500. *Studies on Phosphorylation. Part VIII.* The Production of Tetrabenzyl Pyrophosphate by the Action of Acyl Chlorides on Dibenzyl Hydrogen Phosphate, and a Novel Reaction of Tetraphenyl Pyrophosphate.*

By H. S. Mason and A. R. Todd.

The action of thionyl chloride and other acyl halides on dibenzyl hydrogen phosphate yields tetrabenzyl pyrophosphate rather than dibenzyl chlorophosphonate. With oxalyl chloride the initial product is a mixed anhydride, bisdibenzylphosphoryl oxalate, which yields carbon monoxide, carbon dioxide, and tetrabenzyl pyrophosphate when heated. Tetraphenyl pyrophosphate has been prepared and shown to be a powerful phosphorylating agent. It reacts very readily with dibenzyl hydrogen phosphate in presence of bases, yielding tetrabenzyl pyrophosphate and diphenyl hydrogen phosphate. The implications of these various observations in the general field of nucleotide synthesis are discussed.

The researches which form the subject of this series of papers have as their primary object the development of satisfactory methods for the phosphorylation and polyphosphorylation of nucleosides so as to provide an adequate basis for synthetic work in the fields of nucleotide, nucleotide coenzyme, and polynucleotide chemistry which have been a major topic of interest in these laboratories in recent years. They are indeed an essential part of our nucleotide studies. Broadly speaking, the problem of nucleotide synthesis resolves itself into two parts: (*a*) the phosphorylation and polyphosphorylation of nucleosides and related compounds to yield mono-esters of the oxyacids of phosphorus, *e.g.*, simple monoribonucleotides, adenosine diphosphate, adenosine triphosphate; and (*b*) the linkage of two dissimilar nucleoside molecules through phosphate or pyrophosphate groupings, *i.e.*, the synthesis of unsymmetrical diesters of phosphoric acid, RO·P(O)(OH)·OR′, or pyrophosphoric acid, R·P(O)(OH)·O·P(O)(OH)·OR′, to which types belong the polynucleotides and a number of nucleotide coenzymes such as cozymase. Earlier papers of this series (*J.*, 1945, 382, 660; 1947, 674; 1948, 1106; 1949, 815; 1950, 2023, 2030) have been concerned mainly with part (*a*), and methods have been devised whereby mono-esters of phosphoric and polyphosphoric acids may be prepared. These methods have been applied successfully to the synthesis of various mononucleotides (Baddiley and Todd, *J.*, 1947, 648; Michelson and Todd, *J.*, 1949, 2476; Brown, Haynes, and Todd, *J.*, 1950, 3299), of adenosine diphosphate (Baddiley and Todd, *loc. cit.*), and of adenosine triphosphate (Baddiley, Michelson, and Todd, *J.*, 1949, 582; Michelson and Todd, *ibid.*, p. 2487). The present paper records some of our results in initial approaches to the solution of the second main problem of nucleotide synthesis described under (*b*) above.

The use of a bifunctional phosphorylating agent such as phenyl dichlorophosphinate † ($PhO{\cdot}POCl_2$) offers a fairly obvious route to symmetrical diesters of phosphoric acid and, indeed, Gulland and Smith (*J.*, 1948, 1532) have used it to prepare a diuridine phosphate; since the latter was prepared from benzylideneuridine, it should now be regarded as di(uridine-5′) hydrogen phosphate (Brown, Haynes, and Todd, *loc. cit.*). The reaction of phenyl dichlorophosphinate with one equivalent of an alcohol to give an alkyl phenyl chlorophosphonate, PhO·P(O)(OR)Cl, which can react further with a second alcohol, indicates the possible application of this reagent to the preparation of unsymmetrical diesters. An example of its use in this way is to be found in the preparation of "α-L-glycerylphosphorylcholine" by Baer and Kates (*J. Amer. Chem. Soc.*, 1948, **70**, 1394). For our purposes, this route, although under investigation, is likely to be of limited value since the presence of a phenyl group in the initial product of phosphorylation would be a disadvantage in so far as its removal might be impossible without disruption of a sensitive molecule. Clearly, the simplest solution would be to convert a nucleoside benzyl hydrogen phosphate, which is readily accessible, into a phosphorylating agent by replacement of the acidic hydroxy-group by halogen or some similar grouping and use the product to phosphorylate a second nucleoside. Model experiments have been carried out with this end in view. Another method which might have a limited application would be the

* Part VII, *J.*, 1950, 2030.

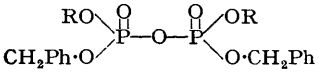
† For the nomenclature now used, see *J.*, 1951, 1868, footnote.

reaction of a nucleoside benzyl hydrogen phosphate with a nucleoside derivative containing an epoxide grouping; experiments bearing on this route will be described in a separate communication.

Deutsch and Fernö (*Nature*, 1945, **156**, 604) stated that reaction of thionyl chloride with potassium dibenzyl phosphate yields dibenzyl chlorophosphonate, although no details were given. Some time ago, Mr. J. J. Michalski in these laboratories tried to repeat this work, assaying the amount of chlorophosphonate produced by reaction of the product with ammonia and weighing the dibenzyl aminophosphonate produced. In no case did his yield of product exceed 50% of the theoretical, and dibenzyl hydrogen phosphate was always obtained in addition to the aminophosphonate. Replacement of the thionyl chloride in this reaction by phosphorus pentachloride or carbonyl chloride gave results similar in nature but much inferior. His tentative assumption that the reaction of thionyl chloride with potassium dibenzyl phosphate yields a pyrophosphate rather than a chlorophosphonate has been found valid. Tetrabenzyl pyrophosphate has been isolated from the reaction product and no dibenzyl chlorophosphonate was detected. A series of experiments was carried out using thionyl chloride alone and in presence of various tertiary bases, in various hydrocarbon solvents, and employing dibenzyl hydrogen phosphate free or as its sodium, potassium, or silver salt; in no case was clear evidence for the formation of chlorophosphonate obtained. That the product obtained from such reactions should be tetrabenzyl pyrophosphate rather than dibenzyl chlorophosphonate was what one would expect, since the standard methods for the preparation of pyrophosphates include reaction of dialkyl chlorophosphonates with metallic salts of dialkyl hydrogen phosphates (cf., *e.g.*, Baddiley and Todd, *loc. cit.*) or with the free acids in presence of tertiary bases (Toy, *J. Amer. Chem. Soc.*, 1950, **72**, 2065).

Oxalyl chloride has been employed for the conversion of acids into acyl chlorides. It was found to react readily with dibenzyl hydrogen phosphate; when a mixture of the two compounds was warmed hydrogen chloride was evolved and a crystalline product was obtained which on the basis of analysis and chemical behaviour is regarded as the mixed anhydride bisdibenzylphosphoryl oxalate (I). The anhydride (I) when heated to its melting point evolved

(I.) $(CH_2Ph\cdot O)_2\overset{O}{\overset{\|}{P}}\cdot O\cdot CO\cdot CO\cdot O\cdot\overset{O}{\overset{\|}{P}}(O\cdot CH_2Ph)_2$



(II.)

carbon monoxide and carbon dioxide, yielding a product which was evidently tetrabenzyl pyrophosphate since it furnished dibenzyl aminophosphonate and dibenzyl hydrogen phosphate on treatment with ammonia. An exactly similar decomposition of the anhydride was effected by treatment with a tertiary base; direct treatment with ammonia yielded oxamide, dibenzyl aminophosphonate, and dibenzyl hydrogen phosphate. Oxalyl chloride appeared to react in the same way with diphenyl hydrogen phosphate. In this instance, the mixed anhydride could not be isolated because of its great sensitivity to moisture, but the reaction product effervesced when heated above 100°, and from the resulting oil diphenyl aminophosphonate could be obtained in fair yield by treatment with ammonia.

The formation of pyrophosphates in the experiments above described is not necessarily a serious drawback from the standpoint of developing methods for the synthesis of unsymmetrical diesters of phosphoric and polyphosphoric acids. Tetraesters of pyrophosphoric acid, being acid anhydrides, can be used as phosphorylating agents; tetrabenzyl pyrophosphate, for example, will phosphorylate alcohols readily in the presence of tertiary bases (Atherton and Todd, *J.*, 1947, 674). The readiness with which such esters act as phosphorylating agents depends *inter alia* on the nature of the ester groups, or, more precisely, on the acid strength of the diesters of phosphoric acid from which they are derived. Thus tetraphenyl pyrophosphate, which can readily be prepared from silver diphenyl phosphate and diphenyl chlorophosphonate, decomposes rapidly in moist air, and it will phosphorylate alcohols even in the absence of bases; the preparation of *p*-nitrobenzyl diphenyl phosphate by this means is described in the Experimental portion. One method, although perhaps an uneconomic one, of achieving our desired aim would be to convert a nucleotide derivative such as benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate into its anhydride, dibenzyl di-(2′ : 3′-*iso*propylidene adenosine-5′) pyrophosphate (II; R = 2′ : 3′-*iso*propylidene adenosine-5′) and bring this substance into reaction with a second nucleoside in presence of a tertiary base; protecting benzyl groups could subsequently be removed by hydrogenation or hydrolysis. Since reaction with a nucleoside would produce not merely the required dinucleotide but also

an equivalent amount of the original benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate, the method might well prove rather cumbersome in operation. Nevertheless, some unsuccessful attempts were made to prepare the pyrophosphate (II; R = 2′ : 3′-*iso*-propylidene adenosine-5′) from benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate or its salts by reaction with thionyl chloride. The silver salt reacted readily with thionyl chloride to produce a metastable soluble complex containing silver, sulphur, and chlorine whose precise structure was not determined. The complex decomposed when heated, with production of silver chloride and sulphur dioxide, but we were unable to isolate any pyrophosphate or chlorophosphonate from the resulting complex mixture of organic materials. The pyrophosphate could not be prepared *via* a mixed anhydride with oxalic acid since benzyl 2′ : 3′-*iso*propylidene adenosine-5′ hydrogen phosphate as well as adenosine and adenine itself reacted with oxalyl chloride to produce stable, bright yellow products from which the original purine derivatives could not be easily regenerated.

A new method for the preparation of certain tetraesters of pyrophosphoric acid has been found during experiments with tetraphenyl pyrophosphate. In the presence of a tertiary base this substance reacts very rapidly with dibenzyl hydrogen phosphate yielding tetrabenzyl pyrophosphate and diphenyl hydrogen phosphate. On the evidence of a series of experiments it appears that triethylamine is a very effective agent for bringing about reaction and dimethylformamide is the preferred solvent; under these conditions the exchange reaction—presumably a two-stage nucleophilic displacement—is almost quantitative. A consideration of this reaction indicates that, appropriately modified, it might have far-reaching applications in the synthesis not only of unsymmetrical diesters of phosphoric acid but also of unsymmetrical pyrophosphates. Exploration of these possible developments and their extension to the nucleotide field are at present in progress.

Experimental.

Action of Thionyl Chloride on Dibenzyl Hydrogen Phosphate : Isolation of Tetrabenzyl Pyrophosphate.—Anhydrous dibenzyl hydrogen phosphate (1·5 g.) was dissolved in benzene (25 c.c.), and remaining traces of moisture were removed by a preliminary distillation of the azeotrope. To the boiling solution freshly distilled thionyl chloride (5 c.c.) was added, and the mixture heated under reflux for 18 hours, then evaporated to dryness under reduced pressure. The residual oil was dissolved in chloroform, and the solution washed with aqueous sodium hydrogen carbonate, dried, and evaporated. The brownish oil was dissolved in a minimum of boiling *cyclo*hexane, treated with charcoal, filtered, and set aside. Tetrabenzyl pyrophosphate separated; recrystallised from *cyclo*hexane, it had m. p. 62°, undepressed on admixture with an authentic specimen (m. p. 62°) (yield, 0·3 g.).

Bisdibenzylphosphoryl Oxalate (I).—Anhydrous dibenzyl hydrogen phosphate (1·0 g.) was dissolved in freshly distilled oxalyl chloride (0·5 c.c.), and the mixture heated to boiling for 4 minutes in absence of moisture, then evaporated to dryness at room temperature under reduced pressure (0·2 mm.), the process being continued for 3 hours to ensure complete removal of oxalyl chloride. The crystalline *anhydride* so obtained (yield, theoretical) had m. p. 107—108° (efferv.) (Found : C, 58·9; H, 4·6. $C_{30}H_{28}O_{10}P_2$ requires C, 59·0; H, 4·6%). On further heating of the molten anhydride decomposition and polymerisation occurred at 185°; the same behaviour was shown by tetrabenzyl pyrophosphate heated above its m. p.

Pyrolysis. The mixed anhydride (from 1 g. of dibenzyl hydrogen phosphate) was kept at its m. p. until effervescence ceased. The product was cooled and dissolved in liquid ammonia, and the solvent allowed to evaporate after 1 hour. The residue (0·3 g.) was washed thoroughly with water, dried, and recrystallised from aqueous ethanol; it then had m. p. 103°, undepressed on admixture with an authentic specimen (m. p. 103—104°) of dibenzyl aminophosphonate.

The presence of carbon dioxide in the gases evolved on pyrolysis was shown by reaction with barium hydroxide solution, and that of carbon monoxide by the appearance of the carbon monoxide-hæmoglobin absorption bands on passing the mixed gases through a solution of hæmoglobin. The mixed anhydride also decomposed when heated in a micro-combustion furnace with use of a cold filling which prevents conversion of carbon monoxide into carbon dioxide; 53·57 mg. treated in this way gave 1·7 mg. of carbon dioxide, *i.e.*, 44·5% of the theoretical for 1 mol. By the hot-tube technique, 48·37 mg. yielded 3·35 mg. of carbon dioxide, *i.e.*, 42% of the theoretical for 2 mols. It is thus concluded that carbon monoxide and carbon dioxide are evolved in equimolar quantities.

Action of ammonia. A solution of the mixed anhydride (0·16 g.) in a small amount of liquid ammonia was allowed to evaporate slowly overnight; some frothing was observed in the early stages of the reaction. The residue was triturated with ethanol; a white residue of oxamide (8 mg., 35%) remained which did not melt below 310°. Precipitation of the ethanolic solution with water yielded dibenzyl aminophosphonate (8 mg.), and acidification of the aqueous ethanolic mother-liquor gave dibenzyl hydrogen phosphate (80 mg.).

Action of Oxalyl Chloride on Diphenyl Hydrogen Phosphate.—Anhydrous diphenyl hydrogen phosphate (0·5 g.) was dissolved in dry chloroform (3 c.c.), freshly distilled oxalyl chloride (0·4 c.c.) added, and the

mixture refluxed for 20 minutes with exclusion of moisture. Solvent was removed at 0·2 mm., and the residue dissolved in hot *cyclo*hexane. On cooling, small rosettes of crystalline material began to deposit, but these were subsequently resorbed by an oil and could not be obtained pure. When the oily product was warmed to about 100°, gas was evolved and a portion of the resulting material (45 mg.), treated with concentrated aqueous ammonia, gave diphenyl aminophosphonate (11 mg., 50%).

Tetraphenyl Pyrophosphate.—Equimolar quantities of silver diphenyl phosphate and diphenyl chlorophosphonate were mixed together in the bulb of a molecular still (pistol type) which was rapidly evacuated, set in a vertical position, and heated in an oil-bath for 10 minutes at 125°. The bulb was then raised to the distillation position, and the bath-temperature raised to 165°, any material distilling below this being discarded. *Tetraphenyl pyrophosphate* distilled at 165° (bath-temp.)/0·1 mm. as a colourless liquid, leaving a residue of silver chloride (Found : C, 59·2; H, 4·4. $C_{24}H_{20}O_7P_2$ requires C, 59·8; H, 4·1%); it is very rapidly attacked by moist air, yielding diphenyl hydrogen phosphate, and it must be stored in sealed ampoules.

Phosphorylation with Tetraphenyl Pyrophosphate : p-*Nitrobenzyl Diphenyl Phosphate.*—A mixture of *p*-nitrobenzyl alcohol (0·2 g.), tetraphenyl pyrophosphate (0·5 g.), and dry chloroform (5 c.c.) was refluxed for 3 hours, then chloroform was removed under reduced pressure and replaced by benzene (5 c.c.). The benzene solution was set aside at 0° for 24 hours; unchanged *p*-nitrobenzyl alcohol (0·025 g.) separated and was removed by filtration. The filtrate was evaporated to dryness, the residue dissolved in ether, and the solution washed with sodium hydrogen carbonate, dried, and evaporated. The residue of p-*nitrobenzyl diphenyl phosphate*, recrystallised from ethanol, had m. p. 60—62° (0·113 g.), undepressed on admixture with a sample (m. p. 65°) prepared by Dr. C. W. Taylor from *p*-nitrobenzyl alcohol and diphenyl chlorophosphonate (Found : C, 59·4; H, 4·2. $C_{19}H_{16}O_6NP$ requires C, 59·2; H, 4·2%).

Exchange Reaction between Tetraphenyl Pyrophosphate and Dibenzyl Hydrogen Phosphate.—(1) *In presence of pyridine.* In a first experiment tetraphenyl pyrophosphate (0·14 g.) was added to a solution of dibenzyl hydrogen phosphate (0·24 g.) in pyridine (1 c.c.), and the mixture left at 0° for 1 minute. Pyridine was removed under reduced pressure at room temperature and concentrated aqueous ammonia (*d* 0·880) was added. The solid product was shown to be dibenzyl aminophosphonate by m. p. and mixed m. p. (102—104°); its yield (21 mg.) was 25·7% of theory based on formation of tetrabenzyl pyrophosphate by the exchange reaction. In a second experiment a mixture of tetraphenyl pyrophosphate (0·25 g.), dibenzyl hydrogen phosphate (0·25 g.), and pyridine (0·5 c.c.) was left for 1 minute at room temperature and worked up in the same way; the yield of dibenzyl aminophosphonate obtained was 40% on the same basis.

In a third experiment, dibenzyl hydrogen phosphate (0·5 g.) was dissolved in pyridine (1 c.c.), and tetraphenyl pyrophosphate (0·5 g.) added. After 1 minute at room temperature pyridine was removed at 0·1 mm. The residue was suspended in water (10 c.c.), and dilute ammonia was added cautiously with shaking until the mixture was weakly alkaline. Excess of ammonia was removed under reduced pressure and the suspension was set aside overnight. The colourless needles (0·12 g.; m. p. 55—60°) which separated yielded pure tetrabenzyl pyrophosphate (m. p. and mixed m. p. 60—62°) on recrystallisation from *cyclo*hexane.

(2) *In presence of triethylamine.* Tetraphenyl pyrophosphate (0·5 g.) was added to a solution of dibenzyl hydrogen phosphate (0·5 g.) and triethylamine (0·2 c.c.) in dimethylformamide (2 c.c.) at room temperature. After 5 minutes, concentrated aqueous ammonia (10 c.c.; *d* 0·880) was added with shaking, and the mixture left for 1 hour. On dilution with water, needles of dibenzyl aminophosphonate separated; recrystallised from carbon tetrachloride, the product had m. p. 102—104°, undepressed by an authentic specimen (m. p. 103—104°). The yield was 0·21 g., or 84% of theory based on tetrabenzyl pyrophosphate.

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