873. *Studies on Phosphorylation. Part XXI.*[1] *Keten Imides as Reagents in Pyrophosphate Synthesis.*[2]

By R. J. Cremlyn, G. W. Kenner, and Sir Alexander Todd.

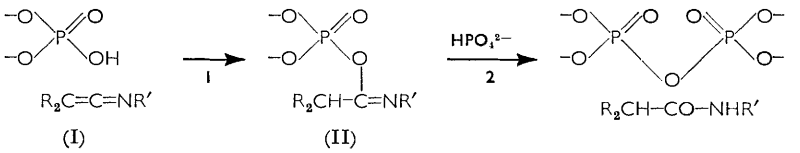
The reactions of diphenylketen *p*-tolylimide and of dimethylsulphonylketen methylimide with phosphates have been studied. With dibenzyl and diphenyl hydrogen phosphates, both reagents gave symmetrical pyrophosphates; however, with monobenzyl and monophenyl dihydrogen phosphates only dimethylsulphonylketen methylimide gave pyrophosphates. In no case was the hypothetical imidoyl phosphate intermediate isolated, but on treatment with an equimolar mixture of dibenzyl and diphenyl hydrogen phosphates both ketenimides gave mainly the unsymmetrical pyrophosphate.

Carbodi-imides are well-established reagents for the condensation of phosphates to pyrophosphates, including nucleotide coenzymes.[3] The chief drawback to their use is that, except where special structural features operate, as, for instance, in the synthesis of cozymase,[4] an unsymmetrical pyrophosphate is only produced in random mixture with the two symmetrical pyrophosphates. Apparently the hypothetical adduct of a phosphoric acid and a carbodi-imide reacts with a second molecule of phosphoric acid faster than it is formed, and hence a two-step process has never been achieved. On the other hand, imidoyl phosphates (*e.g.*, II) are reasonably stable and they can be brought into reaction with phosphoric acid, yielding pyrophosphates.[2] We have therefore investigated reactions between keten imides (I) and phosphates, in case they should proceed in two distinct and separable stages (1 and 2) through imidoyl phosphates. In the event, this possibility has not been realised and the keten imides do not appear to offer practical advantages over carbodi-imides which would offset the labour of their preparation.

Our first experiments were made with diphenylketen *p*-tolylimide (I; R = Ph, R′ = $C_6H_4{\cdot}CH_3$) [5] and dibenzyl hydrogen phosphate. The slow reaction between them (molar ratio 1 : 2) in benzene solution at room temperature was shown by fading of the yellow

[1] Part XX, Brown and Hammond, *J.*, 1960, 4229.
[2] For a preliminary account see Atherton, Morrison, Cremlyn, Kenner, Todd, and Webb, *Chem. and Ind.*, 1955, 1183.
[3] Khorana and Todd, *J.*, 1953, 2257; Todd, *Chem. Soc. Special Publ. No.* 8, 1957, 91.
[4] Hughes, Kenner, and Todd, *J.*, 1957, 3733.
[5] Stevens and French, *J. Amer. Chem. Soc.*, 1953, **75**, 657.

colour of the keten imide, and after four days crystalline tetrabenzyl pyrophosphate could be directly isolated (44%). In most of our experiments, however, unchanged acid was extracted from a reaction mixture with sodium hydrogen carbonate, and then the pyrophosphate was assayed by reaction with cyclohexylamine.[6] The speed of reaction was



(I) (II)

approximately the same in acetonitrile solution, considerably greater in nitromethane solution (70% after 15 hours), and much less in dimethylformamide solution. Similar results were obtained in comparable experiments with diphenyl hydrogen phosphate. Surprisingly, however, an equimolar mixture of dibenzyl hydrogen phosphate, diphenyl hydrogen phosphate, and diphenylketen *p*-tolylimide in nitromethane solution gave mainly the unsymmetrical dibenzyl diphenyl pyrophosphate, as judged by the amounts of dibenzyl *N*-cyclohexylphosphoramidate and cyclohexylammonium diphenyl phosphate isolated after assay.[6] Nevertheless, evidence for the existence of an imidoyl phosphate (II) as such in the products of reaction between diphenylketen *p*-tolylimide and either dibenzyl or diphenyl hydrogen phosphate could not be obtained.

Dimethylsulphonylketen methylimide (I; R = SO_2·Me, R′ = Me) is a representative of a considerably different class of keten imides accessible through action of diazomethane on nitriles,[7] and its behaviour with phosphates was therefore studied. This compound reacted very slowly at room temperature. For example, with dibenzyl hydrogen phosphate in benzene solution practically no pyrophosphate was formed. In acetonitrile the pyrophosphate yield was 12% after 5 days, and in nitromethane, under comparable conditions, 20% after 2 weeks. A similar experiment with diphenyl hydrogen phosphate in nitromethane resulted in a higher pyrophosphate yield (43% after one week). Again, it was observed that treatment with an equimolar mixture of dibenzyl and diphenyl hydrogen phosphates gave chiefly the unsymmetrical dibenzyl diphenyl pyrophosphate. Monobenzyl and monophenyl phosphates also gave moderate yields of pyrophosphates with dimethylsulphonylketen methylimide. In the nucleotide series, adenosine-5′ phosphate, dissolved in dimethylformamide as its tri-n-octylammonium salt, and benzyl dihydrogen phosphate yielded about 30% of the unsymmetrical pyrophosphate, as did the condensation by cyclopentanone oxime *p*-nitrobenzenesulphonate already reported.[8] A more rational synthesis of this unsymmetrical pyrophosphate was sought in preliminary condensation of benzyl dihydrogen phosphate and diphenyl hydrogen phosphate, followed by an exchange reaction [6] with tri-n-octylammonium adenosine-5′ phosphate, but the yield was only 10%.

Although our survey, which included many experiments [9] not recorded here, has not been fully comprehensive, some general conclusions may be drawn. The initial reaction between a keten imide and a phosphoric acid is slow and presumably this step is the transfer of proton from oxygen to carbon. Consequently, the keten imide containing the strongly electron-withdrawing methylsulphonyl groups is generally less reactive than the diphenyl compound; an acidic medium, such as nitromethane, is favourable; and diphenyl hydrogen phosphate reacts more quickly than does the more weakly acidic dibenzyl hydrogen phosphate. The fact that unsymmetrical pyrophosphates, rather than mixtures of the two symmetrical pyrophosphates, are produced from mixtures of phenyl and benzyl

[6] Corby, Kenner, and Todd, *J.*, 1952, 1234.
[7] Backer and Dijkstra, *Rec. Trav. chim.*, 1954, **73**, 575.
[8] Chase, Kenner, Todd, and Webb, *J.*, 1956, 1371.
[9] Cremlyn, Ph.D. Thesis, Cambridge, 1956.

esters of phosphoric acid is probably due to the greater degree of ionization of diphenyl than of dibenzyl hydrogen phosphate in anhydrous nitromethane. This would result in the preferential formation of the imidoyl diphenyl phosphate, and hence, in turn, of the unsymmetrical pyrophosphate. The observation that unsymmetrical pyrophosphates are produced in this way encouraged us to try condensing mixtures of toluene-*p*-sulphonic acid and phosphoric esters with subsequent addition of a salt of a second phosphoric ester, in the hope that a toluene-*p*-sulphonyl phosphate might be formed in the condensation, and that this would undergo an exchange reaction. Preparatively useful results did not emerge from this work or from attempts to catalyse the condensation of phosphoric esters by toluene-*p*-sulphonic acid.

Experimental

Tetrabenzyl Pyrophosphate.—(*a*) Dibenzyl hydrogen phosphate (0·556 g., 2 mmoles) was added to a solution of diphenylketen *p*-tolylimide (0·283 g., 1 mmole) in dry benzene (10 c.c.). After the solution had been kept at room temperature during 4 days, it had become almost colourless and αα-dipheny-*N*-*p*-tolylacetamide (0·123 g., 41%; m. p. 178—180°) had separated. The liquor yielded, by evaporation and recrystallisation of the residue from ether–cyclohexane, tetrabenzyl pyrophosphate (0·239 g., 44%), m. p. 58—60°.

(*b*) Experiment (*a*) was repeated with anhydrous nitromethane instead of benzene. After 15 hr. the amide (56%) was collected and the filtrate was diluted with benzene (200 c.c.) before being washed with ice-cold 10% sodium hydrogen carbonate solution, from which 20% of the dibenzyl hydrogen phosphate was recovered by acidification and extraction with chloroform. The benzene solution was washed with water, dried (Na_2SO_4), and evaporated before being treated with a solution of cyclohexylamine (0·48 c.c., 4 mmoles) in benzene (7 c.c.). Next day, the precipitated cyclohexylammonium dibenzyl phosphate (73%), m. p. 163—165°, was collected and the liquor yielded, after being washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, 70% of dibenzyl *N*-cyclohexylphosphoramidate, m. p. 73—75°.

P^1-*Dibenzyl* P^2-*Diphenyl Pyrophosphate.*—(*a*) The foregoing experiment (*b*) was repeated with an equimolar mixture of dibenzyl hydrogen phosphate (0·278 g.) and diphenyl hydrogen phosphate (0·250 g.) The solution became colourless in a few minutes and next day 89% of amide was collected. The acidic fraction (0·042 g.) did not crystallise. Initially the cyclohexylammonium salt (0·208 g.) had m. p. 187—196°, and recrystallisation of it from methanol–ether afforded pure cyclohexylammonium diphenyl phosphate (0·132 g., 42%), m. p. 195—198°. The neutral product from treatment with cyclohexylamine crystallised directly, and recrystallisation from hexane–chloroform afforded pure dibenzyl *N*-cyclohexylphosphoramidate (0·096 g., 30%).

(*b*) The products of reaction with dimethylsulphonylketen methylimide during 5 days at room temperature were similar.

Di(*cyclohexylammonium*) P^{1}P^2-*Dibenzyl Pyrophosphate.*—A solution of dimethylsulphonylketen methylimide (0·211 g., 1 mmole) in nitromethane (5 c.c.) was added to a solution of benzyl dihydrogen phosphate [8] (0·376 g., 2 mmoles) in nitromethane (5 c.c.). During 4 days at room temperature the solution deposited αα-dimethylsulphonyl-*N*-methylacetamide (0·142 g., 62%), m. p. 210—212°. The liquor was evaporated and the residue was extracted with water, which was then brought to pH 8 by addition of cyclohexylamine. Recrystallisation of the precipitate from aqueous methanol furnished di(cyclohexylammonium) *P*^{1}*P*^{2}-dibenzyl pyrophosphate [10] (0·110 g., 30%), m. p. 213—215° (Found: C, 55·9; H, 7·7; N, 4·9; P, 10·8. Calc. for $C_{26}H_{42}N_2O_7P_2$: C, 56·1; H, 7·6; N, 5·0; P, 11·1%).

Di(*cyclohexylammonium*) P^{1}P^2-*Diphenyl Pyrophosphate.*—An experiment, like the foregoing, with phenyl dihydrogen phosphate (0·348 g., 2 mmoles), including recrystallisation from aqueous acetone, furnished di(cyclohexylammonium) *P*^{1}*P*^{2}-diphenyl pyrophosphate [3] (0·163 g., 46%), m. p. 245—250° (Found: C, 54·3; H, 7·3; N, 4·9; P, 10·9. Calc. for $C_{24}H_{38}N_2O_7P_2$: C, 54·6; H, 7·2; N, 5·3; P, 11·7%).

Di(*cyclohexylammonium*) P^1-*Benzyl* P^2-*Phenyl Pyrophosphate.*—An experiment, like the two foregoing ones, with benzyl dihydrogen phosphate (0·188 g.) and phenyl dihydrogen

[10] Anand, Clark, Hall, and Todd, *J.*, 1952, 3668.

phosphate (0·174 g.), including two recrystallisations from aqueous methanol, furnished *di*(*cyclohexylammonium*) P^1-*benzyl* P^2-*phenyl pyrophosphate* (0·115 g., 31%), m. p. 220—225° (Found: C, 54·7; H, 7·3; N, 5·3; P, 11·0. $C_{25}H_{40}N_2O_7P_2$ requires C, 55·3; H, 7·4; N, 5·2; P, 11·4%).

P^1-*Adenosine-5′* P^2-*Benzyl Pyrophosphate.*—Adenosine-5′ phosphate (0·087 g., 0·25 mmole) and tri-n-octylamine (0·088 g., 0·25 mmole) were dissolved in hot dimethylformamide (6 c.c.), and the cooled solution was mixed with a solution of benzyl dihydrogen phosphate (0·047 g., 0·25 mmole) in nitromethane (2 c.c.). Dimethylsulphonylketen methylimide (0·157 g., 0·75 mmole) was added to the mixture which was kept at room temperature during 2 weeks. The yield of unsymmetrical pyrophosphate, which was converted into adenosine-5′ phosphate by hydrogenolysis, was estimated by the previously described chromatographic techniques [8] to be 30%.

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UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, June 1st,* 1960.]