1197. *Cyclitols. Part XVIII.*[1] *Acetyl Migration: Equilibrium between Axial and Equatorial Acetates*

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In the presence of weak bases acetyl groups migrate readily between all oxygen atoms of partially acetylated derivatives of myoinositol; *cis* and *trans* migrations occur with almost equal ease but there is no transannular migration. At equilibrium there is only a slight preference for either axial or equatorial positions, depending on the conditions. P.m.r. spectroscopy was used to determine the structures and prove the conformations of partially acetylated inositol derivatives.

The migration of acyl groups in partially acylated polyols under mildly alkaline conditions is well known. Originally discovered [2] among partially acylated esters of glycerol, acyl migration was first encountered in carbohydrate chemistry [3] in the conversion of 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose into the 6-*O*-benzoyl isomer under the effect of traces of alkali. Subsequently, numerous instances of acyl migration have been noted; [4,5] they frequently take place during the methylation of partially acylated polyols.[6] It appears that migration always occurs from a secondary to a primary hydroxyl group, if one is available. The generalisation has also been made [7] that, in the cyclic forms of aldohexoses, migration proceeds away from C-1 and towards C-6; exceptions, however, are known.[5,8] The steric factors responsible for the direction of the migration have apparently not yet been defined.

In discussing these migrations it has often been tacitly assumed that they were unidirectional. However, it follows from the nature of the reaction that it is reversible, and therefore must reach equilibrium. In spite of the importance of this rearrangement, no systematic study of the position of the equilibrium appears to have been carried out; this is of particular interest in cyclic systems, such as those of cyclitols and pyranose forms of sugars, in which the equilibrium is determined mainly by conformational effects. We now report a study of the equilibria obtained with partially acetylated cyclitols, and of the experimental conditions which affect the acetyl migrations. Derivatives of myoinositol were used as model compounds for this investigation because of their conformational stability and configurational suitability.

Migration between an Axial and an Equatorial Oxygen Atom.—The monoacetates of 1,4,5,6-tetra-*O*-methylmyoinositol [9] are suitable model compounds for the study of this reaction. Treatment of the tetramethyl ether with one mol. of acetic anhydride in pyridine esterified preferentially the equatorial hydroxyl group, to give 3-*O*-acetyl-1,4,5,6-tetra-*O*-methylmyoinositol (I). Isomerisation of this acetate in aqueous pyridine yielded the isomeric 2-*O*-acetyl-1,4,5,6-tetra-*O*-methylmyoinositol (II) which was separated from the starting material by chromatography on silica gel. The structures of the two monoacetates were deduced from their proton magnetic resonance (p.m.r.) spectra (see below).

The two monoacetates are readily interconvertible in the presence of weak bases. To establish equilibrium, two different methods were used: heating in a mixture of pyridine and water [10] (which allows the treatment of compounds of a wide range of solubility)

[1] Part XVII, S. J. Angyal and S. D. Gero, *J.*, 1965, 5255.
[2] E. Fischer, *Ber.*, 1920, **53**, 1621.
[3] H. Ohle, *Ber.*, 1924, **57**, 403.
[4] J. M. Sugihara, *Adv. Carbohydrate Chem.*, 1953, **8**, 2.
[5] W. A. Bonner, *J. Org. Chem.*, 1959, **24**, 1388.
[6] S. J. Angyal and G. J. H. Melrose, following Paper.
[7] F. Brown, L. Hough, and J. K. N. Jones, *J.*, 1950, 1125.
[8] A. S. Perlin, *Canad. J. Chem.*, 1963, **41**, 555.
[9] Y. C. Lee and C. E. Ballou, *J. Biol. Chem.*, 1964, **239**, 1316.
[10] G. I. Drummond and L. Anderson, *J. Amer. Chem. Soc.*, 1956, **78**, 1750.

and shaking with silver oxide in chloroform (which reproduces the conditions used in methylation and in the Königs–Knorr reaction, both of which are frequently accompanied by acetyl migration). To determine the position of the equilibrium, the mixture was propionylated [1] and the resulting acetylpropionyl derivatives were separated by gas–liquid chromatography (g.l.c.); the monoacetates themselves were not separable by this method. As a check, some mixtures were also analysed by p.m.r. spectroscopy (see below). Equilibria were approached from both sides; the results are shown in Table 1.

TABLE 1

Position of equilibrium between the monoacetates (I) and (II)

		Product composition (%)	
Starting material	Reaction conditions	(I)	(II)
(I)	Pyridine–water (1 : 1 v/v) *	50	50
(II)	Pyridine–water (1 : 1 v/v)	55	45
(I)	Silver oxide–chloroform †	60	40
(II)	Silver oxide–chloroform	65	35

* 7 Hr., 100°. † 3 Weeks, room temperature, wet Ag_2O.

The proportion of the two isomers in equilibrium is nearly equal, that is, the conformational interactions of the hydroxyl and the acetoxy-groups do not differ to any large extent. This is to be expected because attachment of other groups to the atom which in turn is axially attached to the ring does not cause any large change in the conformational free energy (unless the number and arrangement of the groups is such that they cannot avoid serious interactions [11]). The slight but significant difference of 0·2 kcal./mole in thermodynamic stability between the positional isomers in chloroform, as compared to pyridine–water, may be due to solvation of the hydroxyl group in the latter solvent. It appears that the interaction of an axial hydroxyl group with other axial groups in non-hydroxylic solvents is somewhat less than that of an acetoxy-group, but that solvation by hydroxylic solvents increases the effective size of the hydroxyl group, and the interactions then become approximately equal.

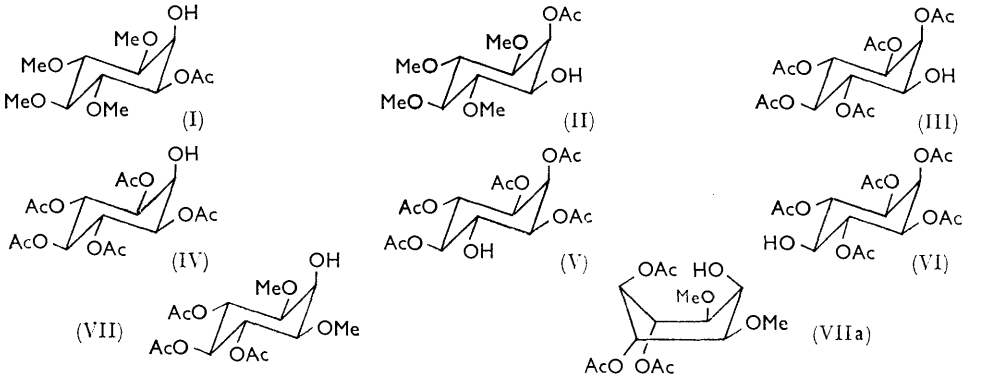
An interesting case of acetyl migration is the complete isomerisation of the 3-acetate of cholestane-3β,4β-diol to the 4-acetate by treatment with basic alumina for 10 hr.[12] Acetyl migration occurred from an equatorial to an axial oxygen atom. This reaction, clearly, does not represent an equilibrium; its outcome is probably determined by the preferential adsorption on alumina of the 4-acetate which has an equatorial hydroxyl group. It appeared of interest to determine the position of the equilibrium in homogeneous solution. Samples of the monoacetates were kindly provided by Dr. S. M. Kupchan, and it was found that in aqueous pyridine the equilibrated mixture contained approx. 70% of the 4-acetate and 30% of the 3-acetate. In this instance the solvated axial hydroxyl group of the 3-acetate interacts to a greater extent with other axial groups than does the axial acetoxy-group of the 4-acetate; however, this interaction involves not only two axial hydrogen atoms (at C-2 and C-6) but also the angular methyl group (at C-10); the approach of solvating molecules to the hydroxyl group is more hindered than in simple cyclohexane derivatives.

Acetyl Migration around the Ring.—Subsequently, migration was studied on the penta-*O*-acetylmyoinositols, of which there are four structurally different isomers, (III)—(VI). Migration proceeded readily, and it was noted with surprise that *trans* migration occurred with nearly the same ease as *cis* migration; in fact, an equilibrium mixture of all four isomers was obtained under comparatively mild conditions.

Analysis of this mixture, however, presented a difficult problem. Although each of the penta-acetates has a characteristically different p.m.r. spectrum, the spectra of mixtures

[11] E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, " Conformational Analysis," Wiley New York, 1965, p. 458.
[12] S. M. Kupchan, P. Slade, R. J. Young, and G. W. A. Milne, *Tetrahedron*, 1962, **18**, 499.

were collectively too complex for the estimation of all their components. The penta-acetates could not be analysed by g.l.c. because their retention times were inordinately long on numerous stationary phases which were tried. After propionylation [1] the 1,3,4,5,6-penta-acetate could be separated from the others which were, however, not resolved. The monomethyl ethers of myoinositol are readily separated by g.l.c.[13] and a method was sought, without success, for converting the penta-acetates quantitatively into the corresponding



methyl ethers. Methylations with methyl iodide were always accompanied by acetyl migration; [6] there was no migration during methylation with diazomethane in the presence of boron trifluoride etherate [14] but the reaction was incomplete even after many repetitions.

Finally, a compromise was adopted. Mixtures of the penta-acetates were divided into two portions. One portion was propionylated and the ratio of 1,3,4,5,6-penta-*O*-acetyl-2-*O*-propionylmyoinositol to the other isomers was estimated by g.l.c. Repeated methylation of the other portion of the mixture with diazomethane–boron trifluoride was followed by gas-chromatographic estimation of the methyl ethers formed. Since the rate of methylation is different for each penta-acetate, the relative rates were determined using known mixtures of pure samples, and were used to calculate the proportion of the penta-acetates (III), (V), and (VI) from the proportion of the methyl ethers. The 1,3,4,5,6-penta-acetate (IV), which has an axial hydroxyl group, was not methylated by diazomethane. By analysing mixtures prepared from pure components it was found that this method of analysis gave product compositions accurate to about 5% of the figures obtained for (III), (V), and (VI) but less than 10% for (IV).

By this method, the results in Table 2 were obtained on mixtures produced by heating the

TABLE 2

Position of equilibrium between the penta-acetates (III)—(VI)

Starting material	Product composition (%)			
	(III)	(IV)	(V)	(VI)
(III)	20	20	30	30
(IV)	17	20	33	30
(V)	20	20	32	28
(VI)	17	20	33	30

TABLE 3

Isomerisation of (IV) in aqueous pyridine solution (28 hr., 15—20°)

Solvent composition (%)		Products (%)			
Pyridine	Water	(III)	(IV)	(V)	(VI)
5	95	35	20	25	20
50	50	35	20	25	20
80	20	30	65	5	0
85	15	15	65	10	10
90	10	15	80	5	0
95	5		>90		

individual penta-acetates in pyridine–water (1 : 1 v/v) for 7 hr. at 100°. It is clear that equilibrium was reached under these conditions. It is to be noted that both (III) and (V) are racemates, that is, mixtures of two different compounds; hence, if all the penta-acetates were of equal stability the equilibrium mixture would consist of (III), (IV), (V), and

[13] Z. S. Krzeminski and S. J. Angyal, *J.*, 1962, 3251.
[14] M. Neeman, M. C. Caserio, J. D. Roberts, and W. S. Johnson, *Tetrahedron*, 1959, **6**, 36.

(VI) in the ratio 2 : 1 : 2 : 1. Actually, the proportion of (III) is lower, and that of (VI) higher, than expected. An explanation of these results is not apparent.

Owing to the ease of acetyl migration, the penta-acetates of myoinositol are not readily obtained pure, nor is it easy to check their purity. Determination of the melting point is of little value, *e.g.*, the melting point of 1,3,4,5,6-penta-*O*-acetylmyoinositol is notoriously variable.[15,16] The melting point of the penta-acetate is lower and over a wider range when observed in soda-glass rather than in a Pyrex capillary.[17] This variability of the melting point is caused by acetyl migration which occurs even in Pyrex glass on heating. Thus, a sample of 1,3,4,5,6-penta-*O*-acetylmyoinositol (IV) after being heated for 5 min. to 180° with an equal amount of powdered Pyrex glass was found to consist of 45% (III), 20% (IV), 15% (V), and 20% (VI); after being heated with soda-glass the composition was 45, 15, 15, and 25%, respectively. The m. p. in Pyrex glass was 167—168°, and in soda-glass, 160—163°; the highest recorded [18] melting point is 177—179°. After heating with soda-glass, g.l.c. showed the presence of some tetra- and hexa-acetates, an indication of some intermolecular acetyl migration.

The rate of migration depends on the composition of the pyridine–water mixture, as shown in Table 3. No migration occurs in anhydrous pyridine at room temperature. It is noteworthy that even a low concentration of pyridine in water will promote migration; this is important because partially acetylated polyols are often handled in aqueous pyridine solution, *e.g.*, in the preparation [19] of 1,3,4,5,6-penta-*O*-acetylmyoinositol from the 1,4,5,6-tetra-acetate, partial acetylation is carried out in the presence of pyridine. During the subsequent working up, involving aqueous solutions, acetyl migration was sometimes found to have occurred; to prevent it we found it necessary to acidify the aqueous solution with acetic acid. Even crystallisation of the penta-acetate may be accompanied by some migration owing to basic impurities in the solvent; it is advisable to add a few drops of acetic acid.

Conditions could not be found under which *cis* migration would occur without concurrent *trans* migration. These observations are in contrast to the finding [20] that *cis* acyl migration occurs when 1-*O*-benzoyl-β-arabino-pyranose and -furanose are dissolved in aqueous pyridine (1 : 4) whereas the corresponding α-anomers, which can only undergo *trans* migration, remain unchanged under the same conditions.

The unexpected ease of acetyl migration around the ring, which involves several *trans* migrations, made it desirable to investigate whether there was any transannular migration, that is, migration across the ring, not involving adjacent positions. A suitable model compound was prepared by partial acetylation of 1,3-di-*O*-methylmyoinositol [21] (dambonitol) which gave a triacetate. It was expected that the axial hydroxyl group on C-2 would have remained unacetylated, and p.m.r. spectroscopy (see below) confirmed that the product was 4,5,6-tri-*O*-acetyl-1,3-di-*O*-methylmyoinositol (VII). In this compound the free hydroxyl group has no acetylated neighbours but in a boat form (VIIa) the acetyl group on O-5 is ideally placed for migration to O-2. The compound, however, was stable in aqueous pyridine solution, indicating that transannular migration does not occur under these conditions.

Determination of Structure and Conformation by P.m.r. Spectroscopy.—In the preceding discussions it has been assumed that derivatives of myoinositol occur in the chair conformation shown in the formulæ (I)—(VII). This conformation occurs in the crystals of myoinositol as shown by *X*-ray diffraction analysis; [22] the evidence for its predominance in solution

[15] S. J. Angyal and L. Anderson, *Adv. Carbohydrate Chem.*, 1959, **14**, 193.
[16] N. Z. Stanacev and M. Kates, *J. Org. Chem.*, 1961, **26**, 912.
[17] L. Anderson and A. M. Landel, *J. Amer. Chem. Soc.*, 1954, **76**, 6130.
[18] B. Iselin, *J. Amer. Chem. Soc.*, 1949, **71**, 3822.
[19] J. H. Davies and T. Malkin, *Nature*, 1959, **184**, 789.
[20] S. Tejima and H. G. Fletcher, jun., *J. Org. Chem.*, 1963, **28**, 2999.
[21] S. J. Angyal, P. T. Gilham, and C. G. Macdonald, *J.*, 1957, 1417.
[22] I. N. Rabinowitz and J. Kraut, *Acta Cryst.*, 1964, **17**, 159.

is, however, only circumstantial.[15] Direct proof of the conformations prevalent in solution can be obtained by p.m.r. spectroscopy of suitably substituted derivatives. In the spectra of unsubstituted or fully acetylated inositols the ring protons are poorly separated and require second-order analysis, similar to that carried out on 5-*O*-methylmyoinositol.[23] In partially acetylated inositols, like the compounds discussed in this Paper, some of the protons are clearly separated from the others because acetylation of a hydroxyl group shifts the signal of the adjacent proton to lower field. The proton on C-2 is recognisable as a triplet with $J_{1,2} = 2{\cdot}5$—$3{\cdot}0$ c./sec. at about δ 5·6 when the adjacent hydroxyl group is acetylated, or at 4·2—4·5 when it is not; H-1 (and the equivalent H-3) appears as a pair of doublets, $J_{1,2} = 2{\cdot}5$—$3{\cdot}0$, $J_{1,6} = 9$—10, at δ 4·7—5·05 and 3·15—3·25, respectively. The coupling constants show that this hydrogen atom has a *cis*- and a *trans*-neighbour, and therefore identify it unambiguously; the larger coupling constant, which corresponds [24] to a dihedral angle of about 180°, defines the hydrogen atom as axial.

By this method it is possible to determine the structures of the two monoacetates formed from 1,4,5,6-tetra-*O*-methylmyoinositol. In the spectrum of one isomer the proton at lowest field appears as a pair of doublets with $J = 10{\cdot}0$ and 2·5; this must be H-3 and, since the shift of the signal to low field is due to the acetoxy-group's being on the same carbon atom, the compound is the 3-acetate (I). The proton at lowest field in the spectrum of the other isomer appears as a triplet with $J = 2{\cdot}5$; the compound is therefore the 2-acetate (II).

The spectrum of the partially acetylated dambonitol (VII) shows H-4, H-5, and H-6 at low field, but the triplet of H-2 appears at higher field; therefore the hydroxyl group on C-2 is unacetylated. Additional evidence is offered by the presence of only two different acetyl signals, of relative intensity 1 : 2. In the spectrum of this compound every proton is clearly resolved, and a first-order analysis of the coupling constants is possible. The conformation is fully defined by the spectrum: H-4 (H-6) and H-5 appear as triplets with $J = 9{\cdot}5$ and are therefore all axial; H-1 (and H-3) as a pair of doublets, $J = 2{\cdot}75$ and 9·5, and are therefore also axial; H-2, a triplet with $J = 2{\cdot}75$, is *cis* to its neighbours and therefore equatorial. The same coupling constants were found in many other derivatives of myoinositol which appears to retain its shape in all (except bicyclic) derivatives.

Details of the p.m.r. spectra are given in the Experimental part.

Experimental

All the asymmetric compounds described in this Paper are racemates. M. p.s. were determined between soda-glass plates on a Kofler hot-stage apparatus and are corrected. Pyridine was stored over, and distilled from, potassium hydroxide pellets; other solvents were dried over anhydrous calcium sulphate. Thin-layer chromatography was performed on silica gel supported on microscope slides [25] the compounds were detected by placing the slides into iodine vapour [26] or by forming ferric hydroxamates from compounds containing acetate groups.[27]

Proton magnetic resonance spectra were recorded at 60 Mc./sec. on a Varian A60 spectrometer using a spinning Pyrex sample tube of 4·5 mm. diameter, in deuterochloroform solution with tetramethylsilane (δ 0·00) as internal reference. The sweep rate was varied. P.m.r. data are given as follows: chemical shift (δ, p.p.m.), multiplicity (t = triplet, pd = pair of doublets, singlet if not indicated), coupling constants (c./sec.), identification. Only those signals which were clearly resolved are listed.

1,4,5,6-*Tetra*-O-*methylmyoinositol*.—Lee and Ballou [9] prepared this compound by methylation of 1,2-*O*-isopropylidenemyoinositol; the method here described was originally developed by Mrs. V. J. Bender. To a well-stirred solution of 1,2-*O*-cyclohexylidenemyoinositol [28,29] (1·09 g.) in a mixture of dimethylformamide (25 ml.) and methyl iodide (10 ml.), silver oxide

[23] N. V. Riggs, *Austral. J. Chem.*, 1964, **17**, 603.
[24] M. Karplus, *J. Chem. Phys.*, 1959, **30**, 11; *J. Amer. Chem. Soc.*, 1963, **85**, 2870.
[25] J. J. Peifer, *Mikrochim. Acta*, 1962, 529.
[26] M. E. Tate and C. T. Bishop, *Canad. J. Chem.*, 1963, **41**, 1801.
[27] M. E. Tate and C. T. Bishop, *Canad. J. Chem.*, 1962, **40**, 1043.
[28] S. J. Angyal, M. E. Tate, and S. D. Gero, *J.*, 1961, 4116.
[29] S. J. Angyal, G. C. Irving, D. Rutherford, and M. E. Tate, *J.*, 1965, 6662.

(14 g.) was added gradually, with cooling. Further amounts of oxide (3 g.) and iodide (5 ml.) were added after 18 hr., and again after another 6 hr. Stirring was continued for 20 hr. The mixture was centrifuged, the supernatant liquid was diluted with chloroform, washed with aqueous potassium cyanide solution (5%) and water, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was dissolved in light petroleum (50 ml.) and the solution extracted with water (25 ml.) to remove incompletely methylated products. The oil which remained on evaporation of the solvent was extracted with methanol (10 ml.); evaporation of the solution gave a product (630 mg.) which failed to crystallise. It was dissolved in aqueous acetic acid (80% v/v; 6 ml.) and heated on a steam-bath for 3 hr. The solution was evaporated and the residue dissolved in benzene from which the tetramethyl ether (440 mg., 48%) separated as rectangular crystals, m. p. 102—104°. Further crystallisation raised the m. p. to 105—106° (lit.,[9] 97—99°) (Found: C, 51·1; H, 8·3. Calc. for $C_{10}H_{20}O_6$: C, 50·85; H, 8·55%).

Partial Acetylation of 1,4,5,6-*Tetra*-O-*methylmyoinositol*.—To a solution of the tetramethyl ether (2·0 g.) in dry benzene (100 ml.) and pyridine (5 ml.) a mixture of acetyl chloride and benzene (1 : 10 v/v; 9·0 ml.) was added. After 2 days, excess acetyl chloride was destroyed by the addition of ethanol, the solution was acidified with acetic acid, and the pyridinium salts were removed by filtration. Evaporation *in vacuo* left an oil (2·88 g.); g.l.c., after propionylation, showed it to contain starting material (25%), 1-acetate (47%), 2-acetate (3%), and diacetate (25%). Attempts to crystallise the material from hydroxylic solvents caused extensive acetyl migration. Subsequently, a sample (1·4 g.) of the oil was chromatographed on silica gel (350 g.) with chloroform as irrigant. Fractions (25 ml.) were examined by thin-layer chromatography or by g.l.c. after propionylation. Fractions 120—150 contained the diacetate (201 mg.) which failed to crystallise. The content (360 mg.) of fractions 173—341, when crystallised from light petroleum containing a drop of acetic acid, gave 1-O-*acetyl*-3,4,5,6-*tetra*-O-*methylmyoinositol*, m. p. 87—89° (Found: C, 51·9; H, 7·9. $C_{12}H_{22}O_7$ requires C, 51·8; H, 7·95%). P.m.r. data: 4·70 pd 10·5, 2·5 (H-1), 4·21 t 2·5 (H-2), 3·49, 3·56, 3·61, 3·63 (Me), 2·16 (Ac.)

The content (357 mg.) of fractions 572—611, on crystallisation from light petroleum containing a drop of acetic acid, gave 2-O-*acetyl*-3,4,5,6-*tetra*-O-*methylmyoinositol*, m. p. 109—111° (Found: C, 51·7; H, 7·8%). P.m.r. data: 5·58 t 2·5 (H-2), 3·42, 3·60 (Me), 3·64 (2Me), 2·16 (Ac).

Penta-O-*acetylmyoinositols*.—1,2,4,5,6-Penta-*O*-acetylmyoinositol (III), m. p. 163—166°, was prepared by debenzylation of 1,2,4,5,6-penta-*O*-acetyl-3-*O*-benzylmyoinositol.[30] P.m.r. data: 5·60 t 2·5 (H-2), 1·99 (Ac), 2·02 (2Ac), 2·08, 2·22 (Ac).

1,2,3,4,6-Penta-*O*-acetylmyoinositol (VI), m. p. 178°, was prepared by the deamination of penta-*O*-acetyl-2-amino-2-deoxyneoinositol.[31] P.m.r. data: 5·04 pd 2·6, 10·5 (H-1, H-3), 5·58 t 2·6 (H-2), 2·01 (2Ac), 2·11 (Ac), 2·21 (2Ac).

1,2,3,4,5-Penta-*O*-acetylmyoinositol (V), m. p. 85—86°, was similarly prepared by deamination of penta-*O*-acetyl-2-amino-2-deoxyepi-inositol.[32] P.m.r. data: 5·58 t 2·75 (H-2), 4·02 t 9·5 (H-6), 1·99, 2·03, 2·07, 2·10, 2·17 (Ac).

1,3,4,5,6-Penta-*O*-acetylmyoinositol (IV) is best prepared by Davies and Malkin's method.[19] To a solution of 1,4,5,6-tetra-*O*-acetylmyoinositol [28] (4·0 g.) in dry pyridine (10 ml.) a mixture of dry benzene (200 ml.) and freshly distilled acetyl chloride (1·8 ml.) was added. After 2 days, ethanol (0·4 ml.) was added, followed by sufficient glacial acetic acid to give a distinct acid reaction. The solution was filtered and evaporated to dryness. The residual syrup was extracted with a boiling mixture of water (200 ml.) and acetic acid (2 ml.); the solution, after evaporation under reduced pressure to 30 ml., deposited the penta-acetate (2·45 g., 55%), m. p. 159—162°. Best solvent for recrystallisation is methanol or methanol–water (1 : 1 v/v) acidified with a little acetic acid. The purity of the compound was checked by g.l.c. after propionylation; isomeric penta-acetates were absent but myoinositol hexa-acetate was often found as an impurity. P.m.r. data: 4·35 t 2·5 (H-2), 2·02 (3Ac), 2·11 (2Ac).

4,5,6-*Tri*-O-*acetyl*-1,3-*di*-O-*methylmyoinositol* (VII).—A mixture (14·6 ml.) of acetyl chloride and anhydrous benzene (1 : 9 v/v) was added to a solution of dambonitol (1·0 g.) in anhydrous pyridine (50 ml.). After 2 days ethanol was added to destroy the remaining acetyl chloride; the solution was filtered and evaporated under reduced pressure. Thin-layer chromatography

[30] S. J. Angyal and M. E. Tate, unpublished results.
[31] S. J. Angyal and M. E. Tate, *J.*, 1961, 4122.
[32] S. J. Angyal and J. S. Murdoch, unpublished results.

showed that the residue was a complex mixture consisting mainly of mono- and di-acetyl derivatives. The product was dissolved in anhydrous pyridine (30 ml.), and a mixture of acetic anhydride and pyridine (4 ml., 1 : 9 v/v) was added. After 4 days the mixture was poured into ice-cold dilute hydrochloric acid, which was extracted with ether (which was discarded), and then with chloroform (3 × 80 ml.). Evaporation of the solvent gave rectangular plates (190 mg.); after three crystallisations from ethanol the *triacetate* melted at 194° (Found: C, 50·3; H, 6·6. $C_{14}H_{22}O_9$ requires C, 50·3; H, 6·6%). P.m.r. data: 3·25 pd 2·75, 9·5 (H-1,3), 4·45 t 2·75 (H-2), 5·48 t 9·5 (H-4,6), 5·01 t 9·5 (H-5), 3·46 (2Me), 1·99 (Ac), 2·03 (2Ac).

The compound was recovered unchanged after being heated at 100° for 5 hr. with pyridine–water (1 : 1 v/v).

Acetyl Migrations.—(*a*) The compound (25 mg.) was dissolved in aqueous pyridine (0·35 ml.) of known composition. After the required period at room temperature or at 100° the solution was evaporated under reduced pressure.

(*b*) The compound (80 mg.) in anhydrous chloroform (5 ml.) was stirred with silver oxide (250 mg.). During isomerisations extending over more than 3 days, more silver oxide (250 mg.) was added after every third day. After a given time the mixture was centrifuged and the supernatant liquid was evaporated.

(*c*) In one case, 1,3,4,5,6-penta-*O*-acetylmyoinositol (12 mg.) was dissolved in aqueous dimethylformamide (0·1 ml.; 1 : 1 v/v) containing 1% sodium hydrogen carbonate. After 1 hr. the solution was evaporated under reduced pressure and the residue was extracted with chloroform (10 ml.). After evaporation of the solvent, analysis indicated that the residue consisted of 30% of (III), 45% of (IV), 10% of (V), and 15% of (VI).

Analytical Method.—Gas–liquid chromatographic analysis was carried out as previously described.[13] For the separation of *O*-acetyl-*O*-propionyltetra-*O*-methylmyoinositols a column of 3% w/w of a polyester resin (LAC 1-R-296, Cambridge Industries Co., Cambridge, Mass.) on 60—100 mesh kieselguhr (Embacel, May and Baker Ltd.) was used at 180°, while the propionates of the penta-acetylinositols were separated at 220° on 100—120 mesh kieselguhr coated with 1·5% of polyester.

Propionylation was carried out by heating a sample (*ca.* 25 mg.) of the inositol derivative with a mixture of pyridine and propionic anhydride (3 ml.; 3 : 2 v/v) at 100° for 2 hr. The solution was then evaporated under reduced pressure and the residue, dissolved in chloroform, was analysed by g.l.c.

For methylation, a sample (15 mg.) dissolved in pure chloroform (15 ml.) was treated with boron trifluoride solution (0·25 ml., prepared by adding 0·4 ml. of commercial etherate to 50 ml. of anhydrous ethyl ether) and with an ethereal solution of diazomethane to give a permanent yellow colour. After 2 hr. at 0° the treatment was repeated twice. Excess diazomethane was expelled by boiling, the mixture was filtered, washed with saturated sodium hydrogen carbonate solution and with water, dried (Na_2SO_4), and evaporated. The mixture was propionylated (to make the penta-acetates more volatile) and then analysed by g.l.c.; the penta-acetates of 1-*O*-methyl-, 4-*O*-methyl, and 5-*O*-methyl-myoinositol were revealed. Analyses of a mixture consisting of equal amounts of (III), (V), and (VI) gave the corresponding methyl ethers in the ratio 1 : 3·94 : 2·64 (owing to different rates of methylation); hence, results of analyses were divided by 1, 3·94, and 2·64, respectively. The penta-acetates were methylated to an extent of not more than 10%.

Equilibration of the Cholestane-3β,4β-diol Monoacetates.—A sample (15 mg.) of each monoacetate (obtained from Dr. S. M. Kupchan) was heated to 100° with pyridine (1·6 ml.) and water (0·4 ml.) for 7 hr.; the mixture was then evaporated to dryness, and the p.m.r. spectrum was recorded. The 3-acetate shows multiplets at δ 4·7 (H-3) and 3·8 (H-4), and a singlet at 2·06 (Ac); the 4-acetate at 3·6, 5·1, and 2·08, respectively. The composition of the mixture was estimated from the intensities of these peaks but, because the ring protons give diffuse peaks and those of the acetyl groups are very close together, the estimation is not accurate (*ca.* ±10%).

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