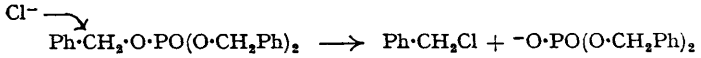
416. *Studies on Phosphorylation. Part VII. The Action of Salts on Neutral Benzyl Esters of the Oxy-acids of Phosphorus. A New Method of Selective Debenzylation.*

By V. M. Clark and A. R. Todd.

The observation that monodebenzylation of tribenzyl phosphate can be accomplished by reaction with a base hydrochloride (Clark and Todd, preceding paper) has been extended to include reaction with a quaternary ammonium salt and with a variety of inorganic salts. Benzyl esters of various phosphorous, phosphoric, and aminophosphonic acids have been shown to undergo the reaction with ease. In the majority of cases an ethoxyethanol solution of lithium chloride is most effective and this reagent is recommended for use in monodebenzylation.

The investigations described in this series of papers have had as their primary objective the development of phosphorylation methods which, while mild enough in operation to be applied to sensitive molecules, would at the same time be sufficiently flexible to permit their extension to the production of pyro- and poly-phosphoric esters. For many purposes the reagent of choice has been dibenzyl chlorophosphonate (Atherton, Openshaw, and Todd, *J.*, 1945, 382) and this has proved to be of great value in the nucleotide field (cf. *J.*, 1947, 648; 1949, 582, 2476, 2487) because of the ease with which the benzyl groups may be removed from the initial products of reaction and because it is possible in various ways to remove one benzyl group selectively, thereby providing suitable starting materials for polyphosphate synthesis. One method of selective monodebenzylation which can be applied to neutral benzyl esters of phosphorous, phosphoric, and pyrophosphoric acids is the reaction with a tertiary base (Baddiley, Clark, Michalski, and Todd, *J.*, 1949, 815). Studies on the range of applicability of this reaction with respect to the nature of both the base and the substrate ester have led to a correlation of certain aspects of the chemistry of the oxy-acids of phosphorus and sulphur and to a general interpretation of the reaction (Clark and Todd, Part VI, preceding paper). A further outcome of these studies was the demonstration that monodebenzylation could also be brought about by the action of base hydrochlorides, and, following up this observation, we investigated the action of a quaternary ammonium salt. 4-Benzyl-4-methylmorpholinium chloride and tribenzyl phosphate at 100° for 2 hours in ethoxyethanol gave a moderate yield of dibenzyl hydrogen phosphate. It was then clear that debenzylation was occurring in accordance with a mechanism of the type :



$$\overset{Cl^{-}\curvearrowright}{Ph{\cdot}CH_2{\cdot}O{\cdot}PO(O{\cdot}CH_2Ph)_2} \longrightarrow Ph{\cdot}CH_2Cl + {}^{-}O{\cdot}PO(O{\cdot}CH_2Ph)_2$$

This is, of course, the type A mechanism of Clark and Todd (*loc. cit.*) in which, as previously suggested, the anion causing debenzylation can be supplied from an external source. The present communication deals with the extension of the reaction to anions in general and the development of a very convenient practical procedure for monodebenzylation.

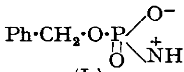
When tribenzyl phosphate was heated with three molecular proportions of lithium chloride in ethoxyethanol at 100°, lithium dibenzyl phosphate crystallised from the reaction mixture and after 2 hours the yield was almost quantitative. This simple monodebenzylation method seems to be generally applicable to neutral phosphorous and phosphoric esters containing benzyl groups. Under the same conditions dibenzyl phosphite gave the crystalline lithium monobenzyl phosphite which was very sparingly soluble in ethoxyethanol and began to separate after a few minutes' heating. Phenyl dibenzyl phosphate and 2-hydroxy*cyclo*hexyl dibenzyl phosphate were similarly converted in excellent yield into the corresponding monobenzyl esters (isolated as *cyclo*hexylamine salts).

This process of anionic debenzylation using metallic salts is confined neither to lithium salts nor to chlorides. Potassium acetate was almost as effective as lithium chloride in converting tribenzyl phosphate into dibenzyl hydrogen phosphate although in this case no crystalline salt separated during the reaction. Lithium acetate, on the other hand, was less effective owing to its low solubility, when anhydrous, in the preferred reaction media (ethanol or ethoxyethanol). Lithium nitrate was much less effective than lithium chloride, and gave dibenzyl hydrogen phosphate in 38% yield under conditions where the latter gave 80—90%. These findings suggest that we are dealing here with an equilibrium reaction, the results obtained depending ***inter alia*** on the position of equilibrium, the rate of attainment of that position, and the relative solubilities of the various salts present :

$$X^{-} + Ph{\cdot}CH_2{\cdot}O{\cdot}PO(OR)_2 \rightleftharpoons Ph{\cdot}CH_2X + (RO)_2PO{\cdot}O^{-}$$

If all components remain in solution the position of equilibrium will be in favour of debenzylation if the attacking anion is less " stable " in solution than the substituted phosphate anion, *i.e.*, if it is the anion of a weaker acid. In other words, the stronger an acid the less effective will be its anion in the debenzylating reaction. This is well illustrated in the series trifluoroacetate, nitrate, acetate; under comparable conditions (2 hours at 100° in ethoxyethanol) the yields of dibenzyl hydrogen phosphate from tribenzyl phosphate with these anions were 23%, 38%, and 75% respectively. It must be borne in mind, however, that these considerations apply only when all components are in solution; they do not apply in the case of lithium chloride, for example, since lithium dibenzyl phosphate crystallises from the reaction solution thereby displacing the equilibrium in the direction of debenzylation. Lithium salts of substituted phosphoric acids are frequently rather sparingly soluble in ethoxyethanol so that, from a practical standpoint, lithium chloride in ethoxyethanol is usually the preferred reagent for the monodebenzylation of phosphates. This tendency of lithium salts to separate can also be turned to advantage in the monodebenzylation of dibenzyl esters of aminophosphonic acids.

Attention was drawn in earlier papers (*locc. cit.*) to the difficulty of debenzylating aminophosphonates and to our inability to bring about reaction between dibenzyl aminophosphonate and tertiary bases. On heating dibenzyl aminophosphonate with lithium chloride in ethoxyethanol under the conditions used for monodebenzylating tribenzyl phosphate, only a small quantity of a lithium salt separated and the high water-solubility of the debenzylated product made its isolation from the reaction solution very difficult. When however a saturated solution of lithium chloride in ethoxyethanol was used as the debenzylating reagent, the high concentration of chloride displaced the equilibrium in the desired direction while the high lithium ion concentration facilitated precipitation of the desired lithium benzyl aminophosphonate. With this method the yield of precipitated lithium salt was 57% after 2 hours, rising to a maximum of 78% after 6 hours (reaction temperature 100°); the variation of yield with time supports the contention that an equilibrium reaction is involved. It is of interest that the precipitated lithium benzyl aminophosphonate in these experiments was completely free from chloride ion. The free benzyl aminophosphonic acid prepared from the lithium salt formed a crystalline hemihydrate. The anhydrous acid has m. p. *ca.* 200° which suggests that it exists as the zwitterion (I). It will be seen that (I) is in fact a "base–metaphosphate complex" (cf. Langheld, *Ber.*, 1911, **44**, 2076) and when heated it loses ammonia yielding, presumably, benzyl metaphosphate. Under the conditions employed for dibenzyl aminophosphonate, dibenzyl anilinophosphonate was also monodebenzylated in good yield; lithium benzyl anilinophosphonate separated from the reaction mixture and the free acid was characterised as its *cyclo*hexylamine salt.



$Ph{\cdot}CH_2{\cdot}O{\cdot}P(=O)(O^-)\overset{+}{N}H_3$

(I.)

An interesting effect has been observed when using a combination of the tertiary base and anionic debenzylation methods. It was reported in Part VI (preceding paper) that 2-picoline, for steric reasons, is an ineffective debenzylating agent for tribenzyl phosphate and, as pointed out above, the trifluoroacetate anion likewise has but little effect. However, a mixture of 2-picoline and potassium trifluoroacetate, dissolved in ethoxyethanol, is quite an effective debenzylating system; this is probably to be attributed to a displacement of the anionic equilibrium by interaction of the tertiary base with the benzyl trifluoroacetate formed.

In all the examples of lithium chloride debenzylation we have studied, an increase in the temperature of reaction has led to an increase in yield; this will not necessarily be true in all cases, of course, since although an increase in temperature will always increase the rate of attainment of equilibrium its effect on the position of equilibrium may vary. The anionic fission of esters of the phosphorus oxy-acids here described applies generally to the removal of benzyl or substituted benzyl groups. Triphenyl phosphate as might be expected is unaffected by lithium chloride, but the successful application of the method to dibenzyl esters of aminophosphonic acids makes it not improbable that, under appropriate conditions, secondary or even primary alkyl groups may be removed by anionic fission; this is under investigation. Should anionic dealkylation prove to be possible in aqueous solution the method might have some application in the degradation of polynucleotides. It will be observed that all the reactions described in this paper have been formulated as bimolecular displacements. A unimolecular solvolytic mechanism may also play a part, however, since a transient yellow colour has been observed in certain cases and Roberts and Hammett (*J. Amer. Chem. Soc.*, 1937, **59**, 1063) have pointed out that the benzyl cation is probably yellow. Although the part played by the unimolecular mechanism would be very small in the examples here reported, it would doubtless become more important in solvents of higher dielectric strength—conditions which would affect the bimolecular mechanism adversely; for this reason the overall effect of water on the debenzylation process cannot be accurately predicted.

EXPERIMENTAL.

Action of Various Salts on Tribenzyl Phosphate.—(a) 4-*Benzyl*-4-*methylmorpholinium chloride*. The quaternary salt (910 mg., 1 mol.) and tribenzyl phosphate (1·46 g., 1 mol.) were dissolved in freshly distilled ethoxyethanol (25 c.c.) and the solution was heated at 100° for 2 hours. Solvent was removed under reduced pressure and the oily residue which smelt strongly of benzyl chloride was worked up for dibenzyl hydrogen phosphate in the usual manner. The yield was 0·48 g. (44%), 0·7 g. (48%) of unchanged tribenzyl phosphate being recovered.

(b) *Lithium chloride*. Anhydrous (fused) lithium chloride (1·38 g., 3 mols.), and tribenzyl phosphate (3·68 g., 1 mol.) were dissolved in freshly distilled ethoxyethanol (25 c.c.) and the solution heated at 100° for 2 hours. During the reaction colourless needles of *lithium dibenzyl phosphate* separated and benzyl chloride was formed. Aqueous sodium hydroxide (40 c.c. of 5%) was added to the cooled mixture and the whole extracted with ether (2 × 50 c.c.). The alkaline layer was acidified with sulphuric acid, so precipitating a colourless oil which quickly solidified. This was taken up in chloroform and the solution dried (Na_2SO_4), filtered, and evaporated, to leave a crystalline mass of dibenzyl hydrogen phosphate (2·74 g.; almost quantitative). Recrystallised from chloroform–light petroleum it formed colourless needles (2·24 g., 80%), m. p. 78—79° alone or mixed with an authentic specimen. In a second experiment the crystalline lithium dibenzyl phosphate was filtered off directly from the reaction mixture and recrystallised from aqueous acetone, forming colourless needles, m. p. 292—293° (decomp.) (Found : C, 59·0; H, 5·1. $C_{14}H_{14}O_4PLi$ requires C, 59·1; H, 5·0%). When ethanol was used as a solvent and the reaction carried out by refluxing the solution for 4½ hours the yield of dibenzyl hydrogen phosphate was only 38%.

(c) ***Lithium acetate.*** Lithium acetate dihydrate was fairly readily soluble in ethanol, but the anhydrous salt was much less so and was very sparingly soluble in ethoxyethanol. On heating an ethoxyethanol solution of the dihydrate above 40° the salt—presumably in anhydrous form—separated from solution. When the dihydrate (3 mols.) was heated under reflux in ethanol with tribenzyl phosphate (1 mol.) for 2 hours and worked up as in the lithium chloride experiment the yield of dibenzyl hydrogen phosphate was 13%, a large amount (61%) of tribenzyl phosphate being recovered unchanged.

(d) *Potassium acetate.* After a solution of tribenzyl phosphate in ethoxyethanol (0·4M.; 1 mol.) had been heated with anhydrous potassium acetate (3 mols.) at 100° for 2 hours, no crystalline material separated but by working up as described above dibenzyl hydrogen phosphate (75%) was obtained.

(e) *Lithium nitrate.* Under the same conditions (3 mols. of salt and 1 mol. of ester in ethoxyethanol at 100° for 2 hours) the yield of dibenzyl hydrogen phosphate was 38%.

(f) *Potassium trifluoroacetate.* Under the same conditions in ethoxyethanol at 100° potassium trifluoroacetate and tribenzyl phosphate gave after 2 hours dibenzyl hydrogen phosphate in 23% yield, together with 69% of unchanged ester.

Phenyl Benzyl Hydrogen Phosphate.—Phenyl dibenzyl phosphate (1·59 g., 1 mol.) (Baddiley, Clark, Michalski, and Todd, *loc. cit.*) and anhydrous lithium chloride (580 mg., 3 mols.) were heated in ethoxyethanol (20 c.c.) for 3 hours at 100°. No crystalline salt separated but the mixture smelt strongly of benzyl chloride. When the products were worked up for dibenzyl hydrogen phosphate as described above, phenyl benzyl hydrogen phosphate (1·054 g., 89%) was obtained as a viscous syrup. This syrup was dissolved in ether (30 c.c.), and ethereal *cyclo*hexylamine (685 mg. in 15 c.c.) added. The crystalline *cyclo*hexylamine salt separated rapidly. More ether (20 c.c.) was added and after 5 minutes the salt (1·05 g., 65%) was collected, dried at 80°, and recrystallised from ethanol–acetone. It formed long silky needles, m. p. 147°, undepressed on admixture with an authentic specimen (m. p. 147°; Part V, *loc. cit.*).

2-*Hydroxy*cyclo*hexyl Benzyl Hydrogen Phosphate.*—2-Hydroxy*cyclo*hexyl dibenzyl phosphate (1·24 g., 1 mol.) (Baddiley, Clark, Michalski, and Todd, *loc. cit.*) and anhydrous lithium chloride (570 mg., 3 mols.) were heated in ethoxyethanol (25 c.c.) at 100° for 2 hours. The mixture worked up as before yielded the water-soluble 2-hydroxy*cyclo*hexyl benzyl hydrogen phosphate as a colourless oil (823 mg., 88%). The free acid was characterised by dissolution in ether and conversion into its cyclo*hexylamine* salt in the usual manner. Recrystallised from ethanol the salt formed colourless needles, m. p. 179° (Found: C, 59·2; H, 8·2; N, 3·7. $C_{19}H_{32}O_5NP$ requires C, 59·2; H, 8·4; N, 3·6%).

Lithium Monobenzyl Phosphite.—A hot solution of dibenzyl phosphite (5·48 g., 1 mol.) in ethoxyethanol (10 c.c.) was added to a solution of anhydrous lithium chloride (1·7 g., 2 mols.) in ethoxyethanol (15 c.c.) at 100°. A crystalline precipitate began to form within 1 minute of mixing. The mixture was kept at 100° for 2 hours and the precipitated ***lithium monobenzyl phosphite*** (2·79 g., 76%) then collected, washed with hot solvent, and dried at 100°. The salt obtained in this way was a fine white powder, m. p. >350°; although hydrophobic in this condition it was in fact appreciably soluble in water but sparingly so in ethanol. Recrystallised from aqueous ethanol, it formed colourless nacreous plates (Found : C, 47·4; H, 4·9. $C_7H_8O_3PLi$ requires C, 47·2; H, 4·6%). The lithium salt was converted into the ammonium salt by acidification, extraction of the free acid with chloroform, and saturation of the concentrated extract with ammonia; recrystallised from ethoxyethanol–dioxan it formed colourless needles, m. p. 154°, undepressed on admixture with an authentic specimen (Part V, *loc. cit.*).

Prolongation of the period of heating with lithium chloride to 4½ hours gave no increase in yield. In a series of similar experiments in ethanolic solution at 78° the yields of lithium monobenzyl phosphite were : after 1 hour, 48%; after 2 hours, 54%; after 4½ hours, 65%. In all cases the amount of monobenzyl phosphite which could be obtained by working up the mother-liquor after filtration of the lithium salt was negligible.

Monobenzyl Hydrogen Aminophosphonate.—Selective debenzylation of dibenzyl aminophosphonate was best carried out by using a hot saturated solution of lithium chloride in ethoxyethanol (solubility 16·4 g. per 100 g. of solution at 100°).

Dibenzyl aminophosphonate (5·19 g.) was heated for 10 hours with a saturated solution of anhydrous lithium chloride in ethoxyethanol at 100° (25 c.c.). After 15 minutes the mixture became opalescent and a solid began to separate. The amount of *lithium monobenzyl aminophosphonate* which had separated was estimated by collection on a sintered-glass funnel at various intervals; after 3 hours it weighed 2·42 g. (67%), and after 6 hours 2·82 g. (78%), no further deposition occurring thereafter. A second experiment under the same conditions gave a yield of 57% in 2 hours and 72% in 4 hours. The lithium salt separated from water as colourless needles having no definite m. p. Placed in a bath at 265° and further heated it shrank at 282°, then gradually became translucent, and finally transparent over the range 295—310° (Found : C, 43·3; H, 4·7; N, 7·0. $C_7H_9O_3NPLi$ requires C, 43·5; H, 4·7; N, 7·3%).

When the above lithium salt (1·4 g.) was dissolved in warm water (15 c.c.), acidified with dilute sulphuric acid (7 c.c. of 3N.), and set aside at 0° ***monobenzyl hydrogen aminophosphonate*** separated as colourless plates, apparently of a hemihydrate (Found : C, 43·1; H, 5·7; N, 7·2. $C_7H_{10}O_3NP,\frac{1}{2}H_2O$ requires C, 42·9; H, 5·7; N, 7·2%). When heated, the hemihydrate showed an indefinite m. p., shrinking at 102° and becoming translucent at 106°. If placed in a bath at 180°, however, it melted immediately, then resolidified, and if heated quickly above this temperature melted at 199—200° without apparent decomposition. This somewhat anomalous behaviour is doubtless due to the fact that the acid gradually loses ammonia on heating, thereby precluding its preparation in anhydrous form from the hemihydrate by the usual methods of drying.

Monobenzyl Hydrogen Anilinophosphonate.—Dibenzyl anilinophosphonate (2·86 g.) was dissolved in ethoxyethanol (25 c.c.) saturated with lithium chloride at 100° and the mixture was heated for 1½ hours at 100°. After 30 minutes solid began to separate and a marked odour of benzyl chloride was apparent. Water (20 c.c.) and ether (50 c.c.) were added to the cooled mixture which was then filtered. Aqueous sodium hydroxide (15 c.c. of 10%) was added to the filtrate and the layers were separated. The aqueous alkaline layer was again extracted with ether (2 × 50 c.c.), the filtered solid added to the aqueous phase, and the mixture acidified with dilute sulphuric acid (30 c.c. of 3N.). The opalescent solution was extracted with ethyl acetate (3 × 100 c.c.), and the combined extracts were washed with water, dried (Na_2SO_4), and evaporated under reduced pressure (bath-temp. 40°/0·2 mm.), yielding *monobenzyl hydrogen anilinophosphonate* (1·77 g., 83%) as a pale yellow amorphous solid (Found: C, 58·5; H, 5·3. $C_{13}H_{14}O_3NP$ requires C, 59·3; H, 5·4%), which resinified on attempting to recrystallise it from benzene. It was, therefore, dissolved in ethyl acetate and converted into its cyclo*hexylamine* salt which crystallised from acetone containing a few drops of ethanol, as colourless plates, m. p. 180—181° (Found: C, 62·8; H, 7·8; N, 7·7. $C_{19}H_{27}O_3N_2P$ requires C, 63·0; H, 7·5; N, 7·7%).

Action of a Mixture of Potassium Trifluoroacetate and 2-Picoline on Tribenzyl Phosphate.—Tribenzyl phosphate (1·09 g.) and potassium trifluoroacetate (1·25 g.), dissolved in a mixture of 2-picoline (5 c.c.) and ethoxyethanol (5 c.c.), were heated for 1 hour at 100°. A transient yellow colour appeared, which faded on cooling and reappeared on heating. After 1 hour the mixture was worked up in the usual manner to give dibenzyl hydrogen phosphate (0·4 g., 49%), together with unchanged tribenzyl phosphate (0·36 g., 33%).

Under the same conditions, but with 2-picoline alone, the yield of dibenzyl hydrogen phosphate was only 19%, and, when potassium trifluoroacetate was used alone, the 2-picoline being replaced by an additional 5 c.c. of ethoxyethanol, the yield was 15%. Potassium trifluoroacetate had no beneficial effect on the action of 2 : 6-lutidine as a debenzylating agent for tribenzyl phosphate, some 91% of which was recovered unchanged after 1 hour in ethoxyethanol at 100°. A mixture of pyridine and potassium trifluoroacetate, on the other hand, gave a 93% yield of crystalline dibenzyl hydrogen phosphate under analogous conditions.

Grateful acknowledgment is made to the Department of Scientific and Industrial Research for a Maintenance Allowance, to Gonville and Caius College for the award of the Dunlop Studentship, and to the University of Cambridge for the award of the W. A. Meek Scholarship (to V. M. C.).

UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, May* 1*st*, 1950.]