494. *Studies in Phosphorylation. Part XXVII.* Nucleophilic Displacements on Methyl Hydrogen N-Cyclohexylphosphoramidothioate*

By N. K. Hamer

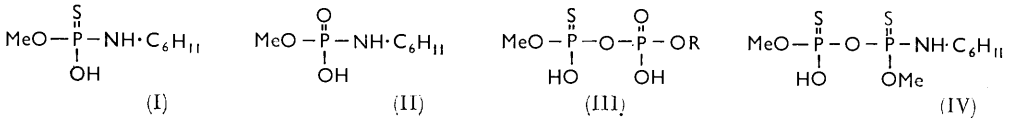
The preparation and resolution of methyl hydrogen *N*-cyclohexylphosphoramidothioate are reported. In reactions with nucleophiles it shows a close resemblance to the corresponding ester of phosphoramidic acid. Some of these reactions were stereospecific and we suggest that they result in inversion of configuration at phosphorus. The relevance of these observations to the mechanism of phosphorylation by derivatives of phosphoramidic acid is discussed.

From investigations [1-4] into the stereochemistry of substitution at four-covalent phosphorus it seems clear that, in the case of the fully esterified phosphates and phosphonates and their thio-analogues, nucleophilic substitution results in inversion of configuration at phosphorus, but no comparable unequivocal result is yet available for partially esterified derivatives. Since the latter include phosphoramidic monoesters which are valuable intermediates in the synthesis of unsymmetrical pyrophosphates ester [5,6] but for which the

* Part XXVI, N. K. Hamer, *J.*, 1965, 46.

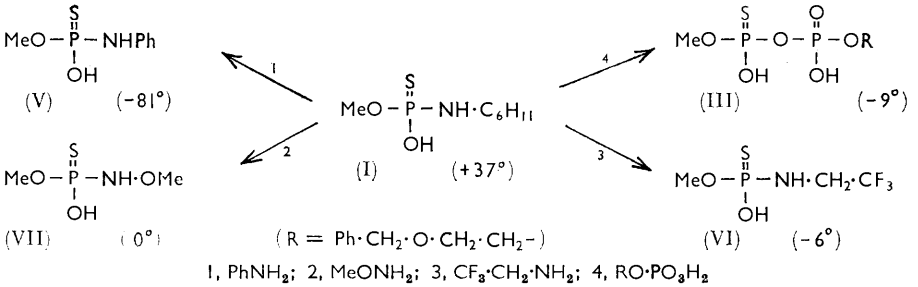
[1] M. Green and R. F. Hudson, *Proc. Chem. Soc.*, 1959, 227; 1962, 307.
[2] J. Michalski and R. Ratajczak, *Chem. and Ind.*, 1960, 1214.
[3] H. S. Aaron, R. F. Uyeda, H. F. Frank, and J. I. Miller, *J. Amer. Chem. Soc.*, 1962, **84**, 617.
[4] M. Green and R. F. Hudson, *Angew. Chem.*, 1963, **2**, 11.
[5] V. M. Clark, G. W. Kirby, and Sir Alexander Todd, *J.*, 1957, 1497.
[6] R. W. Chambers and H. G. Khorana, *J. Amer. Chem. Soc.*, 1958, **80**, 3749.

detailed mechanism of phosphorylation is not fully understood,[7] further information is desirable. Although the partially esterified derivatives of phosphoramidic acid do not possess any intrinsic asymmetry their thio-analogues do and thus provide, in principle, a simple method of investigating the stereochemistry of substitution. The preparation of methyl hydrogen *N*-cyclohexylphosphoramidothioate was therefore attempted, first to investigate how similar in chemical behaviour it was to phosphoramidic monoesters, and then to determine the stereochemical relationship between it and the nucleophilic substitution products.



(I) (II) (III) (IV)

Vigorous alkaline hydrolysis of dimethyl *N*-cyclohexylphosphoramidothioate and subsequent acidification gave the free acid (I) as a stable crystalline solid, whose pK_a was 3·1 [the same as that of methyl hydrogen *N*-cyclohexylphosphoramidate [7] (II)]. When heated in an inert solvent with monoesters of phosphoric acid it gave thiopyrophosphate diesters (III) in high yield. Amine exchange similar to that observed for (II) [7] occurred when (I) was treated with a large excess of a primary aromatic amine. The latter reaction appears to be quite general and occurred also with weakly basic aliphatic amines such as methoxyamine and 2,2,2-trifluoroethylamine. All these reactions were markedly slower than the corresponding reactions of (II) (by a factor of the order of 10) but such decreases in rate are commonly observed when P=S is substituted for P=O in any given class of compound.[8,9] There was evidence from paper chromatography that (I) reacted with itself on prolonged heating in an inert solvent to give a new fast-running compound which, by analogy with (II),[7] was identified tentatively as (IV) but since this was accompanied by several other products (probably resulting from dealkylation) the reaction was not studied further. Hydrolysis of the acid (I) in aqueous solution was complicated by concomitant hydrolysis of the P=S bond with the formation of methyl dihydrogen phosphate and hydrogen sulphide. It does seem clear, however, that under conditions normally employed for phosphorylation, there exists a rather close parallel between the reactions of (I) and (II); experiments with optically active material further support this view. Acid (I) was resolved by fractional crystallisation of the quinine salts; both enantiomers were obtained ($[\phi]_D$ +38° and −37° in ethanol) and the rotations of the ammonium and cyclohexylammonium salts were almost identical with those of the free acid. The molecular rotations (sodium D line) of the products (as their cyclohexylammonium salts) obtained from reaction of (I) with 2-benzyloxyethyl dihydrogen phosphate [10] (chosen because soluble) and with aniline, methoxyamine, and 2,2,2-trifluoroethylamine are annexed.



1, $PhNH_2$; 2, $MeONH_2$; 3, $CF_3 \cdot CH_2 \cdot NH_2$; 4, $RO \cdot PO_3H_2$

[7] N. K. Hamer, *J.*, 1965, 46.
[8] J. A. A. Ketelaar, H. R. Gersmann, and K. Koopmans, *Rec. Trav. chim.*, 1952, **71**, 1253.
[9] D. F. Heath, *J.*, 1956, 3796.
[10] N. K. Hamer, Thesis, Cambridge, 1960.

These results indicate that (I) phosphorylates by a stereospecific mechanism, so methyl metaphosphorothioate cannot be an intermediate since it would, almost certainly, be planar. [A corresponding conclusion was deduced from the reactions of (II) [7] by different methods.] Although the optical purity of the products is undetermined it will be shown that the occurrence of some racemisation is not unexpected and need not affect this conclusion in any way. There was evidence from the optical rotatory dispersion curve of the *N*-phenyl compound (V) that the large observed rotation was due to contributions from transitions of the aromatic system. On the other hand the small rotation of the pyrophosphate ester (III) probably indicates the decrease in asymmetry rather than extensive racemisation. However, a racemic product was obtained when this reaction was carried out in the presence of pyridine. The product (VII) obtained from reaction with methoxyamine had no measurable rotation at any accessible wavelength (nor had that from phenylhydrazine) and probably also some racemisation had occurred with 2,2,2-trifluoroethylamine since a product with smaller rotation was obtained when the reaction was carried

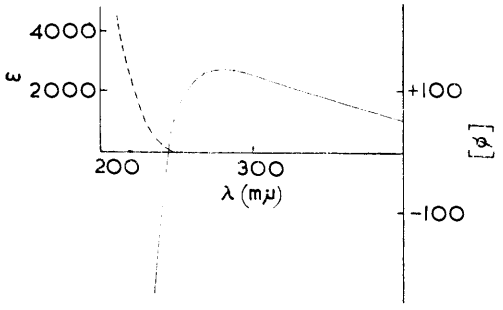


FIGURE 1. Optical rotatory dispersion (——) and absorption (– – – –) of (I)

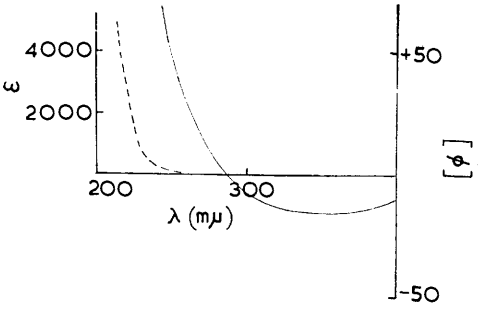
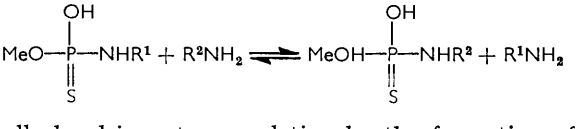


FIGURE 2. Optical rotatory dispersion (——) and absorption spectrum (– – – –) of (VI)

out under more vigorous conditions. Absorption spectra and optical rotatory dispersion curves of (I) (ammonium salt) and (VI) (cyclohexylammonium salt) are shown (Figures 1 and 2) and provide a clue to the stereochemical relationship between these two compounds. The absorption beginning in the region of 250 mμ is not shown by similar derivatives of phosphoramidic acid and may thus be attributed to the thiophosphoryl group. It is likely, therefore, that the change in sign of the rotation in this region is the start of a Cotton effect and this suggests that (I) and (VI) are of opposite configuration.

The fact that, in some cases, a partially or wholly racemic product was obtained may be attributed to racemisation of the product resulting from the initial displacement under the conditions of the reaction. Such racemisation becomes possible if the amine exchange is reversible involving attack by a free base on the free acid (I) which will be in equilibrium with its zwitterion (presumed to be the actual phosphorylating agent as discussed later). When R^1 is cyclohexyl and R^2NH_2 a weak base such as aniline, 2,2,2-trifluoroethylamine or methoxyamine (pK_a of conjugate acids in water 4·6,[11] 5·7,[12] and 4·6,[13] respectively) the

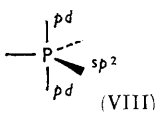


reaction will eventually be driven to completion by the formation of a relatively stable cyclohexylammonium salt. At intermediate stages, however, there will be an equilibrium between the two acids and their conjugate bases and the possibility of further displacements

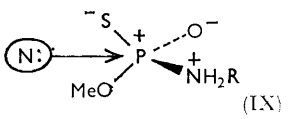
[11] M. Kilpatrick and C. A. Arenberg, *J. Amer. Chem. Soc.*, 1953, **75**, 3812.
[12] T. C. Bissot, R. W. Parry, and D. H. Campbell, *J. Amer. Chem. Soc.*, 1957, **79**, 796.
[13] A. L. Henne and J. J. Stewart, *J. Amer. Chem. Soc.*, 1955, **77**, 1901.

on the product exists. The degree of racemisation of the final product will then depend, among other factors, on the relative ease with which nucleophilic displacements on the product occur. There was evidence from paper chromatography in an acidic solvent system that the relative stabilities were in the order (V) > (VI) ~ (I) > (VII) and these are qualitatively consistent with the experimental findings. It should be added that we cannot yet account for the rather unexpected lability of (VII).

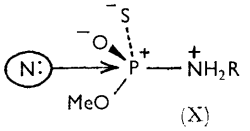
Since in all essential aspects, the chemistry of (I) parallels that of (II) we may assume that their mechanisms of phosphorylation are the same and can combine the present results on the stereochemistry of the displacement with earlier results obtained from (II) on the nature of the phosphorylating species and thus obtain a more complete description of the actual transition state than was hitherto possible. The previous work [7] indicated that with (II) the phosphorylating agent was the zwitterion and this is substantiated by the hydrolysis of phosphoramidic acid.[14] The fact that inversion of configuration was



(VIII)



(IX)



(X)

found in the reaction of (I) with 2,2,2-trifluoroethylamine (and almost certainly occurs in the other cases) can be accommodated if the transition state is assumed to be of the form of a trigonal bipyramid (VIII). Such a transition state has been postulated for other phosphorus compounds which undergo nucleophilic substitution with inversion of configuration [15] but the question as to whether the entering and leaving groups occupy axial (*pd* hybrid bonds) or basal (sp^2 hybrid bonds) is not fully settled (see discussion by Cox and Ramsay [16] in relation to microscopic reversibility). Although the former might seem preferable on general chemical grounds the results of Haake and Westheimer [17] on the acid-catalysed hydrolysis and ^{18}O exchange of ethylene hydrogen phosphate strongly support the latter. The latter view seems preferable also for nucleophilic displacements on these derivatives of phosphoramidic acid since attack by an electron lone pair should be less subject to the unfavourable charge distribution round the phosphorus if it approaches along a twofold axis (IX), which gives rise to a transition state in which both entering and leaving groups occupy basal positions, than if it approaches along the threefold axis (X) to give a transition state in which entering and leaving groups will occupy axial positions.

Experimental

Paper chromatograms were run on Whatman No. 1 paper using the solvent systems: *A*, propan-2-ol–ammonia–water (7 : 1 : 2) and *B*, butan-1-ol–acetic acid–water (5 : 2 : 3) containing 1% of chloroacetic acid.

Dimethyl N-Cyclohexylphosphoramidothioate.—To a solution of cyclohexylamine (22 g.) in chloroform (100 ml.) cooled in ice-water, was added, slowly with stirring, dimethyl phosphorochloridothioate [18] (16 g.) in chloroform (30 ml.). After addition was complete the mixture was kept for 4 hr. at room temperature, then water (100 ml.) added. The chloroform extract was separated, washed with dilute hydrochloric acid (2 × 50 ml.) and finally dried ($MgSO_4$). After removal of the chloroform the *product* (24 g.) was obtained as a slightly yellow crystalline solid. Recrystallisation from methanol gave colourless needles (18·5 g.), m. p. 91° (Found: C, 43·3; H, 7·3; P, 14·5. $C_8H_{18}NO_2PS$ requires C, 43·1; H, 8·1; P, 13·9%).

Methyl Hydrogen N-Cyclohexylphosphoramidothioate.—To a hot solution of potassium hydroxide (8·5 g.) in water (40 ml.) was added a solution of the above dimethyl ester (11 g.) in

[14] J. D. Chanley and E. Feageson, *J. Amer. Chem. Soc.*, 1963, **85**, 1181.
[15] R. F. Hudson, *Adv. Inorg. Chem. Radiochem.*, 1964, **5**, 347.
[16] J. R. Cox and O. B. Ramsay, *Chem. Rev.*, 1964, **64**, 317.
[17] P. C. Haake and F. H. Westheimer, *J. Amer. Chem. Soc.*, 1961, **83**, 1102.
[18] Y. A. Mandelbaum, V. M. Lomakina, and N. N. Melnika, *Doklady Akad. Nauk S.S.S.R.*, 1954, **96**, 1173.

hot methanol (100 ml.) and the mixture heated under reflux for 4—5 hr. After cooling to 0° (filtering if necessary) the solution was neutralised by cautious addition of dilute hydrochloric acid and then evaporated to dryness *in vacuo* at 50°. The residue was extracted with hot ethanol (3 × 50 ml.) and the combined extracts evaporated to dryness to give the crude potassium salt of the product (10·5 g.) as colourless crystals. These were dissolved in water (20 ml.), cooled to 0°, and the solution acidified with hydrochloric acid (20 ml. of 3N). The oil which separated crystallised almost immediately and was filtered off. After drying the *product* was recrystallised from chloroform–light petroleum (b. p. 60—80°) to give colourless needles (6·8 g.), m. p. 115—116°. On paper chromatograms it travelled as a single spot, R_F 0·75 in *A*, 0·90 in *B* (Found: C, 39·7; H, 6·8; N, 6·4. $C_7H_{16}NO_2PS$ requires C, 40·1; H, 7·5; N, 6·7%).

The acid was resolved by fractional crystallisation of the quinine salts, m. p. 176° (+), 158° (−); details will be published separately (with Professor J. Michalski). These were treated with dilute ammonia solution and the liberated quinine extracted with chloroform. Evaporation of the aqueous layer followed by recrystallisation of the residue from ethanol–ether gave the ammonium salts as needles, m. p. 137—138° ($[\alpha]_D$ +16·5° and −16° in ethanol). The optically active free acid was obtained from these by the same procedure as that given above.

Dicyclohexylammonium P^1-*Methyl* P^2-2-*Benzyloxyethyl* P^1-*Thiopyrophosphate*.—A solution of (I) (500 mg., 20% excess) and cyclohexylammonium 2-benzyloxyethyl hydrogen phosphate [10] (640 mg.) in dry acetonitrile (7 ml.) was heated under reflux for 4 hr. Paper chromatography of the reaction mixture showed that all the latter compound had disappeared and had been replaced by a faster-running compound (R_F 0·60 in *A*). Dry ether (25 ml.) was then added and the mixture set aside overnight at −10°. The *product* (890 mg.) which separated was recrystallised from ethanol–ether to give needles (760 mg.), m. p. 148—150° (Found: C, 49·2; H, 7·4; P, 11·5. $C_{22}H_{42}N_2O_7P_2S$ requires C, 49·4; H, 7·9; P, 11·7%).

When the experiment was performed using optically active (+)-(I) the product had $[\alpha]_D$ −1·8° in ethanol.

Ammonium Methyl N-*Phenylphosphoramidothioate*.—A solution of (I) (150 mg.) and aniline (0·6 ml.) in dry benzene was heated at 60° for 18 hr. Chromatography of the solution at this stage indicated the presence of a single phosphorus-containing compound (R_F 0·72 in *A*). The mixture was poured into water (10 ml.) and the aniline and benzene removed by extraction with ether. Evaporation of the aqueous solution gave a colourless crystalline solid but, since elemental analysis does not distinguish between the cyclohexylammonium salt of the product and the anilinium salt of the starting material, this was dissolved in water and passed through a Dowex 50 column (NH_4^+ form). The eluate, on evaporation to dryness, gave the *product* (120 mg.) which was recrystallised from ethanol–ether (Found: C, 38·0; H, 6·2; N, 12·5. $C_7H_{13}N_2O_2P$ requires C, 38·2; H, 6·0; N, 12·6%).

When (I) (+-form) was treated with aniline under the same conditions the cyclohexylamine salt of the product was recrystallised from ethanol–ether to give needles, m. p. 144—145° ($[\alpha]_D$ −27° in ethanol).

Cyclohexylammonium Methyl N-*Methoxyphosphoramidothioate*.—A solution of (I) (200 mg.) and methoxyamine [19] (0·4 ml.; purified by distillation over its hydrochloride to remove all traces of strongly basic impurities) in acetonitrile (1 ml.) were heated at 60° and the mixture analysed by paper chromatography at intervals. After 24 hr. it was found that the reaction mixture gave a single spot on paper chromatograms with R_F 0·60 in *A* (considerable decomposition occurred in *B*). Removal of the solvent and excess of methoxyamine *in vacuo* gave a gum which was taken up in dry ether (6 ml.) and the solution filtered to remove a small quantity of flocculent material. On prolonged standing (several weeks) at −20° the filtrate deposited the *product* as hygroscopic silky needles, m. p. 90—91° (Found: C, 38·0; H, 7·9. $C_8H_{20}N_2O_3PS$ requires C, 37·5; H, 8·2%).

Treatment of (I) (+-form) with methoxyamine under the same conditions gave a product which had no measurable rotation at any wavelength. When the reaction was stopped at an earlier stage the observed rotation was consistent with that expected from the amount of unreacted starting material remaining.

Cyclohexylammonium Methyl N-2,2,2-*Trifluoroethylphosphoramidothioate*.—The reaction of (I) (200 mg.) and 2,2,2-trifluoroethylamine [20] (0·8 ml.) was carried out in the same manner as

[19] E. J. Bourne, S. H. Henry, C. E. M. Tatlow, and J. C. Tatlow, *J.*, 1952, 4014.
[20] C. H. Andrewes, H. King, and J. Walker, *Proc. Roy. Soc.*, 1946, *B*, **133**, 20.

that with methoxyamine. After 30 hr. all the starting material had disappeared and had been replaced by a new compound with R_F 0·75 in *A*, 0·85 in *B* (with slight traces of decomposition). As in the preceding case the crystalline *product* was deposited extremely slowly from an ethereal solution at −20°. It had m. p. 83—84° (Found: C, 35·6; H, 7·1; N, 9·5. $C_9H_{20}F_3N_2O_2PS$ requires C, 35·1; H, 6·5; N, 9·1%).

When (I) (+-form) was used the reaction was terminated after 18 hr. at 60° by which time traces only (<5%) of the starting material remained. After removal of the solvent *in vacuo* the gum was taken up in ethanol (methanol for the optical rotatory dispersion determination), the solution made up to 5 ml., and the rotation determined ($[\alpha]_D$ −2°). When the heating was prolonged or a greater excess of the base used the rotation of the product was smaller.

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UNIVERSITY CHEMICAL LABORATORY, CAMBRIDGE. [*Received, September 14th*, 1964.]