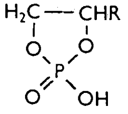
726. *The Hydrolysis of* D-*Carboxyethylene Phosphate* (D-*Glyceric Acid 2,3-Cyclic Phosphate*).

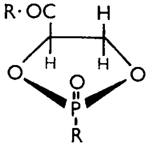
By R. F. WEBB and A. J. DUKE.

Acid-catalysed hydrolysis of D-carboxyethylene phosphate (D-glyceric acid 2,3-cyclic phosphate) gives the two glyceric acid phosphomonoesters in a different ratio from that in which they are obtained on acid-catalysed equilibration of either of them. Basic hydrolysis is not accompanied by appreciable elimination to give phosphoenolpyruvate.

UKITA *et al.*[1] recently showed that the initial product ratio from the acidic hydrolysis of glycerol 1,2-cyclic phosphate (I) is subject to kinetic control, subsequent slower equilibration of the isomeric glycerol phosphates produced leading to thermodynamic equilibrium between them.[2] We therefore record a further example of such behaviour.[3]



(I) R = $CH_2 \cdot OH$
(II) R = CO_2H



(III) R = O^- or OMe

Ammonium D-3-phosphoglycerate was converted into the 2,3-cyclic phosphate of glyceric acid (D-carboxyethylene phosphate) (II) by treatment with trifluoroacetic anhydride[4] and isolated as the trihydrate of the calcium salt. In N-hydrochloric acid at 100°, this ester was completely hydrolysed to a mixture of the isomeric α- and β-phosphoglyceric acids in about 30 seconds, as judged by paper chromatography.[5] Opening of the ring was complete in 0·023N-acid at 100° in about five minutes. In both cases, the hydrolysis and the subsequent acid-catalysed interconversion of the monoesters were followed polarimetrically, selective enhancement of the rotation of the 3-isomer in neutral 12·5% ammonium molybdate solution permitting determination of the isomer ratio in the product.[6] The final equilibrium value for the rotation obtained in N-acid is in good agreement with that found on equilibration of β-phosphoglyceric acid.[6] That the approach to equilibrium by acid-catalysed phosphate migration is much slower than hydrolysis of the cyclic phosphate in N-acid, and that migration is in fact almost negligibly slow at the lower acidity (the isomer ratio remaining almost unchanged at the value initially produced by hydrolysis of the cyclic phosphate), are evident from the variation of the optical rotation with time (Fig. 1). This is demonstrated even more clearly in Fig. 2, where $\log_{10}(\alpha - \alpha_e)$ is plotted against time,[7] α being a measure of the mole fraction of total phosphoglycerate present as the α-isomer, and α_e its value at migration equilibrium. These plots give values of the extrapolated initial isomer ratio (α- : β-phosphoglycerate) of 1·03 ± 0·07 and, 1·24 ± 0·07, respectively, 51% and 55% of α-isomer, as against 81% at migration equilibrium. Hydrolysis in N-acid at room temperature, in which conditions migration is negligibly slow,[6] also gave a product ratio of 1·20, *i.e.*, 54% of α-phosphoglycerate.

The surprising similarity of the percentages of primary and secondary ester found in this work to those found by Ukita *et al.*[1] for the glycerol phosphates (initially 48% of α-isomer, at equilibrium 81%) is probably fortuitous.

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[1] Ukita, Nagasawa, and Irie, *Pharm. Bull.* (*Japan*), 1957, **5**, 127.

[2] Cf. Ingold, "Structure and Mechanism in Organic Chemistry," Bell, London, 1953, p. 670 *et seq.*

[3] A. J. Duke, Ph.D. Thesis, Cambridge, 1957.

[4] Brown, Magrath, and Todd, *J.*, 1952, 2708.

[5] (*a*) Bandurski and Axelrod, *J. Biol. Chem.*, 1951, **193**, 405; (*b*) Cowgill, *Biochim. Biophys. Acta*, 1955, **16**, 614.

[6] Ballou and Fischer, *J. Amer. Chem. Soc.*, 1954, **76**, 3188.

[7] Glasstone, "Textbook of Physical Chemistry," van Nostrand, New York, 1946, pp. 1052—1053.

Hence, it appears that in the interconversion of isomeric 2-hydroxyalkyl phosphates under acidic conditions, the isomer ratio at equilibrium is determined by the relative rates of closure of the isomers to give a cyclic transition state or intermediate, rather than by any tendency of the related cyclic phosphodiester to open at the secondary rather than the primary position.

One of the original purposes of the preparation of D-carboxyethylene phosphate was the hope that its reaction with alkali might afford a convenient preparative method for phosphoenolpyruvate, by the known elimination reaction of β-acyloxy-acids (and their esters).[8] However, treatment of the cyclic ester in various alkaline conditions gave no trace of phosphoenolpyruvate on paper chromatography of the hydrolysates, and comparison of the ultraviolet absorption of the products in the 210—220 mμ region with that of authentic phosphoenolpyruvate and pyruvic acid indicated that less than 0·5% of elimination occurred in the total course of the reaction. Also, the product of methylation of the cyclic ester with diazomethane opened to give a solution showing negligible ultraviolet absorption when compared with a solution of similarly methylated phosphoenolpyruvic acid. Absence of elimination in these cases is presumably due to the inability of the four atoms involved (see III) to adopt the planar configuration generally considered necessary for easy base-catalysed elimination.[9]

FIGS. 1 and 2. *Change of rotation in molybdate solution on hydrolysis of* D-*carboxyethylene phosphate by* (*A*) N-*acid and* (*B*) 0·023N-*acid.*

FIG. 1.

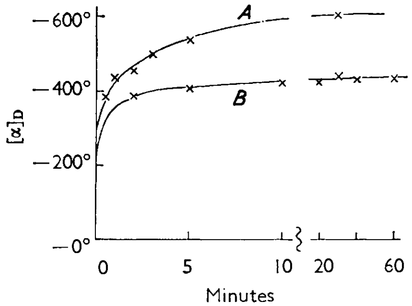
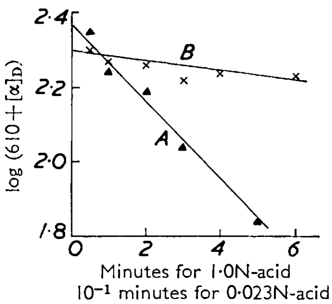


FIG. 2.



[α]D *of α-phosphoglyceric acid* [6] = −740°.

In Fig. 2, *intercepts at t* = 0 *are* 2·37 ± 0·03 *for* (*A*) *and* 2·30 ± 0·02 *for* (*B*).

EXPERIMENTAL

Calcium D-*Carboxyethylene Phosphate* (D-*glyceric acid calcium* 2,3-*phosphate*).—Barium α-D-phosphoglycerate (dihydrate, 5·00 g.) was shaken with ammonium sulphate (1·85 g., one equiv.) in water (50 ml.) for 48 hr. The solution was filtered and evaporated, and the residue dried for 2 hr. at 50—60°/1 mm., then for 4 days over P_2O_5 at 10^{-4} mm. and room temperature, giving anhydrous ammonium α-D-phosphoglycerate, (2·96 g., 96%). This was dissolved with shaking in freshly distilled trifluoroacetic anhydride (50 ml.) and set aside at room temperature overnight. Dry toluene (25 ml.) was then added, and the solution evaporated *in vacuo via* a tube packed with silica gel, a P_2O_5 guard tube being used on the air-leak. The residue was finally heated at 40°, first at 15 mm., then at 0·5 mm. for 2 hr., dissolved in sodium-dried dioxan (15 ml.), and poured into a stirred slurry of lithium carbonate (5·0 g.) previously saturated with carbon dioxide. After 5 minutes' stirring, the solution (pH 8—9) was filtered, evaporated at $\leqslant 40°$ to about 30 ml., and diluted *slowly* with four volumes of ethanol. The precipitate was filtered off and rejected, and the filtrate was evaporated to dryness, again at $\leqslant 40°$. The residue from this evaporation was dissolved in 10% aqueous lithium chloride (25 ml.), and the solution diluted slowly with ethanol (100 ml.) and filtered. The two precipitates from 80% ethanol

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[8] Linstead, Owen, and Webb, *J.*, 1953, 1211, 1218, 1225.

[9] Barton, *J.*, 1953, 1027, and references there cited.

contained all the phosphorus-containing contaminants running more slowly than the desired product on paper chromatograms.[5]

The solution was again evaporated at <40°, and the residue dissolved in water (50 ml.) containing calcium chloride hexahydrate (3·0 g.). This solution was evaporated under reduced pressure to 20 ml., then filtered, and the product precipitated by addition of ethanol (10 volumes) with stirring during 20 hr. and filtered off. As this product contained traces of lithium and chloride ions, it was reprecipitated twice under similar conditions, first from water (8 ml.) containing calcium chloride (150 mg.) on addition of ethanol (80 ml.), then similarly without the addition of calcium chloride. Finally the product was washed with ethanol (15 ml.) and dry ether (30 ml.), giving *calcium* D-*carboxyethylene phosphate trihydrate* (794 mg., 22·7%) which was homogeneous on paper chromatograms,[5] was free from chloride and lithium ions, and had $[\alpha]_D^{20}$ +49° (*c* 1·1 in water), $[\alpha]_D^{20}$ −43° (*c* 0·6 in 12·5% ammonium molybdate solution) (Found: C, 13·6; H, 3·4; P, 12·3; ash 52·7. $C_3H_3O_6PCa,3H_2O$ requires C, 13·8; H, 3·5; P, 11·9; ash 52·4%).

Loss of weight on drying could not be determined, as the compound showed appreciable hydrolysis by its own water of crystallisation at the temperatures required for any significant loss of weight (50—60°).

Acidic Hydrolyses.—(i) *In conditions causing phosphate migration*: N-*acid.* Weighed samples of calcium D-carboxyethylene phosphate (~5 mg.) were heated in tubes at 100°, and preheated N-hydrochloric acid (0·25 ml.) was then added. After the required time, N-sodium hydroxide (0·25 ml.) was added, and the samples were cooled and made to 1·00 ml. with 25% ammonium molybdate solution, and the rotations of the resulting solutions were determined:

Time (min.)	½	1	2	3	5	30
$[\alpha]_D$	−385°	−435°	−455°	−500°	−540°	−610°
$\log_{10}\{610 + [\alpha]_D\}$	2·35	2·24	2·19	2·04	1·84	—

Hydrolysis by N-hydrochloric acid at room temperature gave a value of $[\alpha]_D$ −405° at 30 and 60 min.

Samples were treated in water with one equivalent of ammonium carbonate and then centrifuged, the residues were washed at the centrifuge, and the combined supernatant liquids were heated with half their volume of 3N-hydrochloric acid and analysed as above. The same results were obtained, within experimental error, as in determinations on the calcium salt, indicating the absence of a calcium ion effect on isomer ratio at this acidity.

(ii) *In conditions where migration is negligibly slow*: 0·023N-*acid.* Calcium D-carboxyethylene phosphate (61·0 mg.) in water (5 ml.) was passed down a column of Amberlite IR-120 (H^+ form), and the eluate made up to 10 ml. Aliquot parts (0·5 ml.) were heated in sealed tubes at 100°, and at intervals tubes were cooled and opened, and the contents basified with ammonia. Preliminary experiments with α-phosphoglycerate had shown that exact neutralisation was not critical for its determination by rotation in molybdate solutions, provided that a weak base was used. The samples were made to 1·00 ml. with 25% ammonium molbydate solution, and the rotations determined as before:

Time (min.)	2	5	10	20	30	40	60
$[\alpha]_D$	−385°	−410°	−425°	−430°	−445°	−435°	−440°
$\log_{10}\{610 + [\alpha]_D\}$	2·35	2·30	2·27	2·26	2·22	2·24	2·23

A specific calcium ion effect on the initial isomer ratio was observed in the range pH 1·5—2.

In both the above hydrolyses, the cyclic ester was no longer present on paper chromatograms [5a] after the shortest hydrolysis time, the product consisting of the two isomeric phosphoglycerates, separated in the system ethanol–formic acid–water on ammonium molybdate-treated paper.[5b]

The authors thank Sir Alexander Todd for his interest and encouragement, and the Department of Scientific and Industrial Research for a maintenance award (to A. J. D.).

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[*Received, June 2nd,* 1959.]