1022. *Phospholipids. Part IX.*[1] *Phosphate Elimination from Glycolaldehyde Phosphate.*

By D. M. Brown and J. C. Stewart.

Elimination of orthophosphate from glycolaldehyde phosphate and its esters is fast and complete in weakly acid solution in presence of 1,1-dimethylhydrazine. The reaction is valuable in the degradation of certain phospholipids. In ^{18}O-enriched water the reaction yields isotopically normal phosphate. A mechanism is proposed.

Fleury, Courtois, and Desjobert [2] first drew attention to the lability in acid of glycolaldehyde phosphate (diose phosphate) (I), relative to other alkyl phosphates, and they noticed the marked catalytic effect of phenylhydrazine and its *p*-nitro-derivative on the reaction,[3] but no explanation was offered. We found that the reaction was as effective when applied to esters of glycollaldehyde phosphate.[4] Specifically, an inositol ester gave inositol phosphate in about 50% yield, without phosphoryl migration, when treated with the equivalent amount of phenylhydrazine in aqueous formate buffer at pH 6. The reaction was of considerable value in elucidating the structure of the monophosphinositides,[5] and its more recent application [6] to the degradation of a triphosphoinositide and a glycerol–phosphate polymer has confirmed its generality.

We have studied further both the practical application and the mechanism of the reaction. Glycolaldehyde phosphate, conveniently generated from glycerol 1-phosphate by periodate oxidation, was found to behave in solution in the same way as the isolated barium salt.[3] Increasing the amount of phenylhydrazine to eight equivalents led to 89% elimination of phosphate, in 3—5 hours at room temperature and pH 5·0. The formation of a dark precipitate, also noted by Fleury *et al.*,[3] which carried down phosphate, prevented a full study, but it appeared from chromatograms that the reaction was in fact complete. The nature of the precipitate was not further studied; its infrared spectrum varied with the pH at which the reaction was effected.

As an alternative to phenylhydrazine, 1-1-dimethylhydrazine was found to give a cleaner reaction and higher yield. With one equivalent of the reagent, elimination of phosphate reached 93% at pH 4·0 in 6 hours, and only at pH values below this was a precipitate formed. Some further results are collected in Table 1 which show that the reaction will go to completion under very mild conditions.

Table 1.

Elimination (%) of orthophosphate from glycolaldehyde phosphate.

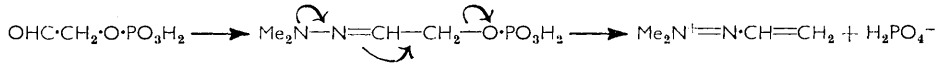
$Me_2N{\cdot}NH_2$:	1 equiv. (room temp.) pH					2 equiv. (5°) pH		
Time (min.)	2·5	4·0	4·5	5·2	6·3	4·0	5·0	6·0
5	22	41	37	36	33	57	60	44
30	—	61	53	53	46	83	84	75
180	21	86	79	76	68	98	97	95
360	—	93	86	84	77	—	—	—

In a degradative study of beef-brain triphosphoinositide, oxidation of the intermediate glycerol inositol triphosphate by periodate, followed by treatment of the glycolaldehyde derivative formed with dimethylhydrazine at pH 4·5, gave 93% conversion into inositol

[1] Part VIII, Brown and Clark, *J.*, 1963, 1475.
[2] Fleury and Courtois, *Bull. Soc. chim. France*, 1941, **8**, 75; Fleury and Courtois, *ibid.*, 1942, **9**, 570; Fleury, Courtois, and Desjobert, *ibid.*, 1948, **15**, 694.
[3] Fleury, Courtois, and Desjobert, *Bull. Soc. chim. France*, 1952, **19**, 458.
[4] Brown, Hall, and Letters, *J.*, 1959, 3547.
[5] Brown, Clark, and Letters, *J.*, 1961, 3774; Brown and Clark, *Nature*, 1962, **194**, 1081.
[6] Brockerhoff and Ballou, *J. Biol. Chem.*, 1961, **236**, 1907; Critchley, Archibald, and Baddiley *Biochem. J.*, 1962, **85**, 420.

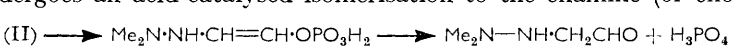
triphosphate.[7] The periodate–dimethylhydrazine reaction sequence has since proved effective, too, in the degradation of a variety of lecithins and cephalins [8] and of cardiolipin.[9]

It seems likely that the reaction depends on the initial formation of a hydrazone (II) and its subsequent decomposition. If this is so, the hydrogen-ion dependence would be expected to be complex and so it has not been further studied. A possible mechanism for the reaction may involve elimination of phosphate from the hydrazone (II), as shown. This is effectively equivalent to a β-elimination, and such reactions are known to proceed



(I) (II) (III)

easily, with phosphate as the leaving group.[10,11] The product (III) would be highly reactive and it is unlikely that isolation of the organic product would help to clarify the course of the reaction. In support of this mechanism we found that cyclohexylamine and diphenylamine were not catalytic (cf. ref. 10). Moreover, when run in ^{18}O-enriched water, the orthophosphate formed was isotopically normal, showing that alkyl–oxygen fission had occurred. Both these observations argue against a mechanism whereby an ester (II) undergoes an acid-catalysed isomerisation to the enamine (or enol phosphate)



(IV)

(IV) which then decomposes in the manner of enol esters,[12] with acyl–oxygen fission. The evidence available supports, but does not establish, a mechanism involving elimination as in (II).

The above reaction is analogous to that in which an α-bromo-ketone is converted into an αβ-unsaturated ketone by 2,4-dinitrophenylhydrazine in acetic acid.[13] The mechanism of this reaction has been discussed, both in terms of intermediates from which the hydrazone was specifically excluded on grounds other than experimental,[14] and in terms equivalent to our own. It has been shown that the hydrazone is a probable intermediate in the case of steroidal α-bromo-ketones,[15] and that the reaction can lead to substitution at C-4 of a 3-keto-4-bromo-steroid under suitable conditions.[16]

We therefore suggest that our reaction proceeds in the same way as does the elimination of halogen from an α-halogeno-ketone. If this is so, a further study of other hydrazines might lead to a useful development of the latter reaction.

Experimental

Analyses for phosphorus were carried out by Allen's method.[17] Periodate uptake was measured spectrophotometrically as described by Dixon and Lipkin.[18]

Disodium Glycerol 1-Phosphate.—The crystalline material (L. Light and Co.) was sufficiently pure, analytically (Found: C, 11·7; H, 6·0; P, 9·6, 10·0. Calc. for $C_3H_7O_6Na_2P,5H_2O$: C, 11·75; H, 5·6; P, 10·15%); it had periodate uptake, 0·97 mol./mol.

Elimination of Phosphate from Glycolaldehyde Phosphate.—The values given here are typical. Sodium metaperiodate (163 mg., 1·5 mol.) was dissolved in water (10 ml.) and the optical density at 222·5 mμ of the solution measured. Disodium glycerol 1-phosphate pentahydrate (157·9 mg.)

[7] Stewart, Ph.D. Thesis, Cambridge, 1963.
[8] R. Letters, personal communication.
[9] Le Cocq and Ballou, *Biochemistry*, 1964, **3**, 976.
[10] Bunton and Peterson, *Biochem. J.*, 1960, **75**, 17.
[11] Brown, Fried, and Todd, *J.*, 1955, 2206.
[12] Lichtenthaler and Cramer, *Chem. Ber.*, 1962, **95**, 1971.
[13] Mattox and Kendall, *J. Amer. Chem. Soc.*, 1948, **70**, 882; see also Chattaway and Irving, *J.*, 1930, 88.
[14] Djerassi, *J. Amer. Chem. Soc.*, 1949, **71**, 1003.
[15] Mattox and Kendall, *J. Amer. Chem. Soc.*, 1950, **72**, 2290; McGuckin and Kendall, *ibid.*, 1952, **74**, 3951.
[16] McGuckin and Kendall, *J. Amer. Chem. Soc.*, 1952, **74**, 5811.
[17] Allen, *Biochem. J.*, 1940, **34**, 858.
[18] Dixon and Lipkin, *Analyt. Chem.*, 1954, **26**, 1092.

was added and the reaction followed to completion in the usual way.[18] Periodate uptake was 97% of the theoretical. A 14·3% solution of ethylene glycol (0·1 ml., 0·5 mol.) was then added.

1,1-Dimethylhydrazine (0·19 ml.) was dissolved in water (24 ml.) and the solution then brought to the required pH with formic acid and made up to 25 ml. with water.

Equal quantities (0·5 ml.) of the glycolaldehyde phosphate solution (0·025 mmole) and the hydrazine solution (0·05 mmole) were mixed and incubated. Each reaction was carried out in duplicate. Solutions of pH 2·5—4·0 became yellow, and then brown, and finally a precipitate was formed; the rapidity with which this series of colour changes occurred was greater as pH decreased. At higher pH values, the solutions became yellow but remained clear.

At intervals, aliquot parts (0·05 ml.) of each reaction mixture were removed and analysed for total and ortho-phosphate and the percentage hydrolysis was calculated. Some results are recorded in Table 1; other results were not at variance with these. The amount of ortho-phosphate in the glycolaldeyde solution was determined immediately before each series of reactions and was always less than 1%; it never increased in blank runs to more than 3% in absence of the hydrazine (cf. also Fleury *et al.*[3]). Experiments with phenylhydrazine were carried out in the same way.

Phenylhydrazine Precipitate.—Glycolaldehyde phosphate solution (1·5 ml., 0·075 mmole) and phenylhydrazine solution (1·5 ml., 0·075 mmole), adjusted to pH 2·5 with formic acid, were mixed and kept at room temperature for several hours. The resulting precipitate was collected, washed with water (3 × 1 ml.), and dried (Found: P, 0·5%). The distribution of phosphorus was 86% in the supernatant solution, 5·5% in the washings, and 8·5% in the precipitate.

^{18}O-*Incorporation Experiment.*—A solution of glycolaldehyde phosphate in normal water was prepared in the usual way. 1,1-Dimethylhydrazine (0·05 ml.) was dissolved in ^{18}O-enriched water (4·0 ml.) and the pH of the solution was adjusted to 4·0 with formic acid and made up to 5 ml. with $H_2{}^{18}O$.

Equal quantities (5·0 ml.) of the glycolaldehyde phosphate solution (1·0 mol.) and the hydrazine solution (2·5 mol.) were mixed and kept at room temperature for 16 hr. Some water was then removed by freeze-drying and collected. The remaining solution was diluted with normal water, and phosphate was precipitated as $MgNH_4PO_4,6H_2O$; this precipitate was washed with water and converted into the dipotassium hydrogen salt by the use of Dowex-50 (K^+ form). The latter salt was reprecipitated twice from water with ethanol. Analysis by the method of Bunton *et al.*[19] showed that the water contained 1·14 atom-% above normal, while the phosphate had the normal abundance ($0·00_2$ below).

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[19] Bunton, Llewellyn, Oldham, and Vernon, *J.*, 1958, 3574.