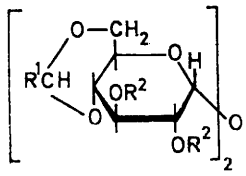
THE abundant disaccharide trehalose (α-D-glucopyranosyl α-D-glucopyranoside) is one of Nature's reserve sugars. It is found in many bacteria and fungi, in plants and in the blood of most insects, where it probably plays an important role in carbohydrate metabolism.[2] Despite its ready availability little work has been carried out on the modification of the stereochemistry and functionality of the molecule. Recently it has been found that two amino-trehaloses, namely, 2-amino-2-deoxy-α-D-glucopyranosyl α-D-glucopyranoside (trehalosamine)[3] and 2-amino-2-deoxy-α-D-glucopyranosyl α-D-mannopyranoside[4] are elaborated by certain strains of *Streptomyces* and possess antibiotic activity. Hence modification of the trehalose molecule (1) is of considerable interest; we report here its conversion into the *galacto*-analogue (10) by inversion of configuration at the 4- and 4′-positions.

We have previously emphasised that trehalose (1) possesses a simple two-fold axis of symmetry through the glycosidic oxygen atom, so that both glucose units are chemically and physically indistinguishable.[1] Hence the reactions of the disaccharide resemble those of simple alkyl α-D-glucopyranosides, making symmetrical substitution, or modification, particularly easy. We have previously described the conversion of methyl α-D-glucopyranoside into the corresponding galactopyranoside[5] and we have now applied a similar series of reactions to trehalose.

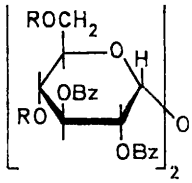
Initially we used the 4,6:4′,6′-di-*O*-ethylidene derivative (2)[6] as starting material but we subsequently found that it was more convenient to proceed by way of the corresponding dibenzylidene derivative (3).[7] Initial difficulties in the preparation of this latter diacetal (yields of only 10—30% could be obtained) were overcome simply by the use of freshly distilled benzaldehyde and a large excess of zinc chloride, which raised the yields to 60—75%.† Each of the diacetals (2) and (3) were benzoylated to give the corresponding 2,2′,3,3′-tetra-*O*-benzoates (4) and (5), both of which were converted into the 2,2′,3,3′-tetra-*O*-benzoate of trehalose (6) by selective methanolysis. The tetrabenzoate (6) was then converted into the desired 4,4′,6,6′-tetra-*O*-methanesulphonate (7) in the usual way. The structures of (6) and (7) were clearly indicated by their ^{1}H n.m.r. spectra in which the H-1 and -1′, H-2 and -2′, and H-3 and -3′



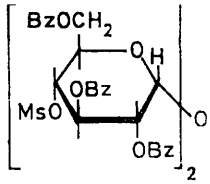
(1)



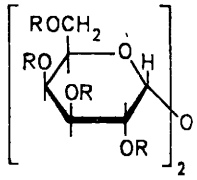
(2) R^1 = Me, R^2 = H
(3) R^1 = Ph, R^2 = H
(4) R^1 = Me, R^2 = Bz
(5) R^1 = Ph, R^2 = Bz



(6) R = H
(7) R = SO_2Me



(8)



(9) R = Bz
(10) R = Ac
(11) R = H

† This observation was originally made at Queen Elizabeth College by Dr. Y. Ali and Mr. P. A. Munroe.

[1] Part I, G. Birch and A. C. Richardson, *Carbohydrate Res.*, 1968, **8**, 411.
[2] G. Birch, *Adv. Carbohydrate Chem.*, 1963, **18**, 201.
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[4] M. Uramoto, N. Otake, and H. Yonehara, *J. Antibiotics, Ser. A*, 1967, **20**, 236.
[5] J. Hill, L. Hough, and A. C. Richardson, *Carbohydrate Res.*, 1968, **8**, 7.
[6] G. Birch, *J. Chem. Soc.* (*C*), 1966, 1072.
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resonances occurred to low field of those of all other ring protons, as a doublet, a quartet, and a triplet respectively (Table). The pattern of these resonances was the same in both cases and was almost exactly duplicated in the spectrum of the dibenzylidene-tetrabenzoate (5) in which the position of the benzoate groups is known. Hence the possibility that benzoate migration could have accompanied the hydrolytic removal of the acetal groups was precluded.

TABLE

N.m.r. spectral assignments (τ values) and first-order coupling constants at 100 MHz for solutions in deuteriochloroform

Compound:	(4)	(5)	(6) *	(7)	(8)	(9)
H-1 and -1′	4·47(d)	4·43(d)	4·48(d)	4·42(d)	4·43(d)	
H-2 and -2′	4·67(q)	4·65(q)	4·79(q)	4·72(q)	4·65(q)	
H-3 and -3′	3·93(t)	3·92(t)	4·00(t)	3·89(t)	3·82(t) †	
H-4 and -4′	6·42(t)	6·20(t)	} *ca.* 6·05(cm)	5·00(t)	4·93(t)	
H-5 and -5′		6·05(cm) ‡		5·92br(d)	5·81br(d)	5·47(t)
H-6a and -6a′						5·85(q)
H-6b and -6b′						5·97(q)
$J_{1,2}$	4·0	4·0	3·7	3·6	3·7	
$J_{2,3}$	10·0	9·5	10·2	10·1	10·0	
$J_{3,4}$	10·0	9·5	8·7	9·5	10·0	
$J_{4,5}$	10·0	9·5		9·7	10·0	*ca.* 1·5
$J_{5,6a}$						6·5
$J_{5,6b}$						6·5
$J_{6a,6b}$						11·0

* In [$^2H_6$]acetone. † Spin decoupling of this resonance caused the H-2 and -2′ and H-4 and -4′ resonances to collapse into doublets. ‡ Cm = complex multiplet.

The tetramethanesulphonate (7) underwent replacement of all four sulphonate groups when treated with sodium benzoate in hexamethylphosphoric triamide at 100° to give *galacto*-trehalose octabenzoate (9) (58%). The fact that all four sulphonate groups were replaced further illustrated that the sulphonate groups could only be situated at the 4-, 4′-, 6-, and 6′-positions, since had they been present at the 2-, 2′-, 3-, or 3′-positions replacement would not have occurred so readily.[8]

An alternative precursor of the *galacto*-trehalose octabenzoate (9) was the highly crystalline 4,4′-di-*O*-methylsulphonyltrehalose hexabenzoate (8). This disulphonate was readily prepared by treatment of the 2,2′,3,3′-tetrabenzoate (6) with benzoyl chloride (2 mol.) in pyridine, which effected 6,6′-dibenzoylation, followed by an excess of methanesulphonyl chloride. This same disulphonate could be prepared less readily from the 4,4′,6,6′-tetramethanesulphonate (7) by selective displacement of the primary sulphonate groups by benzoate anions. The structure of (8) was readily demonstrated by its ^1H n.m.r. spectrum, which was very similar to that of the tetramethanesulphonate (7) (Table). The disulphonate (8) was converted into *galacto*-trehalose octabenzoate (9) (52%) by treatment with sodium benzoate in hexamethylphosphoric triamide. A further 26% could be isolated as the free sugar (11) by de-*O*-benzoylation of the mother liquors.

De-*O*-benzoylation of the octa-*O*-benzoate (9) afforded the highly crystalline *galacto*-trehalose (11) (82%), m.p. 267°, $[\alpha]_D$ +244°, which was further characterised by conversion into the octa-*O*-acetate (10). The structure of this disaccharide was inferred by the fact that only galactose was formed on acid hydrolysis and its molecular rotation (+83,500°) was in reasonable agreement with that predicted by Klyne's rules (+76,100°).[9,10]

This disaccharide has been synthesised twice previously, as an amorphous material, by methods involving coupling of the two galactopyranose units. Bredereck and his co-workers [11] reported that the self-condensation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose in the presence of zinc chloride afforded, after deacetylation, a mixture of αα-, αβ-, and ββ-*galacto*-trehaloses. The αα-isomer (11) was amorphous but they reported m.p. 105° and $[\alpha]_D$ +194·3°. Austin, Hardy, and Buchanan [12] have also described the preparation of (11) as a syrupy by-product in a Koenigs–Knorr condensation and report $[\alpha]_D$ +217°. Neither of these rotations is in close agreement with our value, but both groups prepared the crystalline octa-*O*-acetate (10), which was identical with our sample of this derivative (direct comparison).

The n.m.r. data for compounds (4)—(9) are summarised in the Table. The results show the equivalence of the two pyranose rings and the derived first-order coupling constants prove unequivocally that the pyranosyl rings adopt the *C*1(D) conformation. In (4)—(8) the H-3,3′ resonance occurred to low field of those of all other ring protons because of the deshielding influence of the axial 1-oxygen atom. Similar results were recorded for the corresponding methyl α-D-glucopyranoside derivatives.[5]

EXPERIMENTAL

Unless otherwise stated optical rotations were determined for solutions in chloroform at *ca.* 20° with either a Perkin-Elmer 141 automatic polarimeter or a Bendix photo-

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[11] H. Bredereck, G. Hoschele, and K. Ruck, *Chem. Ber.*, 1953, **86**, 1277.
[12] P. W. Austin, F. E. Hardy, J. G. Buchanan, and J. Baddiley, *J. Chem. Soc.*, 1965, 1419.

electric polarimeter, type 143. Values given are normally the average of several determinations. Alcoholic hydrogen chloride solutions were prepared by the addition of the calculated amount of acetyl chloride to the appropriate alcohol. T.l.c. was performed with microscope slides coated with silica gel G.

2,3-*Di*-O-*benzoyl*-4,6-O-*ethylidene*-α-D-*glucopyranosyl* 2,3-*Di*-O-*benzoyl*-4,6-O-*ethylidene*-α-D-*glucopyranoside* (4).—Di-*O*-ethylidenetrehalose (2) [6] (1·97 g.) was dissolved in dry pyridine (22 ml.) and treated with benzoyl chloride (11·5 ml.). After 2·5 hr. at room temperature the mixture was poured into ice–water; the resulting oily product crystallised slowly during several days. In subsequent preparations this process was accelerated by seeding. The crystals gave the *tetrabenzoate* (2·74 g., 70%). Three recrystallisations from methanol afforded a sample, m.p. 203·5—205·5°, $[\alpha]_D$ +251° (*c* 2·4) (Found: C, 65·2; H, 5·3. $C_{44}H_{42}O_{15}$ requires C, 65·2; H, 5·2%).

2,3-*Di*-O-*benzoyl*-4,6-O-*benzylidene*-α-D-*glucopyranosyl* 2,3-*Di*-O-*benzoyl*-4,6-O-*benzylidene*-α-D-*glucopyranoside* (5).—A solution of di-*O*-benzylidenetrehalose [7] (3) (20 g.) in pyridine (100 ml.) cooled in ice was treated slowly with benzoyl chloride (35 ml.). The mixture was then kept at room temperature for 2 hr. and decomposed with ice–water. The crystalline product was filtered off and washed with water and ethanol, and then recrystallised from methylene chloride–ethanol. The *tetra*-O-*benzoate* (33·8 g., 93%) had m.p. 238—239°, $[\alpha]_D$ +214° (*c* 1·07) and +233° (*c* 1·53 in CH_2Cl_2) {lit.,[7] m.p. 240—241°, $[\alpha]_D$ +239° (*c* 1 in $CHCl_3$)}.

2,3-*Di*-O-*benzoyl*-α-D-*glucopyranosyl* 2,3-*Di*-O-*benzoyl*-α-D-*glucopyranoside* (6).—(*a*) The dibenzylidene-tetrabenzoate (5) (11·8 g.) suspended in 2% ethanolic hydrogen chloride (60 ml.) was heated to boiling. The solid material dissolved rapidly to give a cloudy solution from which crystals began to separate after a few min. After *ca.* 5 min. boiling the mixture was allowed to cool to room temperature and the *tetrabenzoate* (8·1 g., 84%) was filtered off and washed well with ethanol and ether. It had m.p. 271—274°, $[\alpha]_D$ +267° (*c* 1·35 in MeOH). The m.p. slowly changed to 225°, although this varied from sample to sample, in the range 225—240°; however a few crystals often survived until *ca.* 270°. The i.r. spectra of all samples showed no significant differences. In some preparations of this compound the higher-melting form was not obtained, even initially (Found: C, 63·5; H, 5·2. $C_{40}H_{38}O_{15}$ requires C, 63·3; H, 5·1%).

(*b*) The diethylidene-tetrabenzoate (4) (1 g.) was hydrolysed similarly except that 2% methanolic hydrogen chloride was used. The tetrabenzoate (0·6 g.) had m.p. 239—240°, $[\alpha]_D$ +259° (*c* 2·7 in MeOH).

2,3-*Di*-O-*benzoyl*-4,6-*di*-O-*methylsulphonyl*-α-D-*glucopyranosyl* 2,3-*Di*-O-*benzoyl*-4,6-*di*-O-*methylsulphonyl*-α-D-*glucopyranoside* (7).—Methanesulphonyl chloride (10 ml.) was added to a cooled solution of the tetrabenzoate (6) (12·7 g.) in pyridine (75 ml.) and the mixture was kept at room temperature for 2 hr. and then poured into ice–water. The resulting solid was filtered off and washed well with water and ethanol. Crystallisation was best effected by dissolution in acetone (*ca.* 50—100 ml.), addition of ethanol (*ca.* 200—300 ml.) and leaving the mixture to stand in an open beaker for several days. This afforded fine needles (17 g., 95%), m.p. 118—120°. Further recrystallisation from acetone–ethanol gave a sample, m.p. 120—122°, $[\alpha]_D$ +188° (*c* 2·5) (Found: C, 49·3; H, 4·4; S, 12·9. $C_{44}H_{46}O_{23}S_4$ requires C, 49·3; H, 4·3; S, 12·0%).

2,3,6-*Tri*-O-*benzoyl*-4-O-*methylsulphonyl*-α-D-*glucopyranosyl* 2,3,6-*Tri*-O-*benzoyl*-4-O-*methylsulphonyl*-α-D-*glucopyranoside* (8).—(*a*) (With Dr. Y. Ali) To an ice-cold solution of the tetrabenzoate (6) (7·7 g.) in dry pyridine (75 ml.) was added benzoyl chloride (2·5 ml., *ca.* 2·1 moles) dropwise. The mixture was set aside at room temperature for 30 min.; t.l.c. (ether) then indicated that the reaction was complete and that one major product had been formed. Methanesulphonyl chloride (7·5 ml.) was then added, and the mixture was kept for 2 hr. at room temperature and then decomposed with crushed ice. A syrup separated out which slowly solidified and was then filtered off and washed well with water and ethanol. The material was recrystallised by dissolution in the minimum of boiling methylene chloride and slow addition of ethanol while the methylene chloride boiled off. The *disulphonate* separated from the hot ethanolic solution (9·7 g., 86%), m.p. 195—199°, $[\alpha]_D$ +183° (*c* 1·7). Further similar recrystallisation gave a sample, m.p. 198—200°, $[\alpha]_D$ +185° (*c* 2·2) (Found: C, 59·9; H, 5·0. $C_{56}H_{50}O_{21}S_2$ requires C, 60·0; H, 4·5%).

(*b*) The 4,4′,6,6′-tetrasulphonate (7) (1 g.) and sodium benzoate (1 g.) were heated in hexamethylphosphoric triamide (5 ml.) on a boiling water bath for 1·5 hr. The mixture was then cooled and treated with water, and the precipitate was filtered off. Three recrystallisations from methanol–acetone gave the disulphonate (170 mg., 16%), m.p. 200°, $[\alpha]_D$ +199° (*c* 0·7), identical with that obtained in (*a*) (i.r. and n.m.r. spectra).

2,3,4,6-*Tetra*-O-*benzoyl*-α-D-*galactopyranosyl* 2,3,4,6-*Tetra*-O-*benzoyl*-α-D-*galactopyranoside* (9).—(*a*) A mixture of the tetramethanesulphonate (7) (2·5 g.), sodium benzoate (2·5 g.), and hexamethylphosphoric triamide (8 ml.) was heated on a boiling water bath for 43 hr.; t.l.c. then indicated that the reaction was complete. The mixture was cooled and treated with water (25 ml.) and the precipitate was filtered off. Recrystallisation from acetone–methanol gave galacto-*trehalose octabenzoate* (1·58 g.). A further six recrystallisations from methanol gave a sample, m.p. 115—118·5°, $[\alpha]_D$ +221° (*c* 0·4) (Found: C, 68·9; H, 4·8. $C_{68}H_{54}O_{19}$ requires C, 69·5; H, 4·6%).

The reaction was also performed in *NN*-dimethylformamide at reflux temperature for 48 hr., but even after this time it was incomplete and the product needed to be purified by chromatography. The octabenzoate (26%) obtained in this way had m.p. 112—116°, $[\alpha]_D$ +227° (*c* 1·3 in CH_2Cl_2) and was identical with that obtained before (i.r. spectrum).

(*b*) The 4,4′-disulphonate (8) (5 g.) was dissolved in hot hexamethylphosphoric triamide (25 ml.), sodium benzoate (7·5 g.) was added, and the mixture was stirred at 100° for 64 hr. The addition of water produced a solid which was filtered off and then, without being dried, dissolved in ether. The solution was washed well with water, dried ($MgSO_4$), and evaporated to dryness to give a crystalline residue. Recrystallisation, which was difficult, was best effected by adding methanol to an ethereal solution, seeding, and then leaving the solution in an open beaker for several hours, until most of the ether had evaporated off. The crystalline octabenzoate obtained in this way (2·47 g., 52%) had m.p. 110—113°, $[\alpha]_D$ +224° (*c* 1·6). It was recrystallised with ease from methanol (100—125 ml.) to give material (2·4 g.), m.p. 118—120°, $[\alpha]_D$ +224° (*c* 1·0), identical with that prepared in (*a*).

The mother liquors from the first recrystallisation were heated under reflux with 0·05N-sodium methoxide (25 ml.) for about 5 min. After 30 min. at room temperature the

solution was diluted with water and the mixture was extracted with ether to remove the methyl benzoate. The aqueous solution was then neutralised with Amberlite IR-120(H^+) resin, decolourised with charcoal, and evaporated to dryness to give a colourless syrup which crystallised in the presence of aqueous methanol to give α-D-*galactopyranosyl* α-D-*galactopyranoside* (11) (0·4 g., 26% overall). Recrystallisation from aqueous methanol afforded material (0·32 g.), m.p. 255—257°, $[\alpha]_D$ +233° (*c* 1·2 in H_2O), identical with the sample prepared later.

α-D-*Galactopyranosyl* α-D-*Galactopyranoside* (11).—The octabenzoate (9) (0·5 g.) was suspended in boiling methanol (5 ml.), and 0·5N-sodium methoxide (0·5 ml.) was added; the solution was boiled for a few min. A clear solution was obtained initially; crystals which began to separate after a few min. were filtered off but found to be very hygroscopic and collapsed into a gum. The gum was dissolved in water and combined with the mother liquors and the resulting emulsion was extracted with ether to remove methyl benzoate. The aqueous solution was neutralised with Amberlite IR-120(H^+) resin and evaporated to dryness to give a syrup which crystallised in the presence of methanol to give the *disaccharide* (120 mg. 82%), m.p. 262—264°. Several recrystallisations from aqueous methanol gave a sample, m.p. 267—272°, $[\alpha]_D$ +244° (*c* 0·3 in H_2O) (Found: C, 41·9; H, 6·5. $C_{12}H_{22}O_{11}$ requires C, 42·1; H, 6·5%).

The disaccharide formed an octa-*O*-acetate (10) with acetyl chloride–pyridine, m.p. 228—237° (slow heating), 227—229° (fast heating), $[\alpha]_D$ +173° (*c* 1·2). There was a crystal transition from long needles to rhombic and square plates above *ca.* 200°, which was accompanied by a little premelting. The octa-*O*-acetate was identical with an authentic sample [12] which had m.p. 238—241° (slow) and 227—231° (fast) (mixed m.p. and i.r. spectra).

Acid hydrolysis of the disaccharide (45 mg.) in boiling 5N-sulphuric acid (5 ml.) for 5 hr. gave only galactose, which was identified chromatographically.

We thank Bristol Laboratories for financial assistance, Dr. J. G. Buchanan for a sample of *galacto*-trehalose octa-*O*-acetate, and the Physico-Chemical Measurements Unit at Harwell for determination of the 100 MHz n.m.r. spectra.

[9/1795 *Received, October 22nd,* 1969]