268. *Cyclitols. Part V.* Paper Ionophoresis, Complex Formation with Borate, and the Rate of Periodic Acid Oxidations.*

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The ionophoretic mobilities of some 29 cyclitols and related compounds have been determined in sodium tetraborate solutions. In many cases the values are not related to the presence or to the number of *cis*-1 : 2-diol groups, which have always been regarded as necessary for complex formation. In these cases, mobilities are caused by 1 : 1 complexes formed from boric acid and cyclitols which contain *cis*-1 : 3 : 5-hydroxyl groups; the tridentate structure (IV) is assigned to these complexes. Equilibrium constants of complex formation, and the acid dissociation constant of one tridentate complex (that of *cis*inositol), have been determined.

The initial rate of the reaction between cyclitols and periodic acid is found to vary considerably with configuration. It is suggested that the presence of steric strain explains the very fast reaction of several cyclitols.

Cyclitols, like other polyhydroxy-compounds,[1-5] show ionophoretic mobility in aqueous sodium borate solution.[6] As an alternative to paper chromatography, paper ionophoresis is useful in the identification and characterisation of cyclitols and their derivatives, *e.g.*, the methyl ethers of *myo*inositol (I) have very similar R_F values [7] but differ in their ionophoretic mobility.

Every inositol, except the all-*trans*-*scyllo*-isomer, has a high ionophoretic rate of migration (expressed as M_G, the movement relative to that of glucose [4]) and the various isomers are not well separated in 0·15M-borax solution. It was found, however, that approximately ten-fold dilution of the electrolyte changed the M_G values considerably, reversing their order in some cases, causing wide separation of the inositols, and at the same time improving the sensitivity of their detection. On the other hand, some of the slower-moving compounds showed no mobility at the greater dilution. The M_G values, in sodium tetraborate solutions of two different concentrations, of all the inositols, of the known quercitols and inositol methyl ethers, and of some related compounds are given in Table 1, together with Foster's values.[6b]

Ionophoretic mobility has been used by Foster and Stacey [6a] to deduce the likely structure of cyclitols. Complex formation with borate is generally believed to involve *cis*-1 : 2-diol structures.[8] (Frahn and Mills have shown [9] that some aliphatic 1 : 3-diols have ionophoretic mobility, but we have found no migration of *cis*-*cyclo*hexane-1 : 3-diol, or of *trans*-*cyclo*hexane-1 : 2 : 3-triol; complex formation with *cis*-1 : 3-hydroxyl groups is therefore not significant in the ionophoresis of *cyclo*hexane derivatives.) Foster and Stacey [6] have postulated that higher M_G values indicate a larger number of *cis*-1 : 2-diol groups; this assumption, however, did not prove generally valid. Foster's M_G value [6b] for sequoyitol (5-*O*-methyl*myo*inositol)—much lower than that for *myo*inositol (I), although both compounds possess the same three contiguous *cis*-hydroxyl groups—appeared anomalous. The present results reveal numerous anomalies, particularly in the more dilute electrolyte. Thus, sequoyitol and ononitol, each with three adjacent *cis*-hydroxyl

* Part IV, preceding paper.

[1] Consden and Stanier, *Nature*, 1952, **169**, 783.
[2] Kowkabany, *Adv. Carbohydrate Chem.*, 1954, **9**, 338.
[3] Foster, *Chem. and Ind.*, 1952, 828.
[4] *Idem*, *J.*, 1953, 982.
[5] Foster and Stacey, *J.*, 1955, 1778.
[6] (*a*) *Idem*, *Chem. and Ind.*, 1953, 279; (*b*) Foster, *ibid.*, p. 951.
[7] Angyal, Gilham, and McHugh, following Note.
[8] Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189.
[9] Frahn and Mills, *Chem. and Ind.*, 1956, 578.

groups, have widely differing mobility; *epi*quercitol has a much higher mobility in the dilute buffer than *neo*- or *allo*-inositol which contain a larger number of *cis*-1 : 2-diol groups. *scyllo*Quercitol and 2-*O*-methyl*myo*inositol migrate, the latter to a very considerable extent, although each of their adjacent hydroxyl groups is *trans*-situated to one another. It appears, therefore, that a structural feature other than the presence of *cis*-glycol groups is involved in complex formation and that the correct structure would not necessarily be derived from the postulate of a direct relation between M_G value and

TABLE 1. *Ionophoretic mobilities, equilibrium constants of borate complex formation, and rates of oxidation by periodic acid, of cyclitols and related compounds.*

	M_G values in 0·15M-sodium tetraborate soln.	M_G values in 0·012M-sodium tetraborate soln.	M_G values in 0·05M-sodium tetraborate soln. *	K † at 22°	$T_{\frac{1}{2}}$ (min.) ‡ at 21°
Inositols					
scyllo- (1 : 3 : 5/2 : 4 : 6)	Not located	0·02	0·05	—	178
(+)-(1 : 2 : 4/3 : 5 : 6)	0·83	0·28	0·69	—	19
neo- (1 : 2 : 3/4 : 5 : 6)	0·77	0·30	—	—	22
myo- (1 : 2 : 3 : 5/4 : 6)	0·60	0·30	0·53	25	80
muco- (1 : 2 : 4 : 5/3 : 6)	0·97	0·87	0·96	—	31
allo- (1 : 2 : 3 : 4/5 : 6)	0·96	0·54	0·88	—	1·5
epi- (1 : 2 : 3 : 4 : 5/6)	0·74	1·50	0·73	$7·0 \times 10^3$	0·9
cis- (all-*cis*)	0·73	1·60	—	$1·1 \times 10^6$	0·9
Quercitols					
scyllo- (1 : 3 : 5/4 : 6)	0·23	0·05	—	5·0	100
proto- (1 : 3 : 4/2 : 5)	0·31	0·05	—	—	34
vibo- (1 : 2 : 4/3 : 5)	0·31	0·07	—	—	48
epi- (1 : 2 : 3 : 5/4)	0·78	1·17	—	$3·1 \times 10^3$	25
cis- (all-*cis*)	0·80	1·60	—	$7·9 \times 10^3$	1·2
Inositol Me ethers					
1-Me-*myo*- (bornesitol)	0·15	0·02	0·12	—	156
2-Me-*myo*-	0·63	0·29	—	—	162
4-Me-*myo*- (ononitol)	0·60	0·45	—	—	100
5-Me-*myo*- (sequoyitol)	0·24	0·05	0·18	—	73
1-Me-(−)-	0·32	—	—	—	—
2-Me-(−)- (quebrachitol)	0·29	0·14	0·31	—	86
3-Me-(+)- (pinitol)	0·73	0·23	0·66	—	28
1 : 3-diMe-*myo*- (dambonitol)	0·00	0·00	—	—	660
		(by definition)			
***cyclo*Hexanetriols**					
1 : 3/2	0·00	0·00	—	—	—
1 : 2/3	0·20	0·08	0·10	—	—
cis-1 : 2 : 3	0·07	0·05	0·19	—	—
cis-1 : 3 : 5 (phloroglucitol)	Not located	0·11	—	32	—
***cyclo*Hexanediols**					
trans-1 : 2	0·00	0·00	—	—	—
cis-1 : 2	0·11	0·01	—	—	—
trans-1 : 3	0·00	0·00	—	—	—
cis-1 : 3	0·01	0·00	—	—	—

* Foster's values.[6a] Foster refers [4,5] to "0·2M-sodium borate at pH 10," a description which does not define the composition of his electrolyte. From an earlier paper [3] it appears that he used a buffer made from 6 parts of a solution 0·2M in boric acid and 0·1N in sodium hydroxide, and 4 parts of 0·1N-sodium hydroxide. This buffer (which is not 0·2M in borate) is equivalent in borate concentration to 0·05M-sodium tetraborate (19·07 g. of $Na_2B_4O_7,10H_2O$ per l.).

† $K = [\text{complex}^-]/[\text{borate}^-][\text{cyclitol}]$.

‡ $T_{\frac{1}{2}}$ is the time required for the consumption of 0·5 mol. of periodic acid under the conditions given in the Experimental Part.

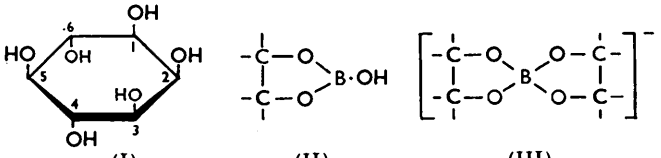
the number of *cis*-1 : 2-hydroxyl groups. It is true, however, that all *cyclo*hexane derivatives which contain *cis*-1 : 2-diol groups show ionophoretic mobility, at least in the more concentrated electrolyte solution. It is seen also from Table 1 that additional *trans*-hydroxyl groups increase the M_G values, probably by an inductive effect on complex formation.

Another anomaly was noted in the ionophoresis of the *cyclo*hexane-1 : 2 ; 3-triols;

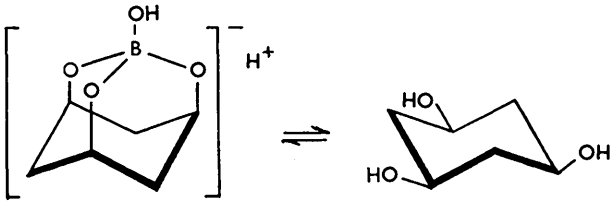
contrary to Foster's statement,[6b] the 1 : 2/3-isomer has a higher mobility than the *cis*-1 : 2 : 3-compound. The identities of the isomers,[10] which were kindly given to us by Professor Th. Posternak, were carefully checked and the ionophoresis carried out repeatedly, leaving no doubt that the isomer with only two *cis*-hydroxyl groups had a higher M_G value than the one with three.

There are four cyclitols (*cis*- and *epi*-inositol, *cis*- and *epi*-quercitol) which have higher M_G values in the dilute than in the more concentrated borate solution. This increase is caused by two factors: (*a*) by a decrease in the mobility of glucose on dilution of the electrolyte (from an average of 14·5 cm. in 0·15M- to 11·7 cm. in 0·012M-sodium tetraborate solution in 4 hours at 11 v/cm.), since the M_G values are relative to the movement of glucose; and (*b*) by an actual increase in the migration distance of these cyclitols on dilution. The rate of migration depends on the extent of complex formation and also on the ionic mobility of the complex; since the extent of complex formation cannot increase on dilution, the greater rate of migration can only be due to higher mobility of the complex anion, there being less interference by other ions in the solution.

According to the extensive studies of Böeseken and his school,[8] 1 : 2-glycols form two different types of chelate compounds with boric acid: simple esters (II) in a 1 : 1 ratio and complexes containing a *spiro*-boron atom (III) in a 2 : 1 ratio. The former [11] are acids comparable in strength to boric acid whereas the latter are strong acids, extensively ionised; it is not known whether they can exist in the undissociated form. Complexes of type (III) cause the increased acidity and conductivity of boric acid solutions after the addition of glycols. It is not known whether complex (II) or (III), or both, are responsible for the ionophoresis of glycols.



(I) (II) (III)



(IV) (V)

The four fast-moving cyclitols have one feature in common: the presence of three *cis*-oriented 1 : 3 : 5-hydroxyl groups. It appears therefore that in these cases complex formation with boric acid occurs by a reaction different from those discussed previously, namely, by a three-point attachment as in (IV): these complexes will be referred to as "tridentate complexes" in contrast to the "Böeseken complexes" formed by *cis*-1 : 2-glycols.[8] On formation of the tridentate complex, the more stable chair form (V) (the one containing the larger number of equatorial hydroxyl groups) must be inverted to the less stable chair form which has the 1 : 3 : 5-hydroxyl groups in axial positions—except in the case of *cis*inositol where both chair forms contain 1a : 3a : 5a-hydroxyl groups.

The data in Table 1 give much support to this hypothesis. The migration of *scyllo*-quercitol, which cannot form a Böeseken complex, is explained by tridentate borate

[10] Posternak and Ravenna, *Helv. Chim. Acta*, 1947, **30**, 441.
[11] Hermans, *Z. anorg. Chem.*, 1925, **142**, 83.

formation. *cis*-Phloroglucitol (V) should also form such a complex and it was indeed found to migrate even in the dilute borate solution (it could not be revealed by our spray reagents in the stronger electrolyte). The behaviour of the *O*-methyl*myo*inositols is highly significant: blocking of the 2- or 4-hydroxyl group has no effect on ionophoretic mobility, although the former destroys the possibility of formation of Böeseken complexes; methylation in the 1- or the 5-position reduces the M_G value considerably although the latter leaves all the contiguous *cis*-hydroxyl groups intact. The low M_G value of the 5-methyl ether (sequoyitol), in fact, indicates the extent to which Böeseken complexes can contribute to the mobility of *myo*inositol.

Tridentate complexes (IV) are formed in a ratio of 1 : 1 from their components and would be expected to be strong acids. Boric acid itself is a weak Lewis acid with little tendency to form the tetrahedral borate ion,[12] $B(OH)_4^-$, because its planar form is considerably stabilised by resonance involving the limiting structures $H^+O{=}B^-(OH)_2$. Brown and Fletcher [13] found it impossible to prepare a compound containing tervalent tetrahedral boron; forcing boric acid out of planarity, as in complex (IV), destroys the resonance and makes it a strong Lewis acid. Tridentate complexes can therefore be distinguished from those of type (II) and (III) by showing that they contain their components in a 1 : 1 ratio and are strongly acidic. It was found that all the cyclitols which show ionophoretic mobility depress the pH of a borate solution; in solutions which were 0·0025M in sodium tetraborate (pH 9·22) and 0·02M in cyclitol the following pH values were found: *scyllo*- 9·20, *myo*- 9·09, (—)- 8·99, *allo*- 8·88, *muco*- 8·85, *epi*- 7·18, and *cis*-inositol 5·04; *scyllo*- 9·21, *epi*- 8·49, and *cis*-quercitol 7·15; quebrachitol 9·17; *cis*-phloroglucitol 9·10; mannitol 7·57.

It was possible to calculate K, the equilibrium constant of complex formation, from the pH changes and to show that the complexes are produced from boric acid and cyclitols containing *cis*-1 : 3 : 5-triol groupings in equimolecular proportions.

Calculation of Equilibrium Constants.—The equilibrium constant of the formation of a 1 : 1 complex is defined as

$$K = [C^-]/[B^-][Cy] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $[C^-]$, $[B^-]$, and [Cy] are the concentrations of the complex anion, borate ion, and cyclitol, respectively.*

If one litre of solution is made up from sodium hydroxide, a moles of boric acid, and na moles of cyclitol, then the total concentration of boric acid in various forms is

$$a = [HB] + [B^-] + [HC] + [C^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

where [HB] is the concentration of boric acid and [HC] that of the conjugate acid of the complex anion, and

$$na = [Cy] + [HC] + [C^-] \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

Since the molarity of anions and cations must be equal, it follows that:

$$[C^-] + [B^-] + [OH^-] = [Na^+] + [H^+] \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Substitution of the value of $[C^-]$ from equation (4) and of the dissociation constants of

* This definition of K does not imply that the complex is formed by the reaction of borate ions with the cyclitol. It is much more likely that the cyclitol reacts with boric acid, but this is not relevant to our discussion. An equilibrium constant defined as $K' = [C^-][H^+]/[HB][Cy]$ would involve inconveniently small quantities in the calculations and would only differ by a constant from K, since it can be shown that $K' = KK_{aB}$. As defined in (1), the value of K allows immediate calculation of the extent of complex-formation in a borate solution of known concentration.

[12] Edwards, Morrison, Ross, and Schultz, *J. Amer. Chem. Soc.*, 1955, **77**, 266.
[13] Brown and Fletcher, *ibid.*, 1951, **73**, 2808.

boric acid and of the complex, $K_{aB} = [B^-][H^+]/[HB]$ and $K_{aC} = [C^-][H^+]/[HC]$, into equation (2) gives:

$$[B^-] = \frac{a - \{[Na^+] + [H^+] - [OH^-]\}\{1 + [H^+]/K_{aC}\}}{[H^+]/K_{aB} - [H^+]/K_{aC}} \quad . \quad . \quad . \quad (5)$$

From equation (4), we have

$$[C^-] = [Na^+] + [H^+] - [OH^-] - [B^-] \quad . \quad . \quad . \quad . \quad . \quad (6)$$

and from equation (3)

$$[Cy] = na - [C^-]\{1 + [H^+]/K_{aC}\} \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

In our experiments, excess of cyclitol ($n > 1$) was added to 0·0025M-sodium tetraborate and the pH measured. In our case, therefore, $a = 0{\cdot}01$ and $[Na^+] = 0{\cdot}005$; K_{aB} is known to be 6×10^{-10}; K_{aC} is not known but, the complex being a fairly strong acid, $[H^+]/K_{aC}$ was neglected at low hydrogen-ion concentrations. From the measured pH values, $[B^-]$, $[C^-]$, and [Cy] were calculated by equations (5), (6), and (7) and substituted into equation (1): for those cyclitols where tridentate complexing was postulated, K proved constant over a wide range of cyclitol concentration, showing that the complexes are formed in a 1 : 1 ratio.

The K values are listed in Table 1, and, as examples, the calculation of K for *epi*- and *myo*inositol is given in Table 2.

*cis*Inositol, which forms the most stable complexes, and therefore lowers the pH of borate solutions to the largest extent, does not give constant K values by the above method of calculation. This indicates that at the low pH values (below 5) produced here, the $[H^+]/K_{aC}$ term in equations (5) and (7) can no longer be neglected. Use of the value of $1{\cdot}8 \times 10^{-4}$ for K_{aC} gives constant K values as shown in Table 2. The calculations show that below pH 5 undissociated complex acid is present in considerable amounts, and give the value of its acid dissociation constant. The structure of this complex acid is uncertain; in view of Brown and Fletcher's results [13] it is unlikely to contain a tervalent boron atom and would probably be hydrated, corresponding to the addition of a proton to (and not to the removal of OH^- from) the complex anion (IV). It is intended to attempt the isolation of tridentate cyclitol complexes later.

TABLE 2. *Calculation of equilibrium constants.*

	*epi*Inositol		*myo*Inositol		*cis*Inositol			(−)-Inositol
						10^6K		
n	pH	10^3K	pH	K	pH	*	†	pH
2	7·18	7·25	9·09	19	5·04	1·0	1·08	8·99
4	6·84	6·95	8·94	24·6	4·70	0·95	1·08	8·89
8	6·50	7·0	8·75	25·6	4·42	0·84	1·10	8·61
12	6·32	6·95	8·63	25·0	4·29	0·74	1·07	8·38
16	6·20	6·8	8·52	25·6	4·20	0·68	1·09	8·20
20	—	—	—	—	4·14	0·62	1·09	—
32	—	—	8·24	27·3	—	—	—	7·76
Average		7·0 (± 0·2)		25·5 (± 1)			1·09	

* Calculated neglecting $[H^+]/K_{aC}$. † Calculated using the value of $1{\cdot}8 \times 10^{-4}$ for K_{aC}.

Cyclitols not possessing *cis*-1 : 3 : 5-triol groupings do not give constant K values by equation (1). Mannitol–borate complexes, according to Antikainen,[14] obey the $K = [C^-]/[B^-][\text{mannitol}]^{1{\cdot}8}$ equation, indicating the presence of both 1 : 1 and 2 : 1 complexes. We have found that (−)-inositol and quebrachitol follow approximately the $K = [C^-]/[B^-][Cy]^{1{\cdot}5}$ equation; the pH variation of the former is shown in Table 2.

[14] Antikainen, *Acta Chem. Scand.*, 1955, **9**, 1008.

The equilibrium constants are in the expected order. *cis*Inositol, with three axial hydroxyl groups on the same side of the ring in either chair form, forms by far the firmest complex. *epi*Inositol and *cis*-quercitol also form firmer complexes than mannitol. *scyllo*Quercitol and *myo*inositol have two axial hydroxyl groups on the same side in the tridentate complexes which increase their free energy and therefore reduce their equilibrium constants. Böeseken and Julius [15] found no complex-formation between *myo*-inositol and boric acid; this is not surprising since calculation, using the value of $K = 25$, shows that in 0·5M-boric acid containing an equimolecular amount of *myo*inositol, complex is formed to an extent of 0·02% only. The configuration of *scyllo*inositol is most unfavourable for tridentate complex formation since it would result in the interaction of three axial hydroxyl groups; tridentate complex formation with two molecules of boric acid would be possible but does not seem to occur. The K values have been used for the calculation of the various repulsive interactions in cyclitol molecules.[16]

Tridentate-complex formation, therefore, explains the anomalies of ionophoresis in borate buffers. The extent of complex-formation, calculated from equation (1) with the use of the K values in Table 1, closely parallels the M_G values. *cis*Quercitol and *cis*- and *epi*inositol exist practically 100% as complex, even in the more dilute electrolyte; glucose so exists to a smaller extent. In the 0·15M-buffer, several cyclitols show greater mobility than the most highly complexed *cis*inositol, which probably indicates that they have, to some extent, combined with a second molecule of boric acid and thereby acquired a double charge; they all possess two pairs of *cis*-1 : 2-diol groups.

Tridentate complexes with borate have not been reported before. It is reasonable to expect that they will be found with compounds other than cyclitols: the ionophoretic migration of pentaerythritol [9] is probably explained by it, and Foster and Stacey's results [5] with glucofuranosides strongly suggest formation of tridentate complexes with the oxygen atoms at $C_{(3)}$, $C_{(5)}$, and $C_{(6)}$.

The Rate of Periodate Oxidations.—For comparison with borate complex formation, the study was extended to another reaction which depends on the relative configuration of hydroxyl groups: oxidative fission by periodic acid. It is believed [17,18] that in this reaction a cyclic intermediate is formed which —like the borate ester or the *iso*propylidene compounds—contains a five-membered ring; but the large iodine atom (I–O distance is 1·93 Å) can bridge the oxygen atoms of *trans-cyclo*hexane-1 : 2-diols whereas boric acid (B–O = 1·38 Å) or acetone (C–O = 1·44 Å) usually reacts with *cis*-glycols only.[19] In the *cyclo*hexane system *cis*-glycols are oxidised more rapidly by periodic acid than their *trans*-isomers; [17] isolated examples of this phenomenon are found in cyclitol chemistry. Thus, Posternak [20] observed that *myo*inositol consumed periodic acid faster than *scyllo*-inositol, and similar measurements have been used to assign configurations to some inosamines.[21] The three *cyclo*hexane-1 : 2 : 3-triols behave similarly,[10] the all-*cis*-isomer being oxidised the fastest and the all-*trans*-isomer the slowest. The fission of the *cyclo*-hexane-1 : 2 : 3 : 4-tetrols has also been investigated.[22] Fleury, Courtois, and Bieder [23] have compared the rates of the reaction of several cyclitols.

Inositols and quercitols consume six mols. of periodic acid by a mechanism which is still under discussion.[24] Obviously, only the rate of the first step, the opening of the ring, will depend on the *cis*- or *trans*-relation of the hydroxyl groups; after the initial ring-fission all the isomers will react at similar rates. Fleury *et al.*[23] measured the consumption

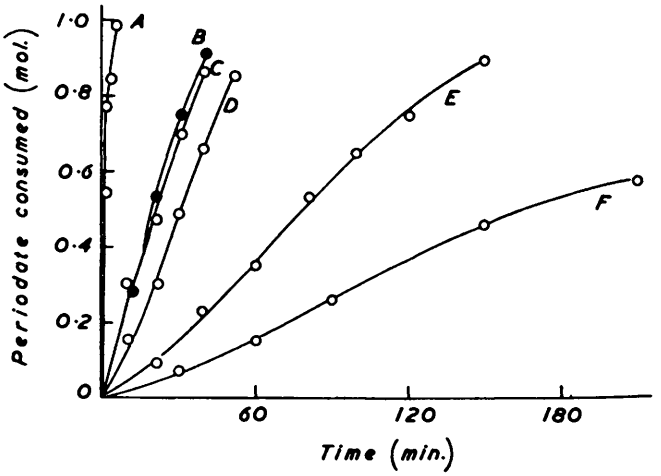
[15] Böeseken and Julius, *Rec. Trav. chim.*, 1926, **45**, 489.
[16] Angyal and McHugh, *Chem. and Ind.*, 1956, 1147.
[17] Price and Knell, *J. Amer. Chem. Soc.*, 1942, **64**, 552.
[18] Buist and Bunton, *J.*, 1955, 1406.
[19] Angyal and Macdonald, *J.*, 1952, 686.
[20] Posternak, *Helv. Chim. Acta*, 1944, **27**, 466.
[21] *Idem, ibid.*, 1950, **33**, 1597.
[22] Posternak and Friedli, *ibid.*, 1953, **36**, 259.
[23] Fleury, Courtois, and Bieder, *Bull. Soc. chim. France*, 1953, 543.
[24] Schwarz, *Chem. and Ind.*, 1955, 1388; Fleury and Le Dizet, *Bull. Soc. Chim. biol.*, 1955, **37**, 1099.

of periodic acid for all compounds *after the same time*, thereby observing them at different stages of the oxidation.

We have determined the initial rate of the periodic acid oxidation by carrying out the reaction at such dilution that several titrations could be performed before one mol. of the oxidant was consumed. Plots of periodic acid consumption *versus* time were drawn and by interpolation the time required for the uptake of 0·5 mol. of periodic acid, $T_{\frac{1}{2}}$, was determined. Several examples of these plots are shown in the Figure, and the $T_{\frac{1}{2}}$ values are listed in the last column of Table 1.

The initial rates show a surprisingly wide range. Whereas Posternak found [20] that *myo*inositol was oxidised about twice as fast as the *scyllo*-isomer, it is now seen that the *cis*-compound reacts about 200 times faster. The reaction of *epi*- and *cis*-inositol is so rapid that only approximate $T_{\frac{1}{2}}$ values could be determined; on the other hand, dambonitol reacts so slowly that the 0·5 mol. consumption of oxidant was not reached and was determined by extrapolation.

The shape of the plots of some slowly reacting cyclitols (initial increase in velocity, see Figure) indicates that the initial ring-fission is accompanied by the—apparently

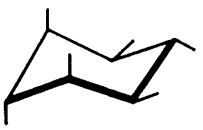


faster—subsequent oxidations, and the measured rate is higher than that of the initial fission. For this reason no attempt was made to calculate rate constants. The difference between the rapidly- and the slowly-reacting cyclitols is therefore even greater than shown by the figures.

The reciprocal of $T_{\frac{1}{2}}$ is an additive quantity and can be used for calculations. For example, from the $10^3/T_{\frac{1}{2}}$ value of *scyllo*inositol (5·6) it follows that the value for *one trans*-hydroxyl pair is 0·9; by using this value and 12·5 for *myo*inositol (in which there are four *trans*- and two *cis*-pairs), the $10^3/T_{\frac{1}{2}}$ value for *one cis*-hydroxyl pair in *myo*-inositol is found to be 4·4. With these values for each *cis*- and *trans*-pair, the following $10^3/T_{\frac{1}{2}}$ values have been calculated for the methyl ethers of *myo*inositol (with the experimentally determined values in parentheses): 1-Me 7·1 (6·4), 2-Me 3·6 (6·2), 4-Me 10·6 (10·0), 5-Me 10·6 (13·7), 1 : 3-Me_2 1·8 (1·5). The agreement is reasonable if it is taken into account that the 2-methyl ether, with its methoxy-group in axial position, has a higher energy than its isomers, which increases the rate of fission (see below).

The same value (4·4) for a *cis*-hydroxyl pair cannot be used for any other cyclitol. Each pair in, for example, (−)-inositol is oxidised considerably faster than in *myo*inositol. It is necessary to point out that the three contiguous *cis*-hydroxyl groups in the latter present a rather unfavourable conformation for the attachment of a five-membered ring

(borate, *iso*propylidene compound, or cyclic periodate); the necessary movement [19] of the central (axial) hydroxyl group towards coplanarity with another hydroxyl group is impeded by the two bulky equatorial neighbours (VI). When the axial hydroxyl group

(VI)   (VII)

is flanked by another axial group [as in (—)-inositol or *proto*quercitol] or only by hydrogen atoms (as in *vibo*quercitol), the deformation required for the attachment of the five-membered ring is more readily achieved. This is also seen in borate-complex formation and explains the behaviour of the *cyclo*hexane-1 : 2 : 3-triols referred to above: the all-*cis*-isomer contains the unfavourable arrangement of the three *cis*-hydroxyl groups. Posternak and Ravenna's data,[10] show that although the all-*cis*-isomer is oxidised faster by periodic acid than the 1 : 2/3-triol, it is not oxidised *twice* as fast, as it should if other conditions were equal. The exceptionally slow reaction of *myo*inositol with acetone to form an *iso*propylidene derivative [25] is another example of this effect.

Four of the cyclitols react very much faster with periodic acid than the others, and all the four contain two axial hydroxyl groups on the same side of the ring, an arrangement introducing extra energy (estimated [16] at 2·8 kcal./mole). Buist and Bunton [18] have shown that in the reaction of ethylene glycol with periodic acid the cyclic complex is formed by a fast reversible reaction, and subsequently breaks down with carbon–carbon bond fission. If the same mechanism is assumed to apply to cyclitols, the rate and the position of the equilibrium of complex formation will depend strongly on the relative position of the two hydroxyl groups. The rate of the fission of the complex, however, may well depend on the strain in the molecule, greater interaction energy lowering the activation energy of this reaction. It is suggested that the interaction of axial hydroxyl groups provides the explanation for the fast rate of glycol fission in *cis*-, *epi*-, and *allo*-inositol and in *cis*quercitol.

The behaviour of *muco*inositol is not fully understood: it is the only cyclitol with two axial hydroxyl groups on the same side which is not rapidly oxidised. Barton [26] has shown that, in many cases, 1 : 3-diaxial substitution renders an axial substituent at $C_{(2)}$ more stable than an equatorial one. *muco*Inositol (VII) has this configuration and may therefore have a lower free energy than the other diaxially substituted cyclitols. (γ-Benzene hexachloride, which has the *muco*-configuration, is the only 1 : 3-diaxially substituted isomer readily formed in the chlorination of benzene.)

Experimental

Materials.—*muco*Inositol was kindly given to us by Dr. Laurens Anderson (Madison), the *cyclo*hexanetriols by Professor Th. Posternak (Geneva), and the *cyclo*hexane-1 : 3-diols by Dr. W. Rigby (Birkbeck College). *allo*Inositol was synthesised by P. T. Gilham (method to be published). The other compounds were prepared or obtained as described in previous papers.[19, 25, 27, 28]

Paper Ionophoresis.—The apparatus and the method used were essentially those described by Foster.[29] The two glass plates were 18″ long and the filter paper (Whatman No. 1), cut to a size of 5 in. × 22 in., dipped about one inch below the surface of the sodium tetraborate solution

[25] Angyal, Gilham, and Macdonald, preceding paper.
[26] Barton, *Chem. and Ind.*, 1953, 664.
[27] Angyal and Matheson, *J. Amer. Chem. Soc.*, 1955, **77**, 4343.
[28] Angyal and McHugh, *Chem. and Ind.*, 1955, 947.
[29] Foster, *ibid.*, 1952, 1050.

in each container. A potential of 550 v was applied, giving a current of 16—17 and 2 milliamp. with 0·15M- and 0·012M-sodium tetraborate, respectively. The rise in temperature was negligible, making cooling unnecessary.

Unfortunately sodium borate interferes with the detection of the cyclitols by the usual reagents,[7] particularly by silver nitrate, and consequently larger amounts are required than in paper chromatography. In our experience Lemieux and Bauer's permanganate–periodate reagent [30] gives the best results. Whilst paper ionophoresis is useful for the identification of cyclitols, it is not particularly suitable for the detection of minor components in a mixture. The difficulties of detection may be obviated in the future by the use of glass filter-paper.[31]

The ionophoretic mobilities are expressed [4] as M_G values, that is, the ratio of the true distance of migration of the substance to that of glucose. The true distances are those corrected for electroendosmotic flow by reference to a non-complexing compound. Foster used 2 : 3 : 4 : 6-tetra-*O*-methylglucose as the reference compound; this is not detected by the Lemieux–Bauer spray and so dambonitol, shown to have the same movement as tetra-*O*-methylglucose (silver nitrate spray) was substituted. It was later found that dambonitol was not an ideal substance for this purpose since its movement with the electroendosmotic flow was somewhat dependent on its concentration and it had to be applied in high concentration to be detected. The outer edge of the dambonitol spot indicates the correct position for the M_G value of 0; a correction has been applied accordingly where necessary. *trans-cyclo*Hexane-1 : 2- or -1 : 3-diol would be better non-migrating markers.

Approx. 40 μg. of a cyclitol were applied to the paper; the non-migrating diols and triols required 200, dambonitol 800, and *cis*-phloroglucitol about 1200 μg. After spraying with the Lemieux–Bauer reagent, the paper was placed between two glass plates to prevent evaporation; the spots appeared after ½—1½ hr., depending on the nature and the concentration of the compound. Surprisingly, *scyllo*inositol was not revealed by this, or any other, reagent in any concentration on paper saturated with 0·15M-borate. The extensive diffusion of *myo*inositol, described by Foster,[6b] was never observed but glucose streaked strongly in 0·012M-borate buffer: its highest concentration (that is, the first appearance of a yellow spot) was taken as its true position.

The M_G values given in Table 1 are the averages of several determinations.

Determination of Equilibrium Constants.—The pH-meter (Cambridge Portable pH Meter) was adjusted with 0·0025M-sodium tetraborate to pH 9·22, the value calculated from the acid dissociation constant of boric acid; this procedure permitted the use of equations (5) to (7) without any correction. The cyclitols were added as solids to the borate solution and the pH was read after each addition.

Rate of Periodic Acid Oxidations.—Periodate solution (1 ml. containing approx. 2·5 mg. of $NaIO_4$) was rapidly added to a mixture of N-sulphuric acid (1 ml.), cyclitol solution (1 ml. containing 1·0 mg. of cyclitol), and water (20 ml.). The time was estimated from the moment the last amount of periodate solution left the pipette. After a suitable time the mixture was treated with N-sodium hydroxide (0·5 ml.), 20% aqueous sodium acetate (1·5 ml.), and a few crystals of potassium iodide. The liberated iodine was immediately titrated with 0·01N-sodium thiosulphate, four drops of 5% starch solution (in formamide) being added near the end-point. A blank determination gave the initial concentration of periodic acid. The titrations were made at appropriate intervals, depending on the rate of oxidation of the cyclitol being measured.

The authors are grateful to Dr. J. A. Mills (Adelaide) for helpful discussions.

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[*Received, September 3rd,* 1956.]

[30] Lemieux and Bauer, *Anal. Chem.*, 1954, **26**, 920.
[31] Briggs, Garner, and Smith, *Nature*, 1956, **178**, 154.