Cyclitols. Part XXII.[1] Synthesis of Some Mannosyl- and Mannosyl-mannosyl-myoinositols, and of Galactinol

By S. J. Angyal and B. Shelton

Two diastereoisomeric α-D-mannopyranosylmyoinositols have been synthesised in poor yield from (±)-1,4,5,6-tetra-*O*-acetylmyoinositol by the Königs–Knorr reaction. Each compound was then converted into the 1 ⟶ 6-linked mannosylmannosyl derivative. Galactinol [1-*O*-α-D-galactopyranosyl-(1*R*)-myoinositol] was synthesised in a similar way. Acid reversion of mannose gives mainly α-(1 ⟶ 6)-linked oligosaccharides.

ONLY a few inositol glycosides have been obtained from natural sources. The best known of these is galactinol,[2] isolated from sugar beet, which is[3] 1-*O*-α-D-galactopyranosyl-(1*R*)-myoinositol (I).* Other inositol glycosides have been isolated as degradation products of inositol-containing phospholipids.

R. J. Anderson and his co-workers first showed that myoinositol is a constituent of a phospholipid present in *Mycobacterium tuberculosis*. By hydrolysis they obtained a product containing myoinositol and 2 mol. of D-mannose, which they named " maninositose "[4] (all later workers used the spelling " manninositose "). More recently Vilkas and Lederer[5] established that in the *Mycobacterium* phospholipid the inositol moiety of " maninositose " is esterified by phosphatidic acid, and they produced evidence which appeared to indicate that the two mannoses are attached to myoinositol, in a position not established, as a 6-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl unit. From the products of the alkaline degradation of the crude *Mycobacterium* phospholipid, Vilkas also obtained[6] an α-D-mannopyranosylmyoinositol, probably related to " maninositose."

Our aim was to synthesise " maninositose " on the basis of this information. Since the point of attachment of the sugars to myoinositol was not known, we hopefully assumed that it might be the same as in galactinol (I), the only related example of established structure. Consequently we synthesised[7] the mono- and di-mannosylinositols of the postulated structure, and we believed, on the basis of similarities in physical properties and of a mixed melting point, that they were identical with the products isolated by Vilkas. Subsequent brilliant work by Lee and Ballou has, however, proved that Vilkas's mannoside is 2-*O*-α-D-mannopyranosyl-myoinositol[8] and that in " maninositose " each mannose unit is attached to myoinositol, and not to another mannose unit.[9] It therefore appears certain that none of the mannosides now fully described is identical with a degradation product of a *Mycobacterium* phospholipid.

The ideal starting material for the synthesis of a 1-*O*-glycosylmyoinositol would be a myoinositol derivative in which every hydroxyl group, except the one on C-1, is blocked; but no such derivative was available. The readily prepared (±)-1,4,5,6-tetra-*O*-acetylmyoinositol[10] was therefore used, on the (correct) assumption that only the equatorial 3-hydroxyl group will take part in the Königs–Knorr reaction. Initially, the yield of mannosylinositol was negligible. It is known that the Königs–Knorr reaction often gives very poor yields when the condensation involves a secondary hydroxyl group of a cyclic compound, *e.g.*, a pyranose sugar.[11] Low yields are obtained because side-reactions undergone by the acetohalogeno-sugars proceed more rapidly than the desired reaction with the comparatively inert hydroxyl group. These side-reactions were studied by Goldschmid and Perlin who found[12] that reactions occur between tetra-*O*-acetyl-α-D-mannopyranosyl bromide and silver oxide even in the absence of a hydroxylic component. Iodine suppresses these side-reactions, as well as the formation of orthoesters,[13] though it also retards, to a lesser extent, the desired Königs–Knorr reaction; it appears that there is an optimum ratio of iodine to silver oxide,[12] and in attempting to find this optimum we obtained a 4·5% yield of mannosylinositols when the ratio was 1 : 5. To achieve this yield requires a reaction period of 16 days. In the absence of iodine no inositol mannoside could be detected. The conditions of the reaction were varied extensively but the yield could not be improved.

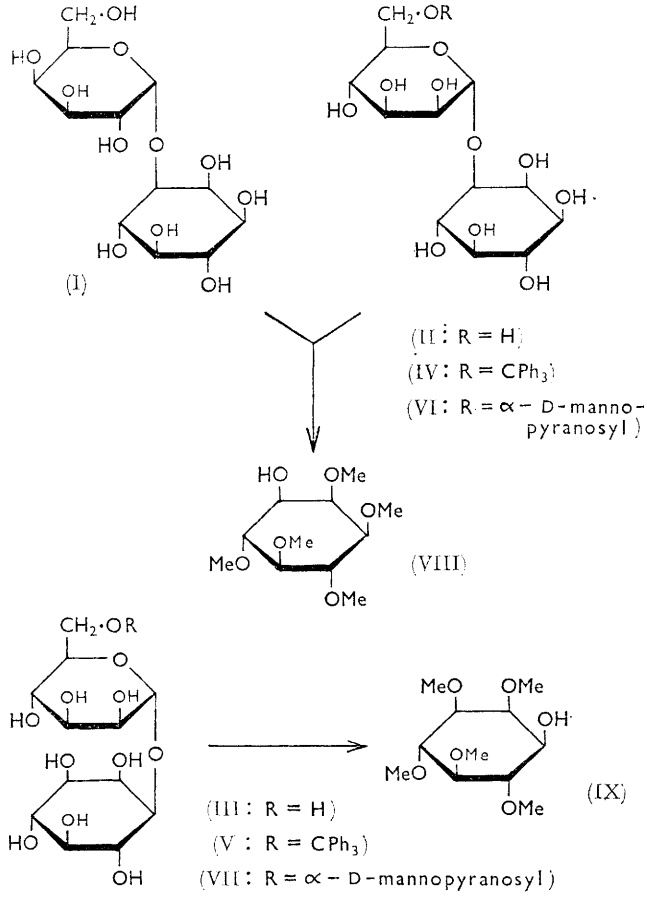
This low yield should be compared with that in another synthesis of an inositol glycoside in which the reaction of tetra-*O*-acetyl-α-D-glucosyl bromide with 1,2:5,6-di-*O*-isopropylidene-3-*O*-methyl-(1*S*)-inositol proceeded in 56% yield.[14] Apparently the free hydroxyl group in this compound is much less hindered by its neighbours

* In this Paper, *R* and *S* are used according to the proposals of S. J. Angyal and P. T. Gilham (*J. Chem. Soc.*, 1957, 3691).

[1] Part XXI, S. J. Angyal and M. E. Tate, *J. Chem. Soc.*, 1965, 6949.
[2] R. J. Brown and R. F. Serro, *J. Amer. Chem. Soc.*, 1953, **75**, 1040.
[3] E. A. Kabat, D. L. MacDonald, C. E. Ballou, and H. O. L. Fischer, *J. Amer. Chem. Soc.*, 1953, **75**, 4507.
[4] R. J. Anderson and E. G. Roberts, *J. Amer. Chem. Soc.*, 1930, **52**, 5023; R. J. Anderson, W. C. Lothrop, and M. M. Creighton, *J. Biol. Chem.*, 1938, **125**, 299.
[5] E. Vilkas and E. Lederer, *Bull. Soc. Chim. biol.*, 1960, **42**, 1013.
[6] E. Vilkas, *Bull. Soc. Chim. biol.*, 1960, **42**, 1005.
[7] S. J. Angyal and B. Shelton, *Proc. Chem. Soc.*, 1963, 57.
[8] C. E. Ballou and Y. C. Lee, *Biochemistry*, 1964, **3**, 682.
[9] Y. C. Lee and C. E. Ballou, *J. Biol. Chem.*, 1964, **239**, 1316.
[10] S. J. Angyal, M. E. Tate, and S. D. Gero, *J. Chem. Soc.*, 1961, 4116.
[11] *E.g.*, P. Bächli and E. G. V. Percival, *J. Chem. Soc.*, 1952, 1243.
[12] H. R. Goldschmid and A. S. Perlin, *Canad. J. Chem.*, 1961, **39**, 2025.
[13] E. A. Talley, D. D. Reynolds, and W. L. Evans, *J. Amer. Chem. Soc.*, 1943, **65**, 575.
[14] K. A. Caldwell, S. P. Raman, and L. Anderson, *Nature*, 1963, **199**, 373; cf. also L. Anderson and M. J. Mohlen-Kamp, Abstracts of Papers, *Amer. Chem. Soc.*, 1965, **149**, 19C.

(*trans*-ether and acetal) than is the one in tetra-*O*-acetylmyoinositol (by a *cis*-hydroxyl and a *trans*-acetoxy-group).

Unfortunately, conditions are not so favourable in the reaction of tetra-acetylmyoinositol. Moreover, the 4·5% yield refers not to one compound but to a mixture of two diastereoisomers, (II) and (III). Fortunately, one of the compounds crystallised readily and the other could then be obtained from the mother-liquors. Under the usual conditions of the Königs–Knorr reaction, tetra-*O*-acetyl-α-D-mannopyranosyl bromide gives predominantly α-mannosides,[15] and both of our diastereoisomers were shown to have this configuration by the value of their molecular rotation: +157 and +149° (methyl α-D-mannopyranoside, +153°); the asymmetrically substituted inositol moiety appears to contribute little to the total rotation. The structures of the diastereoisomers were established by complete methylation, hydrolysis, and isolation of the pentamethylmyoinositols, in the same manner as originally carried out with galactinol.[3] One of the inositol mannosides gave (1*S*)-1,2,4,5,6-penta-*O*-methylmyoinositol (VIII),



and the other yielded its enantiomorph (IX); hence, the two mannosides are 1-*O*-α-D-mannospyranosyl-(1*R*)-myoinositol (II) and 1-*O*-α-D-mannopyranosyl-(1*S*)-myoinositol (III), respectively. The melting point of the nona-acetate of the former compound was not depressed by admixture with a sample, supplied by Mme. Vilkas, of the nona-acetylmannosylmyoinositol [6] isolated from the *Mycobacterium* phospholipid. The acetates of our two diastereoisomers, however, depressed each other's melting points.

In order to synthesise a dimannoside of the type postulated by Vilkas and Lederer it is necessary to attach another α-D-mannopyranosyl unit to the primary hydroxyl group of the inositol mannoside. The method developed by Bredereck *et al.*,[16] in which a trityl derivative is brought into reaction with an acetobromo-sugar in the presence of silver perchlorate, is most suitable for this purpose. Each of the mannosylinositols was converted into the octa-acetate of its 6′-*O*-trityl derivative, (IV) and (V), by treatment with triphenylmethyl chloride in pyridine, followed by acetylation; these derivatives then gave the mannosylmannosylinositols (VI) and (VII) in good yields. The positive molecular rotation (388°) again indicated the formation of α-mannosides, as expected. The melting points and rotations of these trisaccharides and of their acetates are similar to those given for "maninositose" and its acetate, respectively.

Another method was explored for the synthesis of the trisaccharide, namely, condensation of myoinositol with a mannosylmannose. The required 6-*O*-α-D-mannopyranosyl-D-mannose had already been synthesised by Talley *et al.*[13] who originally assigned a β-linkage to the disaccharide; this error was subsequently corrected.[15] We synthesised it by the silver perchlorate method,[16] which gave an improved yield. The acetate of the disaccharide was converted into a non-crystalline bromide which was then subjected to the Königs–Knorr conditions with tetra-acetylmyoinositol. Again it was found that the presence of iodine in the reaction mixture is essential, and that there is an optimum ratio of iodine to silver oxide. By the aid of chromatography a partially acetylated trisaccharide fraction was isolated in 2·2% yield, besides 4·8% of an orthoester of mannosylmannose. The acetylated trisaccharide had one free hydroxyl group on the inositol moiety but its location is not certain because acetyl migration is known to occur under the conditions of the Königs–Knorr reaction.[17]

The trisaccharide, though crystalline, must be a mixture of the two diastereoisomers (VI) and (VII) described above, and its acetate had a lower melting point (132°) than either diastereoisomer. Attempts to separate the components by chromatography or crystallisation were unsuccessful.

In order to find a better method for the synthesis of 6-*O*-α-D-mannopyranosyl-D-mannose, the acid reversion of D-mannose has been re-investigated. Jones and

[15] P. A. J. Gorin and A. S. Perlin, *Canad. J. Chem.*, 1959, **37**, 1930.

[16] H. Bredereck, A. Wagner, G. Faber, H. Ott, and J. Rauther, *Chem. Ber.*, 1959, **92**, 1135.

[17] S. J. Angyal and G. J. H. Melrose, *J. Chem. Soc.*, 1965, 6494.

Nicholson [18] had already shown that this disaccharide is one of the main products of acid reversion. In our hands a 15% yield of this compound was obtained, together with a 2% yield of a trisaccharide which gave a crystalline acetate. It was assumed, by analogy with the disaccharide, that this compound was *O*-α-D-mannospyranosyl-(1 ⟶ 6)-*O*-α-D-mannopyranosyl-(1 ⟶ 6)-D-mannose. To prove it, a compound of this structure was synthesised from 6-*O*-α-D-mannopyranosyl-D-mannose, through its 6′-trityl derivative, by the silver perchlorate method; [16] the compound was identical with the trisaccharide obtained from acid reversion. A tetrasaccharide was also isolated but failed to crystallise; it probably has the analogous α-(1 ⟶ 6)-linked structure, since mannose appears to give mainly oligosaccharides with this type of linkage.

The opportunity was taken to synthesise galactinol (I) in a similar way. Tetra-*O*-acetyl-α-D-galactopyranosyl bromide gives β-galactosides under the usual conditions of the Königs–Knorr reaction; however, Lehmann and Beck [19] recently reported a modification: reaction in nitromethane in the presence of mercuric cyanide, which resulted in a 27% yield of 2-*O*-α-D-galactopyranosyl-D-glucose. As in the foregoing syntheses, we used 1,4,5,6-tetra-*O*-acetylmyoinositol which gave, after nine days, a 5·7% yield of a disaccharide containing galactose and myoinositol. Its melting point (214°) was lower than that of galactinol (222°), and the product is thought to represent a mixture of the two possible diastereoisomers. No attempts were made to separate the components systematically but selective crystallisation by the addition of seed crystals of galactinol gave a 2·1% yield of the naturally occurring isomer.

Acetobromomannose is prone to give orthoesters in the Königs–Knorr reaction; however, the similar low yield of an inositol glycoside obtained with acetobromogalactose indicates that the difficulty lies in the hindered nature of the hydroxyl groups in the partially substituted inositol molecule. Attempts are being made to develop a better method for the synthesis of inositol glycosides.

EXPERIMENTAL

Melting points were determined on a Kofler heated microscope stage. Solutions were concentrated under reduced pressure below 40° unless otherwise stated. Paper chromatography was performed with acetone–water (4 : 1) as mobile phase unless otherwise stated; the mobilities are reported relative to that of myoinositol as R_{myo}. The compounds were detected by dipping the paper into a 1% solution of silver nitrate in acetone, followed by a 2% ethanolic solution of sodium hydroxide; this method of detection is not sensitive to mannosylinositols, and in some cases Tollens reagent was found to be preferable. Silicic acid for chromatography was washed with water, then with acetone, and dried at 110°. Light petroleum had b. p. 40—60°.

1-O-α-D-*Mannopyranosylmyoinositols*.—Silver oxide (4·0 g.) was added to a solution of tetra-*O*-acetyl-α-mannopyranosyl bromide [13] (8·0 g.) in freshly distilled dichloromethane (40 ml.). Carefully dried 1,4,5,6-tetra-*O*-acetylmyoinositol [10] (6·0 g.), m. p. 131—132°, was also dissolved in dichloromethane (40 ml.), and Drierite (10 g.) was added to each mixture. After being shaken briefly, the two suspensions were mixed together, more Drierite (20 g.), solvent (45 ml.), and iodine (0·8 g.) were added, and the mixture was vigorously shaken in the dark for 17 days. After centrifugation the supernatant solution was boiled with charcoal and filtered. The filtrate was evaporated and the residue dissolved in a small amount of anhydrous ethanol. After 24 hr. at 0°, a crop of tetra-acetylmyoinositol, m. p. 131°, was collected by filtration. Ether was added to the mother-liquors, and another crop of the starting material was obtained after 24 hr. at −5°.

The mother-liquors were evaporated and the resulting syrup was treated with sodium methoxide (0·04 equiv.) in methanol. After 2 days at 0°, a precipitate (3·4 g.) was obtained; it was dissolved in boiling 85% ethanol and set aside at room temperature. After 8 days, fine needles (232 mg., 3·3%), m. p. 231°, were obtained; paper chromatography indicated a major component (R_{myo} 0·70) and a small amount of myoinositol. Recrystallisation from 80% ethanol gave chromatographically pure 1-O-α-D-*mannopyranosyl*-(1S)-*myoinositol* (III) as the dihydrate, m. p. 233°, $[\alpha]_D^{22}$ +42° (*c* 2 in H_2O) (Found: C, 37·9; H, 6·8. $C_{11}H_{22}O_{11},2H_2O$ requires C, 38·0; H, 6·9%). After drying *in vacuo* at 145° for 8 hr. the m. p. was 237°, $[\alpha]_D^{23}$ +46° (*c* 1 in H_2O) (Found: C, 42·0; H, 6·4. $C_{12}H_{22}O_{11}$ requires C, 42·1; H, 6·5%). After hydrolysis of a sample with 1N-sulphuric acid at 100°, paper chromatography indicated the presence of myoinositol and mannose.

The other diastereoisomer was recovered from the mother-liquors by chromatography. The solution, diluted to 2·5 l. with water, was poured through a column (14 × 8 cm.) of charcoal, which was then irrigated with water until all the mannose and inositol had been eluted. Final traces of the latter were eluted with 5% ethanol. The solvent was then changed to 10% ethanol, and the fractions containing mannosylinositol were combined and evaporated. The residual syrup crystallised from aqueous ethanol and gave 1-O-α-D-*mannopyranosyl*-(1R)-*myoinositol* (II) (86 mg., 1·1%); after drying at 78° *in vacuo*, the m. p. was 229°, $[\alpha]_D^{23}$ +39° (*c* 1 in H_2O) (Found: C, 39·85; H, 6·6. $C_{11}H_{22}O_{12},H_2O$ requires C, 40·0; H, 6·6%). After drying *in vacuo* at 145° for 8 hr. the m. p. was 233°, $[\alpha]_D^{23}$ +43·5° (*c* 1 in H_2O) (Found: C, 41·6; H, 6·3%). On admixture of the anhydrous forms of the two diastereoisomers the m. p. was 218°.

Nona-O-*acetyl*-1-O-α-D-*mannopyranosyl*-(1S)-*myoinositol*.—Acetylation of the (1S)-diastereoisomer (III) (50 mg.) with acetic anhydride (1·0 ml.) and pyridine (1·5 ml.) at room temperature for 70 hr. and crystallisation from aqueous ethanol gave the *acetate* (52 mg., 55%), m. p. 183°, $[\alpha]_D^{22}$ +24° (*c* 1 in $CHCl_3$) (Found: C, 50·2; H, 5·45. $C_{30}H_{40}O_{20}$ requires C, 50·0; H, 5·55%). On admixture with a sample of "mannosylmyoinositol acetate," m. p. 178—180°, provided by Drs. Lederer and Vilkas, the m. p. was 170°. The infrared spectrum of the compound was indistinguishable from that recorded by Vilkas [20] for her compound.

Nona-O-*acetyl*-1-O-α-D-*mannopyranosyl*-(1R)-*myoinositol*.

[18] J. K. N. Jones and W. H. Nicholson, *J. Chem. Soc.*, 1958, 27.

[19] J. Lehmann and D. Beck, *Annalen*, 1960, **630**, 56.

[20] E. Vilkas-Tenenbaum, D.Sc. Nat. Thesis, University of Paris, 1960, p. 45.

—Acetylation of the (1*R*)-diastereoisomer (II) in the same way gave the *acetate* (68%), m. p. 180°, $[\alpha]_D^{23}$ +20° (*c* 0·5 in $CHCl_3$) (Found: C, 49·8; H, 5·5%). The m. p. of a mixture with the previous compound was 169°, and with the acetate of Vilkas [6] 176—178°. The infrared spectrum was indistinguishable from that recorded by Vilkas,[20] who quotes m. p. 178—180°, $[\alpha]_D^{21}$ +20·5°.

Methylation and Hydrolysis of the Mannosylmyoinositols.—A mixture of the (1*S*)-diastereoisomer (80 mg.), purified [21] dimethylformamide (10 ml.), Drierite (0·5 g.), silver oxide (2·0 g.), and methyl iodide (2·0 ml.) was shaken for 70 hr., and then centrifuged. The supernatant liquid was diluted with chloroform (5 ml.) whereupon a silver complex precipitated; this was removed by washing with 10% potassium cyanide solution. The organic layer was washed with water, dried ($MgSO_4$), and evaporated to a syrup which crystallised after a few days. Recrystallisation from light petroleum gave *nona*-O-*methyl*-1-O-α-D-*mannopyranosyl*-(1S)-*myo-inositol* (58 mg., 58%), m. p. 93°, $[\alpha]_D^{24}$ +49° (*c* 0·5 in H_2O) (Found: C, 53·7; H, 8·5. $C_{21}H_{40}O_{11}$ requires C, 53·8; H, 8·5%).

The methylated compound (46 mg.) was refluxed with 0·5N-hydrochloric acid (5 ml.) for 16 hr. The solution was deionised by Amberlite IR-4B(OH) resin and evaporated. The hydrolysis products were separated, as described by Kabat *et al.*[3] for the galactinol analogue, on a cellulose powder column (35 × 1·4 cm.), using the upper phase of a benzene–ethanol–water (170 : 47 : 15) mixture as irrigant. Recrystallisation from light petroleum gave (1R)-1,2,4,5,6-*penta*-O-*methylmyoinositol* (IX) (20 mg., 81%), m. p. 116—117°, $[\alpha]_D^{24}$ +4° (*c* 0·5 in H_2O). Its enantiomorph (VIII) was prepared from galactinol as described by Kabat *et al.*[6] and had m. p. 117°, $[\alpha]_D^{20}$ −4·5°. The two compounds were indistinguishable by gas chromatography at 190° on a Celite column coated with 25% (w/w) silicone oil but the mixed m. p. was 105—107°.

The (1*R*)-diastereoisomer of mannosylinositol was methylated as described above and gave *nona*-O-*methyl*-1-O-α-D-*mannopyranosyl*-(1R)-*myoinositol* (72%), m. p. 95°, $[\alpha]_D^{21}$ +44° (*c* 1 in H_2O). On admixture with the (1*S*)-diastereomer it melted at 90°. Hydrolysis gave (1*S*)-1,2,4,5,6-penta-*O*-methylmyoinositol (VIII), m. p. 115—117°, $[\alpha]_D^{20}$ −3·5 ± 0·5° (*c* 0·4 in H_2O). The mixed m. p. with the sample prepared from galactinol was 115—117°.

Octa-O-*acetyl*-6′-O-*trityl*-1-O-α-D-*mannopyranosyl*-(1S)-*myoinositol* (V).—Anhydrous mannosyl-(1*S*)-myoinositol (50 mg.) was suspended in dry pyridine (6 ml.) at 100°; triphenylmethylchloride (42 mg.) was added, and the suspension was stirred at 100° for 3 hr.; the disaccharide gradually dissolved. Acetic anhydride was added, the mixture kept at 60° for 1 hr., cooled, and poured slowly into ice–water (5 ml.). The resulting precipitate was recrystallised twice from ethanol, to give the *trityl compound* (63 mg., 47%), m. p. 141—143°, $[\alpha]_D^{17}$ −8° (*c* 1 in $CHCl_3$) (Found: C, 61·1; H, 5·6. $C_{47}H_{52}O_{19}$ requires C, 61·3; H, 5·65%).

Octa-O-*acetyl*-6′-O-*trityl*-1-O-α-D-*mannopyranosyl*-(1R)-*myoinositol* (IV) was prepared from the mannosyl-(1*R*)-myoinositol in the same way in 44% yield; it had m. p. 140—141°, $[\alpha]_D^{17}$ −6° (*c* 1 in $CHCl_3$) (Found: C, 60·9; H, 5·65%).

Synthesis of the Trisaccharides.—(*a*) Finely divided Drierite (100 mg.) was suspended in a solution of the (1*S*)-trityl compound (IV) (150 mg.) and silver perchlorate (40 mg.) in anhydrous nitromethane (3·0 ml.). The mixture was cooled to 0°, and a solution of tetra-*O*-acetyl-α-D-mannopyranosyl bromide (80 mg.) in nitromethane (0·5 ml.) was added with vigorous shaking. A precipitate appeared which was filtered off after 5 min. and washed with nitromethane. The filtrate was washed with sodium hydrogen carbonate solution and with water; it was dried (Na_2SO_4) and evaporated. The residue was fractionally crystallised from ethanol; first triphenylmethanol crystallised, then O-α-D-*mannopyranosyl*-(1 → 6)-O-α-D-*mannopyranosyl*-(1 → 1)-(1S)-*myoinositol dodeca-acetate* (acetate of VII) (35 mg., 21%), m. p. 134—137°, $[\alpha]_D^{18}$ +52·5° (*c* 2 in $CHCl_3$) (Found: C, 50·5; H, 5·6. $C_{42}H_{56}O_{28}$ requires C, 50·0; H, 5·55%).

(*b*) The same procedure with the (1*R*)-trityl compound (IV) gave O-α-D-*mannopyranosyl*-(1 → 6)-O-α-D-*mannopyranosyl*-(1 → 1)-(1R)-*myoinositol dodeca-acetate* (acetate of VII) (22%), m. p. 135—136°, $[\alpha]_D^{19}$ +55·5° (*c* 1 in $CHCl_3$) (Found: C, 49·7; H, 5·4%). Admixture with the preceding compound gave a m. p. of 131—132°. Vilkas reported [6] m. p. 135—137°, $[\alpha]_D^{21}$ +54° (in $CHCl_3$) for acetylated "maninositose."

A sample of the acetate was deacetylated with sodium methoxide at room temperature; recrystallisation from water–ethanol gave an 86% yield of O-α-D-*mannopyranosyl*-(1 → 6)-O-α-D-*mannopyranosyl*-(1 → 1)-(1R)-*myo-inositol*, m. p. 249—250° (decomp.), $[\alpha]_D^{18}$ +77° (*c* 0·6 in H_2O). Vilkas [6] reported decomposition at about 260° and $[\alpha]_D^{21}$ +77° for "maninositose."

(*c*) Octa-*O*-acetyl-6-*O*-α-D-mannopyranosyl-β-D-mannopyranose (see below) (10 g.) was stirred with acetic acid saturated at 0° with hydrogen bromide (30 ml.) for 3 hr. After the addition of chloroform (60 ml.) the solution was washed with ice–water (5 × 50 ml.), dried (Na_2SO_4), and evaporated. The residual syrupy acetobromo-compound was purified in portions (2 g.) by chromatography on a silicic acid column (50 × 4 cm.) irrigated by anhydrous alcohol-free chloroform; fractions of 25 ml. were collected. The acetobromo-compound appeared in tubes 62—70 (Beilstein test). The contents of tubes 65—70 were evaporated to a syrup (total 5·6 g., 54%), $[\alpha]_D^{25}$ +141°. The product failed to crystallise and decomposed within a few days; it was therefore used as soon as possible.

To a solution of well dried 1,4,5,6-tetra-*O*-acetylmyoinositol (2·3 g.) in anhydrous nitromethane (16 ml.), silver oxide (1·5 g.), iodine (0·6 g.), and Drierite (10 g.) were added. The above acetobromo-compound (6·6 g.), dissolved in nitromethane (12 ml.), was then added dropwise with vigorous stirring, which was continued for 10 days in the dark. The mixture was centrifuged, and the supernatant liquid was evaporated to a syrup (8 g.) which was chromatographed on silicic acid with chloroform. Fraction 1 (tubes 12—20; 194 mg.) crystallised on evaporation, m. p. 94°, $[\alpha]_D^{20}$ +27° (*c* 2 in $CHCl_3$), and was identified as 2,3,4,6-tetra-*O*-acetyl-D-mannose; deacetylation gave D-mannose. Fraction 2 (tubes 2—40; 150 mg.) contained octa-*O*-acetylmannosylmannose, m. p. 150—152°. Fraction 3 (tubes 43—78; 301 mg.) gave hexa-*O*-acetylmyoinositol, m. p. 218°, an impurity in the starting material. Fraction 4 (tubes 80—98; 156 mg.) was a mixture. Fraction 5 (tubes 105—113; 2·73 g.) contained a reducing syrup which gave mannosylmannose on deacetylation; it seems to be mainly hepta-*O*-acetylmannosylmannose.

Fraction 6 (tubes 165—175; 269 mg.) crystallised, m. p. 129°, $[\alpha]_D^{19}$ −27° (*c* 2 in $CHCl_3$). Alkaline deacetylation

[21] A. B. Thomas and E. G. Rochow, *J. Amer. Chem. Soc.*, 1957, **79**, 1843.

gave a compound which moved on paper chromatography with the mobility of myoinositol, not as slowly as a mannosylmannosylinositol. When the deacetylated material was treated with methanolic hydrogen chloride (2·5%) for 2 hr. and again chromatographed, mannosylmannose and myoinositol were detected. The compound therefore appears to be an orthoester, 2″,3″,4″,6″,3′,4′,?,4,5,6-deca-*O*-acetyl-6′-*O*-α-D-mannopyranosyl-β-D-mannopyranose 1′,2′-(myoinositol-1) orthoacetate (Found: C, 49·9; H, 5·4. Calc. for $C_{40}H_{54}O_{27}$: C, 49·7; H, 5·6%). The position of the free hydroxyl group on the myoinositol moiety is uncertain.

Fraction 7 (tubes 190—210; 121 mg.) gave a crystalline residue, m. p. 132—134°, $[\alpha]_D^{20}$ +55° (*c* 1 in $CHCl_3$). A deacetylated sample showed R_{man} 0·1 on paper chromatography in butan-1-ol–ethanol–water (3 : 1 : 1), and treatment with methanolic hydrogen chloride (2·5%) at room temperature caused no change; hydrolysis with boiling sulphuric acid (3N) for 24 hr. gave D-mannose, mannosylmannose, and myoinositol. The compound is therefore the *hendeca-acetate* of *O*-α-D-mannopyranosyl-(1 ⟶ 6)-*O*-α-D-mannopyranosyl-(1 ⟶ 1)-(1*RS*)-myoinositol, a mixture of diastereoisomers (Found: C, 49·8; H, 5·4. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%). Acetylation gave the dodeca-acetate, crystals from methanol or ethanol, m. p. 132°, $[\alpha]_D^{19}$ +54° (*c* 0·5 in $CHCl_3$) (Found: C, 50·05; H, 5·6. $C_{42}H_{56}O_{28}$ requires C, 50·0; H, 5·6%). Catalytic deacetylation with sodium methoxide gave the trisaccharide, m. p. 249—250° (decomp.), $[\alpha]_D^{18}$ +80° (*c* 1 in H_2O) (Found: C, 42·75; H, 6·4. $C_{18}H_{32}O_{16}$ requires C, 42·85; H, 6·4%).

6-O-α-D-*Mannopyranosyl*-D-*mannose*.—(a) A solution of silver perchlorate in anhydrous nitromethane (11% w/v) was prepared and stored over Drierite in the dark. 1,2,3,4-Tetra-*O*-acetyl-6-*O*-trityl-β-D-mannose [22] (11 g.) was dissolved in the silver perchlorate solution (40 ml.), Drierite (3 g.) was added, and the mixture was cooled to 0°. A solution of tetra-*O*-acetyl-α-D-mannosyl bromide (8 g.) in a little nitromethane was added with vigorous shaking. After 5 min. the precipitated trityl perchlorate was filtered off and the filtrate was shaken with ice-cold sodium hydrogen carbonate solution, then with ice–water. The organic layer was dried (Na_2SO_4), evaporated to half its volume, and stored at 0°, whereupon triphenylmethanol crystallised out. Evaporation of the mother-liquor and dissolution of the residue in the minimum of anhydrous ethanol gave more triphenylmethanol, which was removed. The mother-liquor was concentrated to a syrup which was dissolved in anhydrous methanol (250 ml.), and a 0·4N-solution of barium methoxide (1·5 ml.) was added. After 2 days at −5° the precipitate which was formed was filtered off and dissolved in water; solid carbon dioxide was added to remove barium, the solution was filtered, and evaporated to a syrup (2·84 g.); this was chromatographed, in two batches, on a column (35 × 4 cm.) of cellulose powder with acetone–water (4 : 1), 25 ml. fractions being collected. D-Mannose was found in tubes 23—47, and the disaccharide in tubes 55—90. Recrystallisation from propan-1-ol gave the disaccharide (1·27 g., 20%), m. p. 195°, $[\alpha]_D^{23}$ +53° (*c* 4·5 in H_2O). Jones and Nicholson [18] reported m. p. 196—197°, $[\alpha]_D^{16}$ +52° (*c* 2 in H_2O); Talley *et al.*[13] claim softening at 70° and caramelisation at 90—95° (probably a misprint). The disaccharide was converted into its osazone, m. p. 121° (lit.,[13] 122°), and into its octa-acetate, m. p. 151°, $[\alpha]_D^{23}$ +20° (*c* 4 in $CHCl_3$) (lit.,[13] m. p. 152—153°, $[\alpha]_D^{25}$ +19·6°).

(b) *By acid reversion*. A solution of D-mannose (80 g.) in 10N-hydrochloric acid (10 ml.) was allowed to stand for 8 days at 32°. The mixture was then de-ionised by passage through a column of Amberlite IR-4B(OH) resin, and was then poured on to a column (125 × 12·5 cm.) of charcoal–Celite (1 : 1). The column was irrigated with water and then with progressively increasing concentrations of methanol (up to 50%). Fractions were collected and tested by paper chromatography in butan-1-ol–ethanol–water (3 : 1 : 1), allowing 4 days for development. Complete separation was not achieved; the fractions were grouped and subjected individually to further chromatography on a charcoal–Celite column (30 × 5 cm.) which was eluted with progressively increasing concentrations of ethanol (5—25%) in water. Apart from those containing mannose, the following fractions were ultimately obtained chromatographically pure. (i) Amorphous material (0·8 g., 1·1%), R_{man} 0·48, $[\alpha]_D^{20}$ −12·5° (*c* 1 in H_2O), apparently 6-*O*-β-D-mannopyranosyl-D-mannose, which is known [18] to have $[\alpha]_D$ −12·7°. (ii) 6-*O*-α-D-Mannopyranosyl-D-mannose (10·5 g., 14·7%), R_{man} 0·36, m. p. 194°, $[\alpha]_D^{20}$ +56° (*c* 2 in H_2O); mixed m. p. with the compound described under (a), 194°. The acetate, m. p. 151°, did not depress the m. p. of the acetate described above. (iii) Amorphous material (1·4 g., 2·0%), R_{man} 0·13, $[\alpha]_D^{20}$ +70° (*c* 2 in H_2O), is *O*-α-D-mannopyranosyl-(1 ⟶ 6)-*O*-α-D-mannopyranosyl-(1 ⟶ 6)-D-mannose. Its acetate, m. p. 163°, $[\alpha]_D^{19}$ +44·2° (*c* 2 in $CHCl_3$), did not depress the m. p. of the acetate described below. (iv) Amorphous material (0·21 g., 0·3%), R_{man} 0·05, $[\alpha]_D^{20}$ +73° (*c* 2 in H_2O), is probably the α-(1 ⟶ 6)-linked tetrasaccharide; it would not crystallise.

O-α-D-*Mannopyranosyl*-(1 ⟶ 6)-O-α-D-*mannopyranosyl*-(1 ⟶ 6)-*mannose*.—1,2,3,4-Tetra-*O*-acetyl-6-*O*-trityl-β-D-mannose [22] (2·95 g.) and silver perchlorate (2·0 g.) were dissolved in anhydrous nitromethane (18 ml.). Drierite (3 g.) was added and the mixture was cooled to 0°, then a solution of acetobromomannosylmannose (3·5 g.; prepared from octa-*O*-acetyl-6-*O*-α-D-mannopyranosyl-β-D-mannose as described above) in nitromethane (5 ml.) was added at once. After being shaken for 5 min. the mixture was filtered, the filtrate was washed with ice-cold sodium hydrogen carbonate solution and with water, dried ($MgSO_4$), and left to stand at 0° for 1 week. Triphenylmethanol crystallised out and was removed by filtration; the filtrate was evaporated and the syrupy residue was dissolved in the minimum amount of ethanol. After 3 days at 0°, another crop of triphenylmethanol was collected. The filtrate was deacetylated with sodium methoxide, then evaporated, and the residue was chromatographed on a column (35 × 5 cm.) of cellulose powder with acetone–water (4 : 1). The main components of the mixture was the *trisaccharide* (515 mg., 20·5%), a clear gum, $[\alpha]_D^{20}$ +69° (*c* 3 in H_2O) (Found: C, 42·6; H, 6·4. $C_{18}H_{32}O_{16}$ requires C, 42·85; H, 6·4%). A crystalline phenylosazone could not be obtained.

The trisaccharide (200 mg.) was dissolved in a mixture of anhydrous pyridine (3 ml.) and acetic anhydride (1 ml.). Next day, the usual working-up and recrystallisation from ethanol gave the *hendeca-acetate* (216 mg., 57%), m. p. 165°, $[\alpha]_D^{23}$ +45·2° (*c* 2 in $CHCl_3$) (Found: C, 50·1; H, 5·6. $C_{40}H_{54}O_{27}$ requires C, 49·7; H, 5·6%).

Synthesis of Galactinol (I).—Thoroughly dried 1,4,5,6-tetra-*O*-acetylmyoinositol (3·1 g.) and tetra-*O*-acetyl-α-D-galactosyl bromide (3·7 g.) were dissolved in anhydrous nitromethane (11 ml.), mercuric cyanide (2·4 g.) was added,

[22] D. D. Reynolds and W. L. Evans, *J. Amer. Chem. Soc.*, 1940, **62**, 66.

and the mixture was shaken for 220 hr. The precipitate was removed by filtration and the filtrate was evaporated. The residue was deacetylated with sodium methoxide (0·04 equiv.) in methanol, and the precipitated mixture of polyols was chromatographed on a column (50 × 4 cm.) of cellulose powder with acetone–water (4 : 1). The eluate was collected in 25-ml. tubes. D-Galactose was found in tubes 25—40, myoinositol in tubes 130—145, and disaccharides in tubes 180—200. The contents (206 mg.) of this last fraction crystallised on evaporation, and were recrystallised twice from aqueous ethanol, to give a mixture (188 mg., 5·7%) of myoinositol *galactosides*, m. p. 214°, $[\alpha]_D^{22}$ +140° (*c* 2 in H_2O) (Found: C, 38·4; H, 7·2. $C_{12}H_{22}O_{11},2H_2O$ requires C, 38·1; H, 6·9%). The mixed m. p. with galactinol (m. p. 222°) was 213—215°.

The product (100 mg.) was dissolved in aqueous ethanol, and the proportion of the solvents was varied until crystallisation commenced after standing for only about 48 hr. Authentic galactinol (3 mg.) was added to this solution 24 hr. after dissolution, and the crystals which gradually formed were filtered off after 96 hr. The galactinol thus obtained (37 mg., 2·1%) had m. p. 220—222°, $[\alpha]_D^{24}$ +137° (*c* 1·5 in H_2O) (lit.,[2] +136°). Acetylation gave the nona-acetate, m. p. 146° undepressed by the following compound.

Nona-O-*acetylgalactinol*.—Anhydrous galactinol (1·0 g.) was stirred for 3 days with a mixture of dry pyridine (3 ml.) and acetic anhydride (2 ml.). The solution was then added dropwise to ice–water (10 ml.) and the mixture was shaken until a precipitate appeared. Recrystallisation from ethanol–water and then from 95% methanol gave *nona*-O-*acetylgalactinol* (1·2 g., 63%), m. p. 146°, $[\alpha]_D^{25}$ +77·7° (*c* 6·5 in $CHCl_3$) (Found: C, 49·8; H, 5·6. $C_{30}H_{40}O_{20}$ requires C, 50·0; H, 5·55%).

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School of Chemistry,
The University of New South Wales,
Kensington, New South Wales,
Australia. [5/1030 *Received, September 27th*, 1965]