Acid-catalysed Hydrolyses of 3,4,6-Tri-*O*-methyl-1,2-*O*-(1-alkoxyethylidene)-α-D-glucopyranoses *

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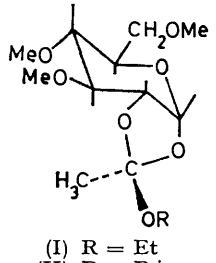
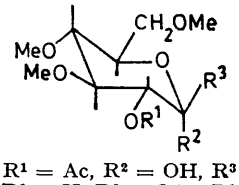
Acid-catalysed hydrolyses of 3,4,6-tri-*O*-methyl-1,2-*O*-(1-alkoxyethylidene)-α-D-glucopyranoses (I) or (II) yield 2-*O*-acetyl- (III) and 1-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose (IV) exclusively as determined by t.l.c., g.l.c., and n.m.r. However, the relative amounts of (III) and (IV) formed, as determined by n.m.r. for hydrolyses of (I), exhibited drastic dependence on the solvent and the acid concentration. Hydrolyses of (I) catalysed by dilute 2,4,6-trinitrophenol (<0·01N) yielded (III) as the major product (93%) in deuterium oxide while in [$^2H_6$]ethanol–deuterium oxide (5 : 1 v/v) or [$^2H_6$]acetone–deuterium oxide (5 : 1, v/v) (IV) was the major product (*ca.* 70%). Hydrolysis of (I) in deuterium oxide with 0·004N-H_2SO_4 yielded predominantly (III) (83%) while 0·8N-H_2SO_4 yielded predominantly (IV) (79%). A reaction mechanism based on the 1,2-acetoxonium ion as the effective intermediate is suggested for the hydrolyses.

In a study of acid-catalysed ethanolyses of 3,4,6-tri-*O*-methyl-1,2-*O*-(1-alkoxyethylidene)-α-D-glucopyranoses (I) and (II) quantitative analysis of unchanged (I) and (II) presented a problem.[1] While accurate analysis of the ethanolysis products was possible by g.l.c., (I) and (II) decomposed appreciably during it. Since glycopyranose 1,2-(alkyl orthoacetates) hydrolyse orders of magnitude more rapidly than glycopyranosides and *O*-acetyl substituents of glycopyranose derivatives,[2] it seemed reasonable that unchanged (I) and (II) could be hydrolysed without altering the ethanolysis products. The concentrations of (I) and (II) could then be estimated by analysis of their hydrolysis products.

In this paper we report the results of a quantitative study of hydrolyses of (I) and (II) and discuss the implications of these results with respect to mechanisms of hydrolysis of D-glucopyranose 1,2-(alkyl orthoacetates).



(I) R = Et
(II) R = Pr^i



(III) R^1 = Ac, R^2 = OH, R^3 = H
(IV) R^1 = H, R^2 = OAc, R^3 = H
(V) R^1 = H, R^2 = OH, R^3 = H
(VI) R^1 = H, R^2 = H, R^3 = OH
(VII) R^1 = Ac, R^2 = H, R^3 = OH

RESULTS

Short-duration acid-catalysed hydrolyses of compounds (I) and (II) in water yielded crystalline 2-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose (III) as the predominant product and, usually, a small amount of crystalline 1-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose (IV). Analysis of the hydrolysis product mixture by t.l.c. showed that only compounds with mobility (R_F) identical with (III) and (IV) could be present; (III) and (IV) were not resolved in the t.l.c. system employed. 3,4,6-Tri-*O*-methyl-α-D-glucopyranose (V) and 3,4,6-tri-*O*-methyl-β-D-glucopyranose (VI) were not detected in the product mixture. Similar hydrolyses of (I) and (II) in water–acetone (9 : 1, v/v) yielded analogous results by t.l.c. analysis.

The yields and relative quantities of (III) and (IV) formed in hydrolyses of (I) and (II) could be ascertained easily by n.m.r. analysis. The resonance frequencies of the anomeric protons of (III) and (IV) are widely separated, *e.g.*, δ 5·32 and 6·19 p.p.m., respectively, in $CDCl_3$, and are easily discernible because of their low-field position. By comparing the integrals of the 1-H signals for (III) and (IV) with an integral of a signal related to the original orthoester, *e.g.*, the methyl triplet of ethyl alcohol generated in the hydrolysis of (I), the absolute yield of (III) and (IV) could be obtained.

Within the limits of the n.m.r. analyses, compounds (III) and (IV) were the only products of hydrolysis of (I) in three solvent systems: deuterium oxide, [$^2H_6$]ethanol–deuterium oxide (5 : 1, v/v), and [$^2H_6$]acetone–deuterium oxide (5 : 1, v/v). This confirmed t.l.c. data which indicated that (V) and (VI) were not hydrolysis products. In addition, it excluded 2-*O*-acetyl-3,4,6-tri-*O*-methyl-β-D-glucopyranose (VII) as a significant hydrolysis product.

Surprisingly, the relative amounts of products (III) and (IV) generated in hydrolyses of (I) were very dependent on the solvent system (Table 1). Hydrolysis of the 1,2-orthoacetate (I) in deuterium oxide catalysed by dilute 2,4,6-trinitrophenol (<0·01N) resulted in *O*-acetyl formation predominantly at C-2; (III) accounted for 93% of the hydrolysed orthoester. However, in [$^2H_6$]ethanol–deuterium oxide (5 : 1, v/v) and [$^2H_6$]acetone–deuterium oxide (5 : 1, v/v) with the same concentration of 2,4,6-trinitrophenol the *O*-acetyl group predominated at C-1; (IV) accounted for *ca.* 70% of the hydrolysed orthoester.

The product distribution for hydrolyses of (I) in deuterium oxide was also very dependent on the acid catalyst concentration (Table 1). Hydrolysis of (I) in deuterium oxide catalysed by very dilute H_2SO_4 (0·004N) resulted in a predominance (83%) of the 2-*O*-acetyl derivative (III) while higher acid concentrations (0·04,

* Presented in part at the 160th American Chemical Society National Meeting in Chicago, Illinois on Sept. 17, 1970.

[1] D. P. Hultman, Doctoral Dissertation, The Institute of Paper Chemistry, Appleton, Wisconsin, June 1970.

[2] E. Pacsu, *Adv. Carbohydrate Chem.*, 1945, **1**, 77.

0·2, and 0·8N-H_2SO_4) resulted in a predominance (60, 71, and 79%, respectively) of the 1-*O*-acetyl isomer (IV).

Although it is known that acetyl migration can occur between C-1 and C-2 in D-glucopyranose derivatives and that the C-2 isomer is the thermodynamically preferred product,[3] such migration did not account for the observed product distribution. In separate experiments it was demonstrated that, in deuterium oxide only, the 1-*O*-acetyl derivative (IV) isomerises to the 2-*O*-acetyl derivative (III) quite rapidly; equilibrium was established in 15—30 min. However, with 2,4,6-trinitrophenol in the deuterium oxide (hydrolysis conditions), compound (IV) was unchanged after 30 min. Also, no isomerisation of the 2-*O*-acetyl derivative (III) was apparent under these conditions in 30 min. Similarly, with the acid catalyst present, no isomerisation of (IV) was detected in the [$^2H_6$]acetone–deuterium oxide system after 30 min. Also, no change was apparent in the relative ratio of (III) and (IV) in any of the solvent systems during repeated analyses. Thus, since hydrolysis of (I) was very fast (<1 min) relative to acetyl migration for (III) or (IV) under conditions of hydrolysis, the observed product distribution can be attributed directly to hydrolysis of (I) and not to subsequent acetyl migration on (III) or (IV).

TABLE 1

Effect of solvent and acid concentration on the product distribution in hydrolyses of 3,4,6-tri-*O*-methyl-1,2-*O*-(1-ethoxyethylidene)-α-D-glucopyranose [a]

Solvent	Catalyst	(III) mol % [b,d]	(IV) mol % [c,d]
Deuterium oxide	<0·01N-TNP [e]	93	7
[$^2H_6$]Ethanol–deuterium oxide (5 : 1, v/v)	<0·01N-TNP [e]	30	70
[$^2H_6$]Acetone–deuterium oxide (5 : 1, v/v)	<0·01N-TNP [e]	28	72
Deuterium oxide	0·004N-H_2SO_4	83	17
Deuterium oxide	0·04N-H_2SO_4	40	60
Deuterium oxide	0·2N-H_2SO_4	29	71
Deuterium oxide	0·8N-H_2SO_4	21	79

[a] Determined by n.m.r.; see text. [b] 2-*O*-Acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose. [c] 1-*O*-Acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose. [d] The chemical shifts (δ/p.p.m.) for 1-H of (III) and (IV) were, respectively, 5·28 and 6·03 in deuterium oxide; 5·23 and 6·03 in [$^2H_6$]ethanol–deuterium oxide; and 5·22 and 6·03 in [$^2H_6$]acetone–deuterium oxide. [e] 2,4,6-Trinitrophenol. The catalyst concentration is not known precisely but is the same for the three indicated hydrolyses.

Quantitative g.l.c. analysis of products resulting from hydrolysis of (I) and (II) in water–acetone (9 : 1, v/v) further substantiated that hydrolyses of the alkyl 1,2-orthoacetates result in quantitative formation of an *O*-acetyl group at either C-1 or C-2. Except for one case in which <1% of 3,4,6-tri-*O*-methyl-D-glucopyranose (V) and/or (VI) was presumably detected, (III) and (IV) were the only measurable products and, within the limits of the analyses, accounted for all of the hydrolysed orthoester (Table 2).

In ethanol–water solutions, hydrolysis of (I) was very fast relative to apparent ethanolysis.* As expected, reaction of (I) in anhydrous ethanol did not yield (III) or (IV) (Table 2). However, in an ethanol solution containing only 5 mol % water hydrolysis products (III) and (IV) accounted for *ca.* 94% of the consumed orthoester. When the water content was increased to 30 mol %, only hydrolysis products were formed (Table 2).

* Ethoxy-exchange with (I) to re-form (I) or the *endo*-OR isomer of (I) would not be detected by the analytical techniques employed.

TABLE 2

Quantitative g.l.c. product analysis

Ortho-ester	Solvent	(III) + (IV) [a] mol fraction	(V) + (VI) [b] mol fraction	Other products,[c] mol fraction	Total measured
(I)	Water–acetone (9 : 1, v/v) [d]	1·015	0·000		1·015
(I)	Water–acetone (9 : 1, v/v) [d]	0·994 [g]	0·006 [g]		1·000 [g]
(II)	Water–acetone (9 : 1, v/v) [d]	0·984	0·000		0·984
(I)	Ethanol (anhydrous) [f]	0·000	0·160	0·813	0·973
(I)	Ethanol–water (95 : 5, molar) [f,g]	0·970	0·014	0·051	1·035
(I)	Ethanol–water (70 : 30, molar) [f,h]	1·026	0·000	0·000	1·026

[a] Analysed as the mono-*O*-propanoyl derivatives; (III) and (IV) both had $T_r = 20·1$ min. The internal standard (XXXII) had $T_r = 33·4$ min. [b] Analysed as the di-*O*-propanoyl derivatives; (V) and (VI) both had $T_r = 28·3$ min. [c] These individually analysed products were ethyl 3,4,6-tri-*O*-methyl-β-D-glucopyranoside (analysed as the 2-*O*-propanoyl derivative, $T_r = 15·6$ min); ethyl 2-*O*-acetyl-3,4,6-tri-*O*-methyl-β-D-glucopyranoside ($T_r = 10·7$ min); and ethyl 3,4,6-tri-*O*-methyl-α-D-glucopyranoside (analysed as the 2-*O*-propanoyl derivative, $T_r = 13·8$ min). Preparative details are given elsewhere.[1] [d] Sulphuric acid catalyst. [e] Normalised data. [f] 2,6-Dichlorobenzoic acid catalyst. [g] Mol water/mol (I) = 11·9. [h] Mol water/mol (I) = 87·6.

DISCUSSION

A recent review by DeWolfe [4] and an earlier review by Pacsu [2] indicate that a limited number of hydrolyses of carbohydrate orthoesters have been investigated and, further, that information relative to the mechanism of hydrolysis is very limited.

1,2-Orthoesters of glycoses such as (I) and (II) can be envisaged as containing both an acetal and an orthoester moiety which have one oxygen atom in common. Thus any mechanistic discussion of reactions of this class of compound should include consideration of both functional groups with special consideration given to possible reactions which involve the common oxygen atom. However, it might be expected that, in hydrolyses, the orthoester character of the molecule would predominate since orthoesters generally hydrolyse considerably more rapidly than acetals.[5]

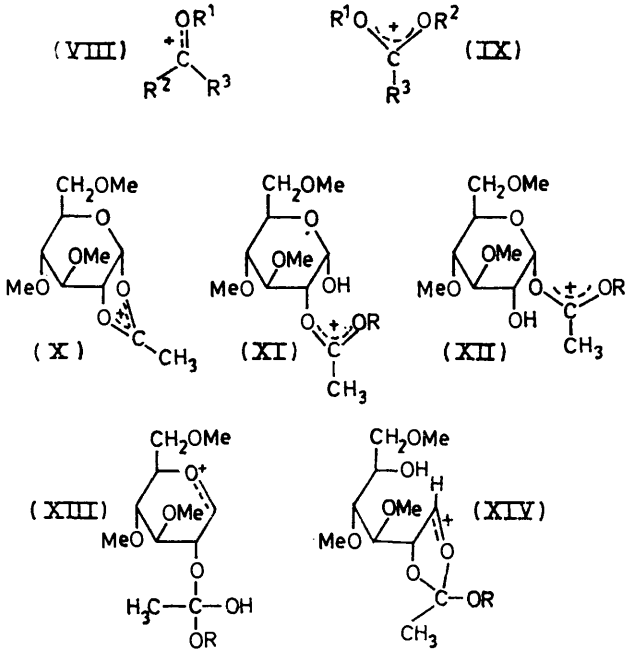
Acid-catalysed hydrolyses of acetals and orthoesters generally occur with cleavage of the carbonyl carbon–oxygen bond rather than the carbinol carbon–oxygen bond.[6] This appears to be typical of hydrolyses of 1,2-orthoesters of glycopyranoses also. Carbonium ion formation (*A*-1 mechanism) or bimolecular nucleophilic displacement (*A*-2 mechanism) at C-2 or C-5 of the pyranose ring does not occur. If this were occurring,

[3] W. A. Bonner, *J. Org. Chem.*, 1959, **24**, 1388.
[4] R. H. DeWolfe, 'Carboxylic Orthoacid Derivatives,' ch. 5, Academic Press, New York, 1970.
[5] Ref. 4, ch. 2.
[6] E. H. Cordes, *Progr. Phys. Org. Chem.*, 1967, **4**, 1.

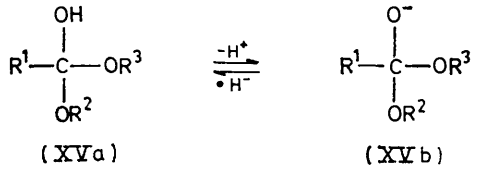
epimeric hydrolysis products would result; such products have not been observed previously [3,4] or in the present investigation.

There is considerable experimental evidence that hydrolyses of acetals and orthoesters proceed *via* carboxonium ions of the type (VIII) and (IX), respectively, rather than reaction pathways involving bimolecular nucleophilic displacement by water.[5,6] Thus, acid-catalysed hydrolysis of (I) or (II) could potentially proceed *via* carboxonium ions (X), (XI), (XII), (XIII), or (XIV). However, all of these ions except the 1,2-acetoxonium ion (X) can be discounted on the basis of the observed products. Any intermediate suggested for the hydrolyses must account for the fact that the exclusive products of the reaction are sugar monoacetates in which the anomeric configuration of the reactant orthoester is retained, *i.e.*, (III) and (IV).

The glucosyl carboxonium ion (XIII) would be expected to react with water to yield an anomeric mixture of products in which the β-anomer probably would predominate. Thus, it cannot account for the retention of anomeric configuration observed in hydrolyses of (I). In addition, quantitative generation of a 2-*O*-acetyl group from the 2-*O*-(1-alkoxy-1-hydroxyethyl) substituent is questionable. Perlin has postulated



this type of substituent as an intermediate to account for formation of glycosides unsubstituted at C-2 in methanolyses of carbohydrate 1,2-orthoesters, *i.e.*, the substituent forms an alkyl acetate rather than the sugar 2-acetate.[7] In contrast to this, DeWolfe, in a postulated mechanism for hydrolysis of carbohydrate 1,2-orthoesters, has credited this type of substituent with quantitative formation of a 2-*O*-acyl derivative of the sugar.[4] In reality, the substituent would probably yield both the alkyl acetate and the sugar 2-acetate since it is a diester of a carboxylic orthoacid. Diesters of carboxylic orthoacids (XVa) have been suggested as intermediates in reversible acid-catalysed transesterifications.[8] In addition, the conjugate bases of the diesters (XVb), which would be expected to be present to some extent, are probable intermediates in reversible alkoxide ion-catalysed transesterifications. If the basicities of the two alkoxy-oxygen atoms are not drastically different,



(XVa) or (XVb) would be expected to yield both possible esters.

Thus, if hydrolysis of (I) or (II) occurred *via* carboxonium ion (XIII), the anomeric 3,4,6-tri-*O*-methyl-D-glucopyranoses (V) and (VI) and the anomeric 2-*O*-acetyl-3,4,6-tri-*O*-methyl-D-glucopyranoses (III) and (VII) would be the expected products. Since compounds (V)—(VII) are definitely not hydrolysis products it is extremely unlikely that (XIII) is an intermediate in the system.

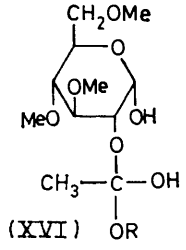
In the case of carboxonium ion (XIV), reaction with water would yield a hemiacetal which would ultimately generate an aldehyde function at C-1. Subsequent reformation of the pyranose ring would result in an anomeric mixture of products rather than the required retention of configuration at the anomeric carbon. The fate of the orthoester moiety would depend on its rate of reaction relative to the rate of formation of the aldehyde from the hemiacetal moiety. However, none of the reactions which can be envisioned would result in formation of an *O*-acetyl group at C-1 and reactions which could generate an *O*-acetyl group at C-2 would proceed *via* a 2-*O*-(1-alkoxy-1-hydroxyethyl) group which would not be expected to yield a 2-*O*-acetyl group exclusively. Thus, the products expected if hydrolysis occurred *via* carboxonium ion (XIV) would also be (III) and (V)—(VII). Since (V)—(VII) have definitely been excluded as hydrolysis products, compound (XIV) cannot be an intermediate in the system.

Carboxonium ions (X)—(XII) can be postulated on the basis of reactions of the orthoester moiety of (I) or (II).

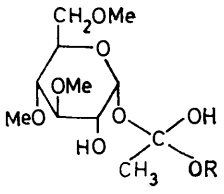
Rapid ethoxy-exchange in [$^2H_6$]ethanolysis of 2-ethoxy-2-methyl-1,3-dioxolan without apparent formation of ethane-1,2-diol suggests that the 2-methyl-1,2-dioxolenium ion [analogous to (X)] is the effective intermediate in the reaction. The 1-ethoxy-1-(2-hydroxyethoxy)ethyl cation [analogous to (XI) and (XII)], if formed, apparently undergoes an intramolecular reaction to re-form 2-ethoxy-2-methyl-1,3-dioxolan rather than react with the alcohol.[9] By

[7] A. S. Perlin, *Canad. J. Chem.*, 1963, **41**, 555.
[8] R. S. Juvet, jun., and F. M. Wachi, *J. Amer. Chem. Soc.*, 1959, **71**, 6110.
[9] L. R. Schroeder, *J. Chem. Soc.* (*B*), 1970, 1789.

analogy, this implies that in the hydrolysis of (I) or (II), compound (X) would be the effective intermediate, and (XI) and (XII), if formed, would react preferentially to re-form (I) or (II) rather than react with water. Re-formation of the 1,2-orthoacetate by (XI) and (XII), rather than reaction with water, would be due to both the forced proximity of the hydroxy-group of (XI) or (XII) to the reaction centre and the larger decrease in entropy when water is bound in the transition state in a reaction with (XI) or (XII) to form the 2-*O*- or 1-*O*-(1-alkoxy-1-hydroxyethyl) derivatives (XVI) and (XVII), respectively, as opposed to the hydroxy-group of (XI) or (XII) being bound in the transition state of an intramolecular reaction to form (I) or (II).



(XVI)

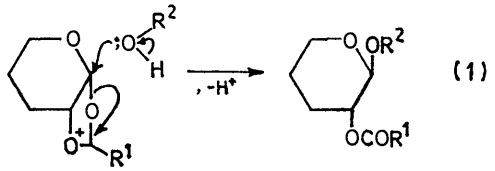


(XVII)

However, even if (XVI) and (XVII) were formed from (XI) * and (XII) it is unlikely that they would form sugar monoacetates exclusively. As discussed earlier for carboxonium ion (XIII), the *O*-(1-alkoxy-1-hydroxyethyl) substituents of (XVI) and (XVII) would be expected subsequently to form some alkyl acetate. Thus, 3,4,6-tri-*O*-methyl-α-D-glucopyranose (V), which has been excluded as a hydrolysis product (Table 2), would probably be formed to some extent from both (XVI) and (XVII).

Another argument against (XI) and (XII) being intermediates which lead to hydrolysis products is the difficulty of accounting for the solvent and acid-concentration dependence of the products if this were the case. Compounds (III) and (IV) are the observed hydrolysis products of (I) and (II). Since (XI) can possibly account for formation of (III) but not (IV), and (XII) can possibly account for (IV) but not (III), (XI), and (XII) would have to be considered as co-intermediates. To account for the drastic change in the relative amounts of (III) and (IV) formed on change of solvent or acid concentration (Table 1), it would have to be assumed that the relative amounts of (XI) and (XII) formed changed similarly. No apparent justification exists for such an assumption.

Nucleophilic attack by alcohols at the anomeric carbon of carboxonium ions analogous to (X) has frequently been suggested to account for formation of 2-acyloxy-glycosides with inverted configuration at the anomeric carbon in alcoholyses of carbohydrate 1,2-orthoesters [equation (1)].[4,7,10,11] However, analogous



(1)

nucleophilic attack by water on (X) in hydrolyses of (I) or (II) can be discounted on the basis that 2-*O*-acetyl-3,4,6-tri-*O*-methyl-β-D-glucopyranose (VII), the expected product of such a reaction, is not a hydrolysis product. In addition, if such a mechanism were operative, hydrolyses in aqueous ethanol systems (Table 2) would generate considerably more ethanolysis products than is observed since the reactivity of ethanol and water toward (X) should not be drastically different.† In aqueous ethanol containing 95 mol % ethanol, ethanolysis products account for only 6% of the consumed orthoester; with 70 mol % ethanol only hydrolysis products are formed.

The observed hydrolysis products, including their solvent and acid-concentration dependence, can be accounted for if it is assumed that the 1,2-acetoxonium ion (X) is the hydrolysis intermediate and that it reacts with water to form the 1,2-*O*-(1-hydroxyethylidene)-α-glycose (XVIII, Figure) which is a diester of orthoacetic acid. The hydroxy-group of (XVIII) should be relatively acidic owing to the two geminal electronegative oxygen atoms and, hence, some ionisation to form the conjugate base (XIX) would be expected. Thus, the potential of both (XVIII) and (XIX) to form the observed hydrolysis products (III) and (IV) must be considered.

Conjugate bases of diesters of carboxylic orthoacids [(XX), equations (2) and (3)], analogous to (XIX) are probable intermediates in reversible alkoxide ion-catalysed transesterification of carboxylic esters. As shown, the diester intermediate (XX) can generate two different carboxylic esters. The ratio of the two esters formed from (XX) would depend on the relative 'leaving ability' of the two alkoxy-groups. Since the two leaving groups are similar, their relative 'leaving ability' can be correlated with their relative basicities; the alkoxy-group which is the weaker base would be the better leaving group.

In the rearrangement of (XIX) (Figure) the two possible esters which can be generated are the sugar monoacetates (III) and (IV). The relative ratio of (III) and (IV) formed will be dependent, at least partly,

* DeWolfe, in a review of hydrolyses of carbohydrate orthoesters, has postulated carboxonium ions analogous to (XI) as the reaction intermediate. However, the suggested mechanism is admittedly based on 'very limited experimental evidence' which indicates that the initial hydrolysis products were 2-acyloxy-carbohydrates with retention of configuration at the anomeric carbon atom.[4]

† A similar assumption has been made concerning the relative reactivity of water and methanol by Cordes in work of a similar nature.[6]

[10] N. E. Franks and R. Montgomery, *Carbohydrate Res.*, 1968, **6**, 286.
[11] N. K. Kochetkov, A. J. Khorlin, and A. F. Bochkov, *Tetrahedron*, 1967, **23**, 693.

on the relative basicity of the C-1 and C-2 oxygen atoms. The C-1 oxygen atom should be less basic, and, hence, a better leaving group, than the C-2 oxygen atom because of the proximity of the electronegative ring oxygen atom to the former. Thus, based on this consideration, rearrangement of (XIX) should yield more of the 2-*O*-acetyl-α-glucosyloxy-anion [(XXI), Figure] than the isomeric 1-*O*-acetyl-2-anion (XXII) and hence, more (III) than (IV).

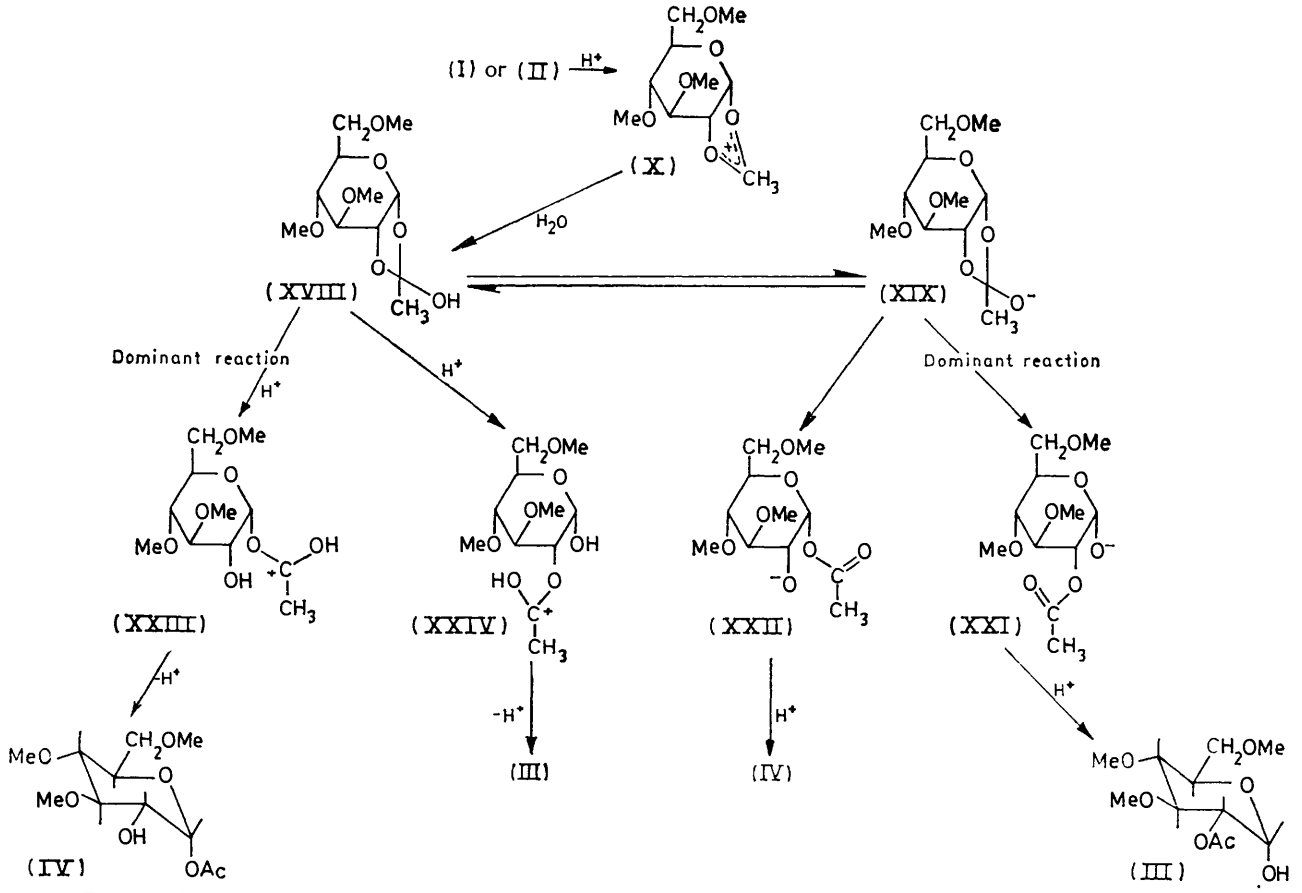
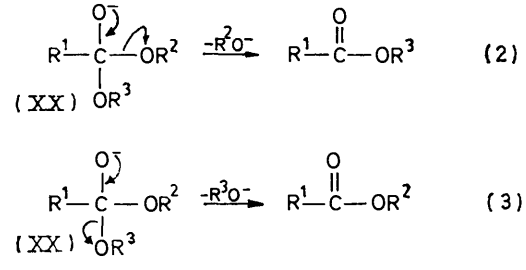


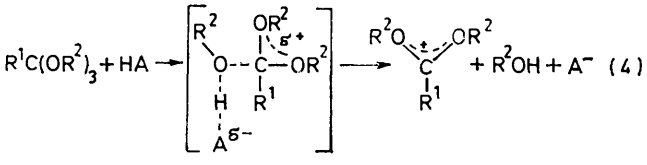
FIGURE Suggested mechanism for hydrolysis of 3,4,6-tri-*O*-methyl-1,2-*O*-(1-alkoxyethylidene)-α-D-glucopyranoses

In contrast to the acyclic carboxylic orthoacid diesters (XVI) and (XVII) discussed previously, (XVIII) should



react to form the sugar monoacetates (III) and (IV) exclusively. However, in contrast to the rearrangement of its conjugate base (XIX), reaction of (XVIII) requires rapid formation of the intermediate carboxonium ion or an $A\text{–}S_E2$ mechanism [equation (4)] in which proton transfer is concerted with carboxonium ion formation.[5,6]

It is reasonable to expect that the diester (XVIII) reacts *via* a similar mechanism and, therefore, that the relative amounts of (III) and (IV) formed from it will



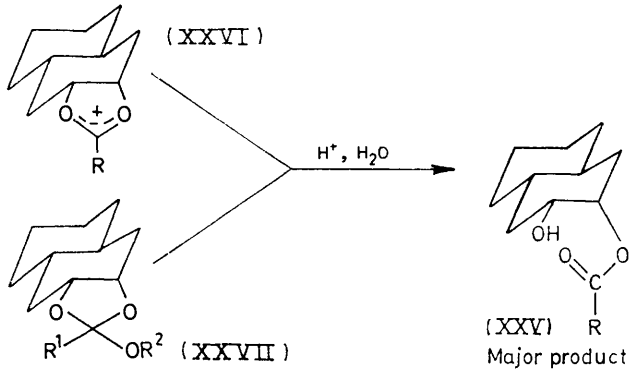
be dictated primarily by the relative basicities of the C-1 and C-2 oxygen atoms. As discussed previously, the C-2 oxygen atom should be more basic than the C-1 oxygen atom. Thus, the dominant reaction of (XVIII)

should be addition of a proton to the C-2 oxygen atom concerted with opening of the dioxolan ring to form (XXIII) (Figure). Loss of a proton from the intermediate carboxonium ion (XXIII), which is the conjugate acid of the ester product (IV), would be very rapid.* The lesser product (III) would similarly form *via* (XXIV) (Figure) when a proton is transferred to the C-1 oxygen atom of (XVIII).

Hence, formation of the 1-*O*-acetyl isomer (IV) should be favoured in reactions of the orthoacid diester (XVIII) whereas reactions of the conjugate base (XIX) should favour formation of the 2-*O*-acetyl isomer (III). If this theory is correct, the product distribution would be expected to show a dependence on acid concentration. An increase in acid concentration should result in a higher concentration of (XVIII) relative to its conjugate base (XIX) and, in turn, the final product distribution should shift toward more (IV) and less (III). Experimental results are in accord with this prediction. Table 1 shows that as the acid concentration was increased from 0·004N to 0·8N the product distribution for hydrolyses in deuterium oxide shifted from predominantly (III) (83%) to predominantly (IV) (79%).

Further, the observed variation in the relative amounts of (III) and (IV) formed with change of solvent (Table 1) can be rationalised on the basis of the relative amounts of the diester (XVIII) and its conjugate base (XIX) formed in the system. A decrease in the polarity or basicity of the solvent system would favour increased formation of the 1-*O*-acetyl-α-glucose (IV) since more of the reaction would proceed *via* the un-ionised diester (XVIII).

King and Allbutt [12] have suggested that the preponderance of axial ester-equatorial alcohol (XXV) formed in hydrolyses of dioxolenium ions and orthoesters of *trans*-decalin-*cis*-2,3-diol [(XXVI) and (XXVII),



respectively] can be rationalised on the basis of stereoelectronic and steric arguments involving maximum stabilisation of the developing carboxonium ion by an unshared electron pair of a dioxolan-ring oxygen atom. Extension of their arguments to carbohydrate systems seems risky in view of the potential effects of the pyranose-ring oxygen atom on the stereochemical and electronic factors they mention. In particular, the solvent-dependence and acid-dependence observed in this work demand that the relative basicities of the C-1 and C-2 oxygen atoms as well as the acidity of orthoacid intermediates [such as (XVIII)] be taken into account in any explanation of the acid-catalysed hydrolyses of carbohydrate 1,2-orthoesters.

The ability to vary the product distribution by changing the solvent or the acid concentration should be of potential use in synthetic work.

EXPERIMENTAL

Analytical Methods.—M.p.s were determined on a Thomas Hoover capillary apparatus which was calibrated against known compounds. Polarimetric measurements were made on a Zeiss–Winkel visual polarimeter.

N.m.r. spectra were determined on a Varian A-60A spectrometer at the normal probe temperature. Sodium 2,2-dimethyl-2-silapentane-5-sulphonate was employed as the internal standard in deuterium oxide solutions; tetramethylsilane was used in all other solutions.

A Varian Aerograph model 1200-1 gas chromatograph equipped with a hydrogen flame ionisation detector was used for g.l.c. Chromatographic response was recorded and integrated with a Honeywell Electronic 16 recorder equipped with a model 227 Disc integrator. The column (5% SE-30 on 60—80 mesh Chromosorb W, 10 ft × 0·125 in o.d., stainless steel) was arranged for on-column injection. Analyses were performed with: column, 162 °C; injector, 205 °C; detector, 265 °C; and N_2 (carrier gas), 45 lb in^{-2}.

3,4,6-*Tri*-O-*acetyl*-1,2-O-[1-(exo-*ethoxy*)*ethylidene*]-α-D-*glucopyranose* (XXVIII).—Tetraethylammonium bromide (164 g, 0·78 mol), 2,4,6-trimethylpyridine (46·2 ml, 0·35 mol), and absolute ethanol (28·5 ml, 0·49 mol) were dissolved in absolute chloroform [13] (1200 ml) and added to 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide [14] (120 g, 0·292 mol). The resulting solution was refluxed for 1·0 h with exclusion of moisture, cooled in an ice-bath, washed with ice–water (3 × 800 ml), dried (Na_2SO_4), and concentrated *in vacuo* to a thick syrup. Crystallisation from di-isopropyl ether (150 ml) containing pyridine (1 ml) yielded (XXVIII) (94 g, 85%), m.p. 93—96 °C. Recrystallisations were from di-isopropyl ether–ethanol (3 : 1, v/v) containing a trace of pyridine. The pure product had m.p. 96—97 °C, $[\alpha]_D$ +29·5 ($CHCl_3$) {lit.,[15] m.p. 96—97 °C, $[\alpha]_D$ +31° ($CHCl_3$)}. The n.m.r. chemical shifts ($CDCl_3$) of the dioxolan 2-methyl protons [δ 1·71 p.p.m. (s)] and the anomeric proton [δ 5·72 p.p.m. (d, $J_{1,2}$ 5·2 Hz)] are indicative of the *exo*-ethoxy-configuration.[16]

3,4,6-*Tri*-O-*acetyl*-1,2-O-[1-(exo-*isopropoxy*)*ethylidene*]-α-D-*glucopyranose* (XXIX).—Compound (XXIX) was prepared in 86% yield by substituting absolute propan-2-ol in the procedure for preparing (XXVIII). Recrystallisations

* Alternatively, direct formation of (III) or (IV) from (XVIII) could potentially result from concerted proton transfer to the C-1 or C-2 oxygen atom, respectively, dioxolan ring-opening, and proton loss from the orthoacid hydroxy-group.

[12] J. F. King and A. D. Allbutt, *Canad. J. Chem.*, 1970, **48**, 1754.

[13] D. D. Reynolds and W. L. Evans, *J. Amer. Chem. Soc.*, 1938, **60**, 2559.

[14] F. J. Bates, 'Polarimetry, Saccharimetry, and the Sugars,' U.S. Govt. Printing Office, Washington, D.C., 1942, p. 500.

[15] M. Schulz and H. Steinmaus, *Z. Naturforsch.*, 1964, **19b**, 263.

[16] R. U. Lemieux and A. R. Morgan, *Canad. J. Chem.*, 1965, **43**, 2199.

were from di-isopropyl ether–propan-2-ol (3 : 1, v/v) containing a trace of pyridine. The pure product had m.p. 120—121 °C, $[\alpha]_D$ +26·9 ($CHCl_3$) {lit.,[16] m.p. 120—121 °C, $[\alpha]_D$ +30° ($CHCl_3$)}. The n.m.r. spectrum was consistent with the *exo*-isopropoxy-assignment.[16]

3,4,6-*Tri*-O-*methyl*-1,2-O-[1-(exo-*ethoxy*)*ethylidene*]-α-D-*glucopyranose* (I).—Compound (XXVIII) (40 g) and powdered sodium hydroxide (120—130 g) were added to tetrahydrofuran (650 ml) in a 2000 ml, round-bottom flask fitted with a dropping funnel and an efficient overhead stirrer and maintained in a water-bath at 24—28 °C. Dimethyl sulphate (65 ml) was added dropwise during 2·5 h to the vigorously stirred mixture. After an additional 2·5 h, triethylamine (200 ml), benzene (475 ml), and enough water to dissolve all solids were added. The mixture was heated (60 °C) for 1·0 h with stirring to decompose excess of dimethyl sulphate. After cooling (0 °C), the usually encountered, three-phase (liquid) system was broken by adding water and benzene. The benzene phase was washed with 1% aqueous potassium iodide (1 × 400 ml), 1% aqueous sodium thiosulphate (1 × 400 ml), and water (2 × 600 ml), dried (Na_2SO_4), and concentrated *in vacuo* to an oil (average yield, *ca.* 90%). The oil was purified by distillation from barium oxide (0·5 g) through a 15 cm Vigreux column at reduced pressure (0·05 mmHg). The purest distillate was the last one-third to one-half of the still charge. The purified *oil* had $[\alpha]_D$ +45° ($CHCl_3$) (Found: C, 53·7; H, 8·3. $C_{13}H_{24}O_7$ requires C, 53·4; H, 8·3%), δ ($CDCl_3$) 1·68 (3H, s, CH_3·C), 5·68 (1H, $J_{1,2}$ 5·2 Hz, 1-H), 4·36 (1H, octet, $J_{2,3}$ 3·3 Hz, $J_{2,4}$ *ca.* 1 Hz, 2-H), 3·42, 3·47, and 3·50 (3 × MeO), and 1·18 p.p.m. (3H, O·CH_2CH_3).

Methanolysis of (I) and subsequent deacetylation of the product mixture yielded known methyl 3,4,6-tri-*O*-methyl-β-D-glucopyranoside (71%), m.p. 51·5—52 °C (from hexane), $[\alpha]_D$ −17·1 ($CHCl_3$) {lit.,[17] m.p. 51·5—52·5 °C, $[\alpha]_D$ −16·5° ($CHCl_3$)}.

Mild hydrolysis of (I) yielded a mixture of 2-*O*-acetyl- and 1-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose (III) and (IV), respectively, which were characterised as part of this study.

Extended hydrolysis of (I) in refluxing 0·1N-H_2SO_4 and crystallisation of the product mixture from hot di-isopropyl ether yielded (with seeding) crude 3,4,6-tri-*O*-methyl-α-D-glucopyranose (V) as fine needles. Recrystallisation to constant m.p. gave (V), m.p. 80·5—81·5 °C, $[\alpha]_D^{22}$ *ca.* +120° ⟶ +77° (*c* 1·6, H_2O) {lit.,[17] m.p. 76—77 °C, $[\alpha]_D^{25}$ +91·9° ⟶ +77·4° (*c* 1·6, H_2O)}. The original mother-liquor subsequently yielded crude 3,4,6-tri-*O*-methyl-β-D-glucopyranose (VI) on cooling. Recrystallisation from di-isopropyl ether yielded (VI) as prisms, m.p. 102—103 °C, $[\alpha]_D^{25}$ +41° ⟶ +76° (*c* 1·3, H_2O) {lit.,[17] m.p. 97—98 °C, $[\alpha]_D^{25}$ +41·1° ⟶ +78° (*c* 1·6, H_2O)}.

3,4,6-*Tri*-O-*methyl*-1,2-O-[1-(exo-*isopropoxy*)*ethylidene*]-α-D-*glucopyranose* (II).—Compound (II) was prepared by methylating (XXIX) and subsequent purification as described for the preparation of (I). The pure *oil* had $[\alpha]_D$ +42° ($CHCl_3$) (Found: C, 55·0; H, 8·6. $C_{14}H_{26}O_7$ requires C, 54·9; H, 8·6%), δ ($CDCl_3$) 1·69 (3H, s, CH_3·C), 5·67 (1H, d, $J_{1,2}$ 5·3 Hz), 4·37 (1H, octet, $J_{2,3}$ 3·1 Hz, $J_{2,4}$ *ca.* 1 Hz), 3·42, 3·46, and 3·50 (3 × MeO), and 1·17 p.p.m. (6H, d, Me_2C·O).

The hydrolysis reactions of (II) were comparable with hydrolyses of (I), yielding the same products under comparable conditions.

2-O-*Acetyl*- (III) *and* 1-O-*Acetyl*-3,4,6-*tri*-O-*methyl*-α-D-*glucopyranose* (IV).—Short-duration, acid-catalysed hydrolyses of (I) and (II) yielded two crystalline monoacetates of 3,4,6-tri-*O*-methyl-α-D-glucopyranose. Physical separation of the crystals and subsequent recrystallisations yielded pure (III) and (IV). A typical hydrolysis is described below.

Crude compound (I) (29 g) was shaken with 0·1N-sulphuric acid (200 ml) for 4 min at room temperature, neutralised with aqueous sodium hydrogen carbonate, and extracted with chloroform (5 × 100 ml). The chloroform extracts were washed with water (100 ml), and the water phase was back-extracted with chloroform (100 ml). Concentration of the combined chloroform extracts *in vacuo* yielded a syrup (25·2 g) which on crystallisation from di-isopropyl ether–light petroleum (b.p. 30—60 °C) yielded first, nearly colourless needles and later, highly coloured (yellow-orange) needles.

Manual separation of the highly coloured needles and recrystallisation from di-isopropyl ether with decolourisation yielded crude (IV). Several more recrystallisations from di-isopropyl ether yielded the *glucopyranose* (IV); m.p. 107·5—108·5 °C, $[\alpha]_D$ +146° ($CHCl_3$) (Found: C, 50·0; H, 7·7. $C_{11}H_{20}O_7$ requires C, 50·0; H, 7·6%). Recrystallisation of the remaining crystals (major product) several times from di-isopropyl ether yielded the *glucopyranose* (III), m.p. 103·5—105 °C, $[\alpha]_D$ +116° ($CHCl_3$) (Found: C, 50·3; H, 7·8. $C_{11}H_{20}O_7$ requires C, 50·0; H, 7·6%).

The structures of (III) and (IV) were assigned on the following evidence: a mixed m.p. of (III) and (IV) gave a depression of 20 °C. Compounds (III) and (IV) both gave positive ferric hydroxamate ester tests. Acetylation of either (III) or (IV) with acetic anhydride in pyridine at 0 °C yielded 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methyl-α-D-glucopyranose (XXX), identical with (XXX) obtained by a similar acetylation of (V). Extended acid hydrolysis of either (III) or (IV) yielded a mixture of (V) and (VI). The above, in conjunction with the specific optical rotations and elemental analyses of (III) and (IV) are consistent with the compounds being distinct monoacetates of 3,4,6-tri-*O*-methyl-α-D-glucopyranose. This was further substantiated by the n.m.r. spectra of (III) and (IV) which were dissimilar but both of which indicated tri-*O*-methyl substitution (δ *ca.* 3·5 p.p.m.) and an *O*-acetyl substituent (δ *ca.* 2·1 p.p.m.).

The position of *O*-acetyl substitution was easily ascertained from n.m.r. data. The anomeric proton of (IV) appears as a doublet [δ 6·19 p.p.m. ($J_{1,2}$ 3·4 Hz)] at relatively low field indicating geminal *O*-acetyl substitution. The magnitude of $J_{1,2}$ confirms the α-configuration indicated by the preceding data.[18] The unsubstituted C-2 hydroxy-group is indicated by a doublet [δ 2·59 p.p.m. ($J_{2,OH(2)}$ 4·5 Hz)] at relatively high field which disappears on addition of deuterium oxide.

The anomeric proton of (III) appears as a triplet [δ 5·32 p.p.m. ($J_{1,2} = J_{1,OH(1)} = 4·0$ Hz)] which collapses to a doublet on addition of deuterium oxide, thus indicating that the anomeric proton is coupled with the proton of a geminal hydroxy-group. The magnitude of $J_{1,2}$ confirms the α-configuration of (III). The unsubstituted anomeric hydroxy-group also accounts for the fact that 1-H of (III) resonates at higher field than 1-H of (IV). *O*-Acetyl

[17] R. L. Sundberg, C. M. McCloskey, D. E. Rees, and G. H. Coleman, *J. Amer. Chem. Soc.*, 1945, **67**, 1080.

[18] R. U. Lemieux and J. D. Stevens, *Canad. J. Chem.*, 1966, **44**, 249.

substitution at C-2 of (III) is indicated by the low-field position of the 2-H octet [δ 4·70 p.p.m. ($J_{1,2}$ 4·0 Hz, $J_{2,3}$ 10·0 Hz, $J_{2,OH(1)}$ *ca.* 1 Hz)]. On addition of deuterium oxide the 2-H octet collapses to a quartet thus confirming long-range coupling of 2-H with the anomeric hydroxy-proton. The anomeric hydroxy-proton appears as a quartet (δ 4·08 p.p.m.) at relatively low field and disappears on addition of deuterium oxide.

1,2-*Di*-O-*acetyl*-3,4,6-*tri*-O-*methyl*-α-D-*glucopyranose* (XXX).—A mixture of (III) and (IV) (46 g) was dissolved in a solution of acetic anhydride (92 ml) and pyridine (184 ml) at 0 °C. After 16 h at 0 °C the solution was poured into ice–water (400 ml) and stirred for 0·5 h. The product was extracted with chloroform (2 × 300 ml); washed successively with N-sulphuric acid, saturated sodium hydrogen carbonate, and water; and concentrated *in vacuo* to a pale yellow syrup. The syrup was dissolved in an equal volume of hot di-isopropyl ether and crystallised at −15 °C to yield 48·5 g (91%) of crude (XXX), m.p. 60—64 °C. Several recrystallisations from di-isopropyl ether yielded the *glucopyranose* (XXX) as prisms, m.p. 64—65 °C, $[\alpha]_D^{22}$ +122° (*c* 1·9, $CHCl_3$) (Found: C, 51·1; H, 7·2. $C_{13}H_{22}O_8$ requires C, 51·0; H, 7·2%).

The sugar (XXX) was also obtained when pure (III), (IV), or (V) were acetylated by the above procedure.

The α-configuration was assigned to (XXX) on the basis of the high positive specific optical rotation, n.m.r. data for 1-H [δ ($CDCl_3$) 6·25 p.p.m. ($J_{1,2}$ *ca.* 3·9 Hz)],[18] and the fact that acetylation under these conditions generally yields a product with the anomeric configurations of the reactant.[19]

2-O-*Acetyl*-3,4,6-*tri*-O-*methyl*-α-D-*glucopyranosyl Bromide* (XXXI).—Compound (XXX) (15 g) was treated with hydrogen bromide (30—32%) in acetic acid (20 ml) for 1·5 h at room temperature. The reaction solution was diluted with chloroform (200 ml) and washed with ice–water (200 ml). The aqueous phase was back-extracted with chloroform (75 ml). The combined chloroform extracts were washed with ice–water (2 × 200 ml), dried ($CaCl_2$), and concentrated *in vacuo* to yield crude (XXXI) (16·3 g) as a pale yellow syrup. Attempts to crystallise the crude bromide failed so it was employed in the following Koenigs–Knorr synthesis without further purification.

3-*Methyl*-1-*butyl* 3,4,6-*Tri*-O-*methyl*-β-D-*glucopyranoside* (XXXII).—Crude (XXXI) was treated with 3-methylbutan-1-ol in a Koenigs–Knorr reaction employing mercuric salts.[20] The product mixture was distilled under reduced pressure (<0·1 mmHg) through a 15 cm Vigreux column at pot and head temperatures of 165 °C and 120 °C, respectively. The first 20 ml of distillate from a pot charge of 30 ml was retained and subjected to a second distillation in which the middle 10 ml of distillate were retained.

The oil was deacetylated with excess of 0·2N-NaOH in aqueous methanol (1 : 1, v/v; 800 ml) at 70 °C for 1 h. After removal of methanol by partial concentration *in vacuo*, the resulting solution was extracted with chloroform (2 × 200 ml). The chloroform extracts were washed with water (4 × 200 ml), treated with carbon, and concentrated *in vacuo* to a colourless oil. Residual solvent was removed at 0·05 mmHg and 75 °C for 2 h.

The purified *oil* had $[\alpha]_D^{25}$ −21·2° (*c* 1·5, $CHCl_3$) (Found: C, 57·5; H, 9·7. $C_{14}H_{28}O_6$ requires C, 57·5; H, 9·7%). Analysis of an *O*-propanoylated sample (propanoic anhydride in pyridine) by g.l.c. indicated *ca.* 1% of an impurity assumed to be the α-anomer and a smaller amount of an unidentified material.

The β-configuration was assigned to (XXXII) on the basis of the specific optical rotation and the n.m.r. data for 1-H [δ 4·20 p.p.m. (d, $J_{1,2}$ *ca.* 7·2 Hz, $CDCl_3$)].

Solvents and Reagents.—Ethanol and methanol were dried by refluxing with magnesium and iodine.[21] Propan-2-ol was dried under reflux with sodium (5 g l^{-1}). All the alcohols were fractionally distilled (40 cm Vigreux column) with the exclusion of moisture into receivers previously dried by storage over potassium hydroxide or by rinsing with fresh distillate. Ethanol used as a reaction solvent was subjected to the drying procedure twice, the second time just before use. Deuterium oxide (99·8%), [$^2H_1$]-chloroform (99·8%), anhydrous [$^2H_6$]ethanol (99%), and [$^2H_6$]acetone (99·5%) were used as received from Stohler Isotope Chemicals, Inc. 2,4,6-Trinitrophenol was crystallised twice from methanol and dried *in vacuo* over potassium hydroxide and had m.p. 121·3—121·8 °C. 2,6-Dichlorobenzoic acid was prepared from 2,6-dichlorotoluene [1,22] with final purification by sublimation and had m.p. 143—144 °C. Propanoic anhydride (97%) was fractionally distilled (100 cm Raschig ring column) from phosphoric oxide (25 g/l). Tetraethylammonium bromide was dried *in vacuo* at room temperature over phosphoric oxide for extended periods before use.

Product Analysis.—T.l.c. Several drops of either (I) or (II) were allowed to react for 5 min at room temperature in aqueous sulphuric acid (*ca.* 0·02N, 3 ml). The reaction products were analysed by t.l.c. on Silica Gel G (Brinkman Instruments, Inc., Cantiague Road, Westbury, N.Y.) with use of double development with hexane–pyridine (4 : 1, v/v). Spot positions were determined by spraying with sulphuric acid (1 : 5, w/w, sulphuric acid–methanol) and subsequent charring. Products identifications were made by running reference compounds on the same plate. The R_F values varied between plates but the following are typical: (I) or (II), 0·69; (III), 0·32; (IV), 0·32; and (V) or (VI), 0·17.

G.l.c. Hydrolyses of (I) and (II) were ultimately incorporated into an analytical procedure used to estimate their concentrations in ethanolyses.[1,23] Thus it was necessary to determine the hydrolysis products quantitatively. To do this aliquot portions of ethanolysis solutions were subjected to the following procedure immediately after preparation of the reaction solution. Details of solution preparation and sampling techniques are given elsewhere.[1,23]

An aliquot portion (1 ml) of an ethanolysis solution of (I) or (II) (*ca.* 6·7 × 10^{-3}M; 5·2 × 10^{-3}N in 2,6-dichlorobenzoic acid) was pipetted into a standard solution (2 ml) of (XXXII) (2·99 × 10^{-3}M at 25 °C) in toluene–triethylamine (7 : 3, v/v). A portion of the resultant solution (1—2 ml) was concentrated to an oil *in vacuo* (bath 45 °C) and an aqueous acetone solution of Universal Indicator * (4 ml) was added. Sulphuric acid (N, one drop) was added and, after 3 min, sodium hydroxide (0·01N) was added to pH 5—7. Buffer (0·4 ml; 0·1M-K_2HPO_4 and 0·1M-KH_2PO_4)

* Harleco Universal Wide Range Indicator (50 ml) was concentrated to dryness to remove the ethanol solvent and the residue was dissolved in water–acetone (2 l; 9 : 1, v/v).

[19] R. Behrend and P. Roth, *Annalen*, 1904, **331**, 359.
[20] L. R. Schroeder and J. W. Green, *J. Chem. Soc.* (*C*), 1966, 530.
[21] H. Lund and J. Bjerrum, *Ber.*, 1931, **64**, 210.
[22] J. F. Norris and A. E. Bearse, *J. Amer. Chem. Soc.*, 1940, **62**, 956.
[23] D. P. Hultman and L. R. Schroeder, in preparation.

was added and the solution was concentrated *in vacuo* to *ca.* 0·5 ml. At this point the reaction products could also be analysed by the preceding t.l.c. system. Drying of the sample was completed by storing the open flask in a vacuum desiccator (*ca.* 20 mmHg) (KOH) for a minimum of 5 h.

The dried sample was treated with pyridine–propanoic anhydride (*ca.* 1·5 ml; 5 : 3, v/v) at room temperature with occasional swirling for 24 h. Water (12 ml) was added and, after 15 min, the solution was extracted with chloroform (3 × 15 ml). The chloroform extracts were washed with 2N-HCl in saturated NaCl (6—7 ml), saturated $NaHCO_3$ in 10% NaCl (6—7 ml), and water (10 ml). After each washing the aqueous phase was back-extracted with a comparable volume of chloroform. The chloroform solutions were then combined for the succeeding stage of the procedure.

The resultant chloroform solution was concentrated *in vacuo* to an oil. In cases where residual propanoic acid was noted, it was removed as its aqueous azeotrope by adding several ml of water and reconcentrating. The oil was further dried (KOH) in a desiccator for several hours, dissolved in acetone (1—2 ml), and analysed by g.l.c.

The response factors required for quantitative g.l.c. were determined by treatment of synthetic mixtures of the necessary compounds in ethanol by the above procedure.

Compound (I) was also allowed to react to completion in anhydrous and aqueous ethanol (0, 5, and 30 mol % water; 0·0085N-2,6-dichlorobenzoic acid) and analysed by the above procedure. The solutions were prepared by weighing water into a volumetric flask, adding an aliquot portion of a standard ethanolic solution of 2,6-dichlorobenzoic acid, diluting to volume with anhydrous ethanol, and reweighing. Compound (I) was then weighed into a volumetric flask and, after thermal equilibration, was diluted to volume with the thermally equilibrated ethanol–water solution. In calculations, it was assumed that the density of (I) is 1·0 g ml^{-1} and that the volumes are additive.

N.m.r. In studies of the effect of solvent, the solvent (0·50 ml) and a saturated solution of 2,4,6-trinitrophenol in deuterium oxide (0·10 ml) were added to an n.m.r. sample tube. Compound (I) (0·25 ml) was added to the tube, the tube was shaken, and the necessary spectra were determined.

In studies of the effect of acid concentration, the appropriate H_2SO_4–deuterium oxide solutions (0·50 ml) were utilised with (I) (0·10 ml) in similar fashion. Deuterium oxide–H_2SO_4 solutions of varying concentration were prepared directly in the n.m.r. sample tubes by diluting appropriate volumes of stock solutions of H_2SO_4 in D_2O (2N and 0·04N) with D_2O by means of microsyringes. Volumes were assumed to be additive.

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