94. *Studies on Phosphorylation. Part I. Dibenzyl Chlorophosphonate as a Phosphorylating Agent.*

By F. R. Atherton, H. T. Openshaw, and A. R. Todd.

Dibenzyl chlorophosphonate has been prepared by the action of chlorine on dibenzyl hydrogen phosphite. Although too unstable to be distilled, it can be used in carbon tetrachloride solution as a convenient phosphorylating agent. It reacts readily with amines to give the corresponding dibenzyl aminophosphonates, with alcohols in the presence of pyridine and with sodium salts of phenols, to give dibenzyl phosphoric esters. The benzyl groups in these products can be removed by hydrogenolysis.

In the course of a search for other mild phosphorylation methods β-hydroxyethyl and β-hydroxypropyl phosphates have been prepared from ethylene and propylene oxides by the action of aqueous disodium hydrogen phosphate.

Esters and amides of phosphoric acid play a vital rôle in many biological processes and they appear to be synthesised and to undergo interconversion in living organisms with great ease. Their preparation in the laboratory under mild conditions, however, is still a matter of some difficulty despite the efforts of many workers during the past 35 years. In recent papers from these laboratories (J., 1943, 383, 386, 571, 574; 1944, 315, 318, 476), the development of synthetic methods for the preparation of purine glycosides has been described; these papers deal with the first part of a more comprehensive programme of research embracing, amongst other objects, efforts to synthesise natural nucleotides which exercise co-enzyme function as well as related compounds which might be of value in a study of conditions governing co-enzyme specificity. A necessary part of this programme is clearly the elaboration of a convenient method for the phosphorylation of alcohols, in particular carbohydrates, and amines. The chief requirements to be met by such a method are that it should proceed under conditions which would not destroy sensitive glycosides and that it should be flexible enough to be applied to the production of pyro- and poly-phosphoric esters. The present communication deals with some of our investigations in this field; as a preliminary to their discussion it is desirable to review briefly the known phosphorylation procedures.

The first practicable synthesis of phosphoric acid esters of carbohydrates was described by Neuberg and Pollak (*Biochem. Z.*, 1910, **23**, 515; *Ber.*, 1910, **43**, 2060), who treated aqueous sugar solutions with phosphoryl chloride in presence of calcium hydroxide or carbonate. E. Fischer (*Ber.*, 1914, **47**, 3193) improved upon this method by working with phosphoryl chloride in anhydrous pyridine at − 20°, and his procedure has since been widely employed in the carbohydrate field, mainly by Levene and his co-workers, and has also been used for the synthesis of the phosphoryl derivatives of other alcohols and of amines. The method has been applied to the phosphorylation of both protected (Levene and Tipson, *J. Biol. Chem.*, 1934, **106**, 113; 1935, **111**, 313; 1937, **121**, 131) and unprotected nucleosides (Jachimowicz, *Biochem. Z.*, 1937, **292**, 356; Gulland and Hobday, J., 1940, 746), but the yields are frequently unsatisfactory, particularly in the cases of adenosine and guanosine. Fischer (*loc. cit.*) showed that the original method of Neuberg and Pollak gave products different from those obtained by working in pyridine solution, and Gulland and his collaborators (*loc. cit.*; Barker and Gulland, J., 1942, 231) have converted uridine, guanosine and adenosine into the 3-phosphates by the use of phosphoryl chloride and aqueous baryta, again in poor yield.

Ethyl metaphosphate was introduced as a phosphorylating agent by Langheld (*Ber.*, 1910, **43**, 1857; 1911, **44**, 2076), and has been employed to some extent (*e.g.*, Abderhalden, Paffrath, and Sickel, *Arch. ges. Physiol.*, 1925, **207**, 241; Plimmer and Burch, J., 1929, 292; McMeakin, *J. Amer. Chem. Soc.*, 1937, **59**, 2383; Neuberg, *Arch. Biochem.*, 1943, **3**, 105). Fischer (*loc. cit.*) found this reagent unsatisfactory for the preparation of carbohydrate phosphates, probably owing to the insolubility of the sugars in the reaction medium. A further objection to its use is the necessity for the subsequent hydrolytic removal of the ethyl group.

Recently, diphenyl chlorophosphonate has been introduced as a phosphorylating agent; it reacts readily in pyridine solution with compounds containing alcoholic hydroxyl groups, and the protecting phenyl groups can be removed from the product by catalytic hydrogenation or by hydrolysis under mild acid or alkaline conditions (Brigl and Müller, *Ber.*, 1939, **72**, 2121; Bredereck, Berger, and Ehrenburg, *ibid.*, 1940, **73**, 269; cf. Zeile and Meyer, Z. *physiol. Chem.*, 1938, **256**, 131). The reagent has been employed successfully for the synthesis of glyceraldehyde phosphate (Baer and Fischer, *J. Biol. Chem.*, 1943, **150**, 213, 223) and of phosphorylcholine chloride (Baer and McArthur, *ibid.*, 1944, **153**, 451); cytidine-3-phosphate was obtained from tritylcytidine, but treatment of diacetyladenosine with this reagent, followed by hydrolysis, afforded only a small amount of adenylic acid (Bredereck *et al.*, *loc. cit.*). Phenyl dichlorophosphinate (Brigl and Müller, *loc. cit.*; Gulland and Hobday, *loc. cit.*), anilinophosphonyl and dianilinophosphinyl chlorides (Zetsche and Büttiker, *Ber.*, 1940, **73**, 47) have also been studied as possible phosphorylating agents.

2 : 3 : 4 : 6-Tetra-acetylglucose-1-phosphate has been synthesised by the interaction of silver dibenzyl phosphate and acetobromoglucose, followed by removal of the benzyl groups by hydrogenolysis (Zervas, *Naturwiss.*, 1939, **27**, 317); deacetylation of the product affords β-glucose-1-phosphate (Wolfrom, Smith, Pletcher, and Brown, *J. Amer. Chem. Soc.*, 1942, **64**, 23). Lynen (*Ber.*, 1940, **73**, 367) has prepared acetyl phosphate in a similar manner, but the applicability of this method is clearly limited by the availability of the necessary halogen compound.

In seeking a more satisfactory method for the phosphorylation of sensitive nucleosides we have prepared dibenzyl chlorophosphonate (II) and examined its application as a phosphorylating agent. Zervas (*loc. cit.*) mentions that this substance can be prepared from dibenzyl hydrogen phosphate but states that it is so unstable as to be of little use. We have prepared it by the action of chlorine on *dibenzyl hydrogen phosphite* (I) in an inert solvent such as carbon tetrachloride (cf. McCombie, Saunders, and Stacey, preceding paper). Evaporation of the solvent gives the chlorophosphonate as a colourless oil which decomposes on standing or on attempted distillation; of its identity there can be no doubt, since it is easily hydrolysed to dibenzyl hydrogen phosphate, isolated as its silver salt, and reacts with amines to give the corresponding aminophosphonates in almost theoretical yield.

For phosphorylation purposes isolation of the free ester need not be attempted; the solution in an inert solvent obtained by chlorinating dibenzyl hydrogen phosphite reacts readily with amines, preferably under anhydrous conditions, or with alcohols in presence of pyridine, and the products can be debenzylated by hydrogenolysis with a palladised charcoal catalyst.

In this way by reaction with the appropriate amine we have prepared *dibenzyl aminophosphonate* (III; R = H), *dibenzyl anilinophosphonate* (III; R = Ph), *dibenzyl benzylaminophosphonate* (III; R = CH_2Ph), *dibenzyl α-phenylethylaminophosphonate* (III; R = CHMePh), and *dibenzyl* cyclo*hexylaminophosphonate* (III; R = C_6H_{11}). A carbon tetrachloride solution of dibenzyl chlorophosphonate reacted readily with alcohol in presence of pyridine to give dibenzyl ethyl phosphate, hydrogenated to ethyl dihydrogen phosphate (isolated as its barium salt). An observation which may be of significance in developing methods for polyphosphorylation is that the hydrogenation of dibenzyl *iso*amyl phosphate could be interrupted after 1 mol. of hydrogen had been absorbed and benzyl *iso*amyl hydrogen phosphate was then isolated as its *silver* salt.

Dibenzyl chlorophosphonate does not appear to react under ordinary conditions with phenols, but it reacts with their sodium salts with great ease; for example, with sodium β-naphthoxide a thick oil is formed which on hydrogenation gives β-naphthyl dihydrogen phosphate in excellent yield. These preliminary studies with dibenzyl chlorophosphonate are being extended in various directions.

$$\underset{\text{(I.)}}{(CH_2Ph{\cdot}O)_2P{\cdot}OH} + Cl_2 \xrightarrow{-HCl} \underset{\text{(II.)}}{(CH_2Ph{\cdot}O)_2POCl} \xrightarrow{NH_2R} \underset{\text{(III.)}}{(CH_2Ph{\cdot}O)_2PO{\cdot}NHR}$$

In the course of our search for mild phosphorylation methods we devoted some attention to the observation of Bailly (*Ann. Chim.*, 1916, **6**, 133) that α-glyceryl dihydrogen phosphate is formed from glycide alcohol and disodium hydrogen phosphate in aqueous solution at room temperature. Although very attractive on account of the mild conditions of reaction, this method is limited by the availability of ethylene oxide derivatives. Using it, we have, however, prepared β-hydroxyethyl dihydrogen phosphate and β-hydroxypropyl dihydrogen phosphate from ethylene oxide and propylene oxide respectively and we intend to examine its applicability to certain carbohydrate derivatives.

Experimental.

Dibenzyl Hydrogen Phosphite.—To an ice-cold solution of phosphorus trichloride (147·5 g.; 1 mol.) in dry benzene (750 c.c.) a mixture of dimethylaniline (242 g.; 2 mols.) and benzyl alcohol (216 g.; 2 mols.) was added dropwise with continuous stirring during 2 hours. Stirring was continued for a further ½ hour, more benzyl alcohol (108 g.; 1 mol.), then added in like manner (20 mins.), and the mixture left overnight at room temperature. Water (500 c.c.) was now added to dissolve precipitated dimethylaniline hydrochloride, and the benzene layer separated, washed in turn with water (2 portions of 500 c.c.), 5N-ammonia (2 portions of 500 c.c.), and water (2 portions of 500 c.c.), and dried over anhydrous sodium sulphate. Evaporation of the benzene under reduced pressure left a pale yellow oil, from which remaining traces of benzyl chloride were removed by stripping at $100°/10^{-2}$ mm. The residue (190 g.) was now subjected in portions of 40—50 g. to slow distillation under 10^{-3} mm., from an electrically heated retort. The main bulk of *dibenzyl hydrogen phosphite* (149 g.; 57%) distilled at 110—120° as a colourless oil, $n_D^{18°}$ 1·5521 (Found: C, 64·3; H, 5·8; P, 12·4. $C_{14}H_{15}O_3P$ requires C, 64·2; H, 5·7; P, 11·8%). At − 5° the pure ester set to a mass of colourless crystals which melted between 0° and 5°.

Dibenzyl hydrogen phosphite decomposes when heated to 160°, but prolonged heating at lower temperatures (*ca.* 120°) may at times have the same effect. For this reason care should be taken as regards purity of starting materials and distillation should be carried out in a high vacuum, not more than 50 g. being distilled in one operation. Distillation of larger amounts is a capricious and at times rather dangerous operation, since decomposition of the ester is strongly exothermic and leads to the production of a white resinous polymer and considerable quantities of phosphine.

Dibenzyl Chlorophosphonate.—Dibenzyl hydrogen phosphite (39·3 g.), dissolved in dry carbon tetrachloride (200 c.c.), was cooled to − 15°, and a solution of chlorine in carbon tetrachloride (200 c.c. of 1·4N) added dropwise with stirring, the temperature being kept below − 10°. Dry nitrogen was now passed through the liquid to remove excess of chlorine and hydrogen chloride (*ca.* 1½ hours). For phosphorylation purposes the carbon tetrachloride solution thus obtained was normally used directly without isolation of the ester. Evaporation of the solution in a vacuum at room temperature gave dibenzyl chlorophosphonate as a thick oil which decomposed on standing or on attempted distillation. Inability to distil and adherent hydrogen chloride made analysis of the ester unsatisfactory, but its reaction with amines to give the corresponding dibenzyl aminophosphonates (see below) in nearly quantitative yield leaves no doubt as to its identity. The same product is obtained when chlorination is carried out in ether or chloroform solution.

Silver Dibenzyl Phosphate.—Water (10 c.c.) and pyridine (30 c.c.) were added to a solution of dibenzyl chlorophosphonate (from 4 g. of dibenzyl hydrogen phosphite) in carbon tetrachloride (100 c.c.), and the mixture kept at room temperature for several days. The liquid was now evaporated, and the residue dissolved in water (50 c.c.), neutralised (phenolphthalein) with sodium hydroxide, and extracted with ether to remove pyridine. Acidification of the aqueous layer with sulphuric acid gave an oil, which was taken up in chloroform (120 c.c.), and the solution dried and evaporated. Barium hydroxide solution was added to the residue until neutral to phenolphthalein, and the milky liquid made up to 200 c.c. with water, heated to boiling, filtered, and concentrated to 50 c.c. Aqueous silver nitrate (5 g. in 10 c.c. of water) was added to the hot solution; on cooling, silver dibenzyl phosphate (2·8 g.) separated as colourless crystals (Found: Ag, 27·8. Calc. for $C_{14}H_{14}O_4PAg$: Ag, 28·0%).

Dibenzyl Aminophosphonate.—A solution of dibenzyl chlorophosphonate (from 6·55 g. of dibenzyl hydrogen phosphite) in carbon tetrachloride (125 c.c.) was cooled to − 10° and saturated with ammonia. The mixture was kept at − 10° for 5 mins., then at room temperature overnight, and the excess of ammonia removed by a stream of nitrogen. The mixture was now heated to boiling, filtered, the filter residue extracted with hot carbon tetrachloride, and the combined filtrate and extract concentrated and allowed to cool. *Dibenzyl aminophosphonate* (6·3 g.; 91%) separated as colourless needles. The crude product had m. p. 98—100°, raised to 103·5—104·5° by recrystallisation from carbon tetrachloride (Found: C, 60·1; H, 5·8; N, 5·0. $C_{14}H_{16}O_3NP$ requires C, 60·6; H, 5·8; N, 5·0%). Dibenzyl aminophosphonate was also obtained in slightly lower yield by shaking a carbon tetrachloride solution of dibenzyl chlorophosphonate with aqueous ammonia.

Dibenzyl Anilinophosphonate.—Dibenzyl chlorophosphonate (from 13·1 g. of dibenzyl hydrogen phosphite) in carbon tetrachloride (250 c.c.) was cooled to − 10°, and excess of aniline (20 c.c.) added with shaking. After 5 mins., the mixture was removed from the cooling bath and kept at room temperature overnight. After washing with dilute hydrochloric acid and water the carbon tetrachloride solution was dried over anhydrous sodium sulphate and evaporated. The thick syrup which remained set to a mass of plates (17·3 g.), m. p. 80—86°. Recrystallisation first from hexane–ethylene dichloride and then aqueous alcohol gave *dibenzyl anilinophosphonate* as colourless plates, m. p. 91—92·5° (Found: C, 68·2; H, 5·5; N, 4·3. $C_{20}H_{20}O_3NP$ requires C, 68·0; H, 5·7; N, 4·0%).

By exactly similar methods the following substances were prepared.

Dibenzyl cyclo*hexylaminophosphonate,* colourless needles from hexane, m. p. 79—80° (Found: C, 67·1; H, 7·4; N, 4·0. $C_{20}H_{26}O_3NP$ requires C, 66·8; H, 7·2; N, 3·9%). Yield, 95%.

Dibenzyl benzylaminophosphonate, colourless needles from hexane–benzene or hexane–ethylene dichloride, m. p. 84—85° (Found: C, 68·4; H, 5·9; N, 4·0. $C_{21}H_{22}O_3NP$ requires C, 68·7; H, 6·0; N, 3·8%). Yield of crude product (m. p. *ca.* 80°), theoretical.

Dibenzyl α-phenylethylaminophosphonate, colourless prisms or needles from hexane or *cyclo*hexane, m. p. 81—82° (Found: C, 69·1; H, 6·1; N, 3·7. $C_{22}H_{24}O_3NP$ requires C, 69·3; H, 6·3; N, 3·7%). Yield crude (m. p. 75—80°), 98%.

Barium Ethyl Phosphate.—Absolute alcohol (20 c.c.) and dry pyridine (50 c.c.) were added to a carbon tetrachloride solution (200 c.c.) of dibenzyl chlorophosphonate (from 8 g. of dibenzyl hydrogen phosphite), and the mixture left for 4 days at room temperature. It was now evaporated under reduced pressure, dilute hydrochloric acid (200 c.c.) added to the residue, and the mixture extracted with ether. Evaporation of the dried ethereal solution gave crude dibenzyl ethyl phosphate as a thick oil, which was dissolved in alcohol (150 c.c. of 95%), refluxed for 1 hour with Raney nickel (0·5 g.), filtered, and shaken with hydrogen at atmospheric pressure in presence of palladised charcoal (10% Pd) till no more hydrogen was absorbed. The catalyst was removed, the filtrate evaporated, and the residue taken up in water (200 c.c.) and neutralised (phenolphthalein) with barium hydroxide. Precipitated barium phosphate was removed, and the filtrate concentrated in a vacuum to small bulk (25 c.c.). On addition of alcohol (100 c.c.) the monohydrate of barium monoethyl phosphate crystallised (4·3 g.; 50%) (Found: Ba, 48·8. Calc for $C_2H_5O_4PBa,H_2O$: Ba, 49·2%).

Silver Benzyl iso*Amyl Phosphate.*—The solution of dibenzyl chlorophosphonate employed was prepared from dibenzyl hydrogen phosphite (13·1 g.) by diluting with carbon tetrachloride (5 c.c.) and passing in chlorine diluted with nitrogen. The product was diluted with chloroform (15 c.c.), added to a mixture of *iso*amyl alcohol (8·8 g.) and dry pyridine (15 c.c.), and left overnight. More chloroform (60 c.c.) was added, and the mixture washed successively with dilute hydrochloric acid, sodium bicarbonate solution, and water, dried over anhydrous sodium sulphate, and evaporated. The crude dibenzyl *iso*amyl phosphate obtained was dissolved in aqueous methanol (250 c.c. of 70%), *n*-butylpiperidine (9 g.) added, and the solution shaken with hydrogen in presence of palladised charcoal (10% Pd). Absorption of hydrogen, at first fairly rapid, slowed after 1 molecular proportion had been taken up; the hydrogenation was stopped, the catalyst removed, and the filtrate evaporated in a vacuum. The residue was dissolved in water (300 c.c.), and *n*-butylpiperidine removed by steam-distillation under reduced pressure, barium hydroxide being added gradually during the distillation to keep the solution weakly alkaline. The resulting solution was made just acid (phenolphthalein) with sulphuric acid, filtered through charcoal, and silver nitrate (50 c.c. of 20%) added. After filtration and concentration to small bulk under reduced pressure *silver benzyl* iso*amyl phosphate* (5·5 g.) separated as colourless needles (Found : C, 39·5; H, 5·1; Ag, 29·4. $C_{12}H_{18}O_4PAg$ requires C, 39·5; H, 4·9; Ag, 29·6%).

β-Naphthyl Dihydrogen Phosphate.—A suspension of sodium β-naphthoxide (from 8·64 g. of β-naphthol) in toluene (300 c.c.) was cooled to — 5°, and a solution of dibenzyl chlorophosphonate (from 7·86 g. of dibenzyl hydrogen phosphite) in carbon tetrachloride (96 c.c.) added slowly with stirring so that the temperature was maintained at — 5°. After being stirred for a further 2 hours, the mixture was left overnight at room temperature. Water was added, and the phases separated by addition of more carbon tetrachloride (200 c.c.). The lower layer was tapped off, washed with sodium hydroxide solution and water, dried over anhydrous sodium sulphate, and evaporated. The residual oil was dissolved in chloroform and chromatographed on activated alumina. On development with chloroform, dark-coloured impurities were retained on the adsorbent and dibenzyl β-naphthyl phosphate passed through and was isolated as an orange-coloured oil (11 g.) on evaporation of the solvent. The oil was dissolved in methanol (300 c.c.), refluxed with Raney nickel, filtered, and hydrogenated at atmospheric pressure in presence of *N*-methylmorpholine (10 c.c.) and a palladised charcoal catalyst (10% Pd). Hydrogenation in this instance was slow owing to poisoning of the catalyst, which had to be renewed during the operation; it ceased after absorption corresponding to 2 mols. of hydrogen had occurred. The solution was now filtered and evaporated. The residue was evaporated twice with water, dissolved in water again, and traces of oily impurities removed by ether extraction. When the solution was acidified, a gummy precipitate was obtained, which was collected and dissolved in absolute alcohol, the solution evaporated, and the residue brought to crystallisation with a small amount of chloroform. The crude product (5·4 g.; 80%) had m. p. 165—166° and on recrystallisation from chloroform–alcohol gave colourless plates of β-naphthyl hydrogen phosphate, m. p. 172—173°, undepressed by an authentic specimen prepared by hydrolysis of β-naphthyl dichlorophosphinate.

Disilver β-Hydroxyethyl Phosphate.—A solution of ethylene oxide (8·8 g.; 1 mol.) in water (54 c.c.) was added to aqueous disodium hydrogen phosphate (71·6 g.; 1 mol. in 500 c.c. of water), and the mixture kept for 21 days. A solution of barium acetate (82 g.) in water (200 c.c.) was added, precipitated barium phosphate removed, and the filtrate concentrated to small bulk. On cooling, barium β-hydroxyethyl phosphate separated as colourless plates (38 g.; 64·5%). This product was converted into the *silver* salt by solution in the minimum quantity of boiling water and addition of silver nitrate (Found : Ag, 60·0. $C_2H_5O_5PAg_2$ requires Ag, 60·6%).

Disilver β-Hydroxypropyl Phosphate.—Prepared in similar fashion by the action of disodium hydrogen phosphate on propylene oxide, β-hydroxypropyl phosphate gave a *silver* salt crystallising in colourless needles (yield, 41%) (Found : C, 9·4; H, 1·9. $C_3H_7O_5PAg_2$ requires C, 9·7; H, 1·9%).

Our thanks are due to Roche Products, Ltd., for a scholarship held by one of us (F. R. A.).

UNIVERSITY OF MANCHESTER.
UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE.

[*Received, February 19th*, 1945.]