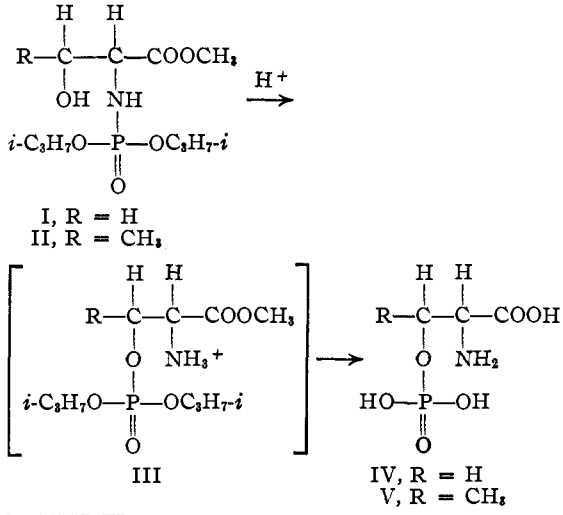
A Nitrogen-to-Oxygen Phosphoryl Migration: Preparation of *dl*-Serinephosphoric and Threoninephosphoric Acid

BY ROBERT E. PLAPINGER AND T. WAGNER-JAUREGG

RECEIVED JULY 10, 1953

Previous publications have dealt with the isolation of serinephosphoric acid from the acid hydrolysates of the diisopropylphosphoryl derivatives of chymotrypsin and purified cholinesterase.[1] In connection with these findings, it was advisable to determine whether O-phosphorylated hydroxyamino acids can be formed by migration of a phosphoryl group from nitrogen to oxygen.

It is a well established fact that certain N-acyl-1,2-aminoalcohols rearrange on treatment with mineral acid to the corresponding amine salt of the O-acyl-1,2-aminoalcohol.[2] We found that a similar transformation was accomplished easily with N-diisopropylphosphoryl derivatives of *dl*-serine, *dl*-threonine and ethanolamine.[3] These findings now provide a simple method for the preparation of both serinephosphoric and threoninephosphoric acids.[4] Treatment of N-diisopropylphosphoryl derivatives of *dl*-serine methyl ester (I) and *dl*-threonine methyl ester (II) with boiling aqueous hydrochloric acid gave *dl*-serinephosphoric (IV) and "threoninephosphoric acid" (V), respectively, the former in 25% yield and the latter[5] in 50% yield. Ethanolaminephosphoric acid was obtained similarly from its N-diisopropylphosphoryl derivative, in 18% yield.



I, R = H
II, R = CH_3

III

IV, R = H
V, R = CH_3

An unsuccessful attempt was made to isolate the intermediate III as the hydrochloride, by treatment of I or II with dry hydrogen chloride gas in either dioxane or absolute methanol. The product obtained in each case was a sirupy, ether-insoluble hydrochloride, which would not crystallize. In the case of the intermediate derived from N-diisopropylphosphoryl-*dl*-serine methyl ester, a small amount of *dl*-serine methyl ester hydrochloride was isolated. This indicated that some hydrolysis of the phosphoramide linkage had occurred. When I and II were first treated with dry hydrogen chloride gas, as described above, and then hydrolyzed with boiling aqueous hydrochloric acid, the yields of the corresponding phosphoramino acids (IV and V) were identical with those obtained by direct treatment of I and II with boiling aqueous hydrochloric acid.

An attempt to synthesize cysteine-phosphoric acid from N-diisopropylphosphoryl-1-(+)-cysteine methyl ester was unsuccessful.

Experimental

All melting points were taken on a Fisher–Johns block and are uncorrected.

N-Diisopropylphosphoryl Derivatives of *dl*-Serine, *dl*-Threonine and *l*-(+)-Cysteine Methyl Esters.—The serine and threonine derivatives were prepared from diisopropylphosphoryl chloride (DClP) and the appropriate amino acid ester by the procedure described earlier.[6] The serine derivative, which had been reported[6] as a sirup, has since been obtained in crystalline form, as a white waxy solid of m.p. 48–50°.

Anal. Calcd. for $C_{10}H_{22}O_6NP$: N, 4.94; P, 10.94. Found: N, 4.94; P, 11.0.

The cysteine derivative, which has not been previously characterized, was similarly prepared from DClP and *l*-(+)-cysteine methyl ester hydrochloride,[7] in the presence of 2 moles of triethylamine. This product was isolated as a viscous sirup, soluble in ether, benzene and chloroform, and insoluble in petroleum ether. It solidified when kept overnight in the ice-box, and softened at approximately 22°. This substance gave a positive test for a sulfhydryl group with alkaline sodium nitroprusside.

Anal. Calcd. for $C_{10}H_{22}O_5NPS$: N, 4.68; P, 10.35. Found: N, 4.70; P, 10.65.

Standard Van Slyke amino nitrogen determinations indicated the absence of free amino nitrogen in the above mentioned phosphoramides.

N-Diisopropylphosphorylethanolamine.—This substance was prepared from DClP and ethanolamine in the presence of triethylamine, using dry chloroform as a solvent. It boiled at 151° (0.8 mm.), decomposition setting in after about one-half of the material distilled, $n^{23}D$ 1.4400.

Anal. Calcd. for $C_8H_{20}O_4NP$: N, 6.2; P, 13.75. Found: N, 6.05; P, 13.85.

A standard Van Slyke amino nitrogen determination indicated the absence of free amino nitrogen in this compound.

Preparation of *dl*-Serinephosphoric, "Threoninephosphoric" and Ethanolaminephosphoric Acids.—Approximately 0.01 mole of N-diisopropylphosphoryl-amino acid ester or -ethanolamine was placed in a 100-cc. round-bottom flask containing 40 cc. of 5–7% hydrochloric acid. After refluxing for 6 hours, the solution was concentrated *in vacuo* to dryness. The oily residue was dissolved in water, and ethanol was added until turbidity was reached. The turbid solution was allowed to stand overnight, and the solid which precipitated was then separated from the mother liquor by centrifugation. This solid was washed several times with alcohol, then ether, and dried over phos-

(1) (a) N. K. Schaffer, S. C. May, Jr., and W. H. Summerson, *J. Biol. Chem.*, **202**, 67 (1953); (b) *Federation Proc.*, **12**, 264 (1953).

(2) Some recent papers are: A. P. Phillips and R. Baltzly, THIS JOURNAL, **69**, 200 (1947); L. H. Welsh, *ibid.*, **71**, 3500 (1949); G. Fodor and J. Kiss, *ibid.*, **72**, 3495 (1950); E. E. van Tamelen, *ibid.*, **73**, 5773 (1951); D. P. Elliot, *Biochem. J.*, **50**, 542 (1952).

(3) Presumably the mechanism postulated by Welsh (see footnote 2) for acyl migrations of derivatives of ephedrine and ψ-ephedrine can be interpreted to be operative in these phosphoryl migrations.

(4) Our attempts to prepare these substances by the procedures of P. H. Levine and A. Schormuller, *J. Biol. Chem.*, **105**, 547 (1934), and R. H. A. Plimmer, *Biochem. J.*, **35**, 461 (1941), were unsatisfactory with respect to both purity and yield. R. E. Ferrel, H. S. Olcott and H. Fraenkel-Conrat, THIS JOURNAL, **70**, 2106 (1948), experienced similar difficulties.

(5) In the light of Welsh's work (see footnotes 2 and 3) this product could be *dl*-threoninephosphoric acid, *dl*-allothreoninephosphoric acid or a mixture of both of these substances. No attempt was made to determine the stereochemical uniformity of our preparation.

(6) T. Wagner-Jauregg, J. J. O'Neill and W. H. Summerson, THIS JOURNAL, **73**, 5202 (1951).

(7) Prepared from *l*-(+)-cysteine hydrochloride, absolute methanol and gaseous hydrogen chloride; m.p. 145°. *Anal.* Calcd. for $C_4H_{10}O_2NSCl$: N, 8.16. Found: N, 8.30.

phorus pentoxide. In order to induce precipitation, it was sometimes necessary to keep the turbid solution at −20° overnight. Addition of ether to the turbid solution often proved helpful. The alcohol and ether washings, when added to the original mother liquor, usually yielded additional amounts of the aminophosphoric acid.

N-Diisopropylphosphoryl-*dl*-serine methyl ester (4.0 g.) yielded 0.540 g. (21%) of ***dl*-serinephosphoric acid,**[8] melting at 166–167°. When the reflux time was reduced to 4 hours, *dl*-serinephosphoric acid was isolated in 26% yield.

Anal. Calcd. for $C_3H_8O_6NP$: C, 19.47; H, 4.36; N, 7.57; P, 16.74. Found: C, 19.4; H, 4.43; N, 7.45 (Van Slyke), 7.80 (Dumas); P, 16.6.

N-Diisopropyl-*dl*-threonine methyl ester (3.0 g.) yielded 1.04 g. (52%) of **threoninephosphoric acid,**[9] melting at 184°.

Anal. Calcd. for $C_4H_{10}O_6NP$: C, 24.13; H, 5.06; N, 7.04; P, 15.56. Found: C, 24.2; H, 5.30; N, 7.1 (Van Slyke), 7.0 (Dumas); P, 15.8.

N-Diisopropylphosphorylethanolamine (3.0 g.) yielded 350 mg. of **ethanolaminephosphoric acid** (18.6%), melting at 242°.[10]

Anal. Calcd. for $C_2H_8O_4NP$: C, 17.03; H, 5.72; N, 9.93; P, 21.96. Found: C, 16.8; H, 5.6; N, 9.55 (Van Slyke), 9.65 (Kjeldahl); P, 22.10.

Attempts were made to isolate the O-diisopropylphosphorylated esters of serine and threonine by treatment of the corresponding N-phosphorylated isomer, in either methanol or dioxane, with gaseous hydrogen chloride gas. When these attempts proved unsuccessful, the organic solvent was removed under vacuum and the residue hydrolyzed with boiling aqueous hydrochloric acid. The yields of phosphoroamino acids were identical with those obtained by direct treatment of the phosphoramide with boiling aqueous acid. N-Diisopropylphosphoryl-*l*-(+)-cysteine methyl ester yielded only cysteine hydrochloride when subjected to treatment with gaseous and then boiling aqueous hydrochloric acid.

Acknowledgment.—The authors wish to express their sincere appreciation to Pfc. Patrick Tetta, of this Branch, and to the Analytical Branch, Chemical and Radiological Laboratories, Army Chemical Center, Md., for the microanalyses of the compounds encountered in this investigation.

(8) R. H. A. Plimmer (see footnote 4), reports a m.p. of 165–166° for this substance.

(9) R. H. A. Plimmer (see footnote 4), reports a m.p. of 169° for *dl*-threoninephosphoric acid. However, his material is a monohydrate. C. H. de Verdier, *Nature*, **170**, 894 (1952), reports a m.p. of 194° for *l*-threoninephosphoric acid isolated from bovine casein.

(10) E. L. Outhouse, *Biochem. J.*, **31**, 1454 (1937), reports a m.p. of 244° for this compound.

MEDICINAL CHEMISTRY BRANCH
CHEMICAL CORPS MEDICAL LABORATORIES
ARMY CHEMICAL CENTER, MD.

Some Pyrimidine Derivatives[1]

BY JOSEPH L. RABINOWITZ AND SAMUEL GURIN

RECEIVED JULY 10, 1953

During the course of an antimetabolite project to be reported elsewhere, several new pyrimidine derivatives were prepared. This note concerns their synthesis in addition to modifications or improvements in the preparation of a number of previously known substances.

Experimental[2]

(a) Thymine-1-acetic Acid.—To 12.6 g. (0.1 mole) of thymine and 9.6 g. (0.2 mole) of KOH in 75 ml. of H_2O was added slowly 7.85 g. (0.1 mole) of chloroacetic acid in 30 ml. of H_2O.[3] (The corresponding ester can be used with equal success.) The *p*H of the solution was adjusted to and kept at 10 by the dropwise addition of a KOH solution. After refluxing for two hours, the solution was cooled, and acidified to *p*H 2 by the addition of concd. HCl. The resulting precipitate was filtered, washed with a little cold water, dissolved in a saturated $KHCO_3$ solution and reprecipitated with HCl; crude yield 16 g. (*ca.* 85%); recrystallized *ca.* 50% yield, m.p. 260–261°.

Anal. Calcd. for $C_7H_8O_4N_2$: C, 45.65; H, 4.38; N, 15.21. Found: C, 45.64; H, 4.41; N, 15.21.

(b) 1,3-Diethylthymine.—To 13 g. (*ca.* 0.1 mole) of thymine in a solution of 10 g. of NaOH in 60 ml. of water, was added dropwise 30 ml. of ethyl sulfate.[4] The solution was stirred at room temperature for one hour, then kept stirring for another hour just below its boiling temperature. After cooling, the solution was extracted several times with $CHCl_3$; after drying the $CHCl_3$ with $MgSO_4$, it was filtered and evaporated to dryness. The resulting 1,3-diethylthymine can be recrystallized from petroleum–ethyl ether, m.p. 56–57°, b.p. 140–143° (7 mm.), yield 6.5 to 7.8 g. (*ca.* 40%).

Anal. Calcd. for $C_9H_{14}O_2N_2$: C, 59.31; H, 7.74; N, 15.37. Found: C, 58.99; H, 7.61; N, 15.27.

(c) 2,4-Diethoxy-5-nitro-6-methylpyrimidine.—To a cold mixture consisting of 15 ml. of red fuming nitric acid (d. 1.5) and 15 ml. of concd. H_2SO_4 was added slowly 2.5 g. (0.02 mole) of 2,4-diethoxy-6-methylpyrimidine.[12] The solution was kept at 80° for one hour, then poured onto cracked ice. The mixture was first neutralized with KOH, then acidified to *p*H 2 with HCl. The solution was chilled, filtered and the precipitate washed with cold water, the residue was extracted with 50 ml. of ether, decolorized with charcoal and treated with 50 ml. of MeOH. The ether was removed by warming on a water-bath and the remaining solution treated with cold water to faint turbidity. The suspension was chilled, and fine yellow needles collected. The compound sublimes, m.p. 38°, yield 2.7 g. (*ca.* 60%).

Anal. Calcd. for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.76; N, 18.49. Found: C, 47.93; H, 5.71; N, 18.53.

(d) 2,4-Diethoxy-5-nitropyrimidine.—Twenty-five grams (0.15 *M*) of 2,4-diethoxypyrimidine[5] was added dropwise to a mixture of 150 ml. of red fuming nitric and 150 ml. of concd. sulfuric acids (prepared by slow addition of chilled sulfuric to chilled nitric). After standing for one hour at room temperature, the solution was placed in warm water (60°) and stirred. The temperature was maintained at 60° for one hour. The solution was then cooled to room temperature and decomposed cautiously with 500 g. of cracked ice. After removal of the first crop by filtration, additional material was recovered from the filtrate by neutralization with concd. KOH to *p*H 7.5 followed by the addition of NaCl and chilling.

All of the precipitated material was combined, dissolved in hot absolute EtOH and decolorized with charcoal. Fine, pale yellow needles were obtained after chilling, m.p. 45°, yield 9.5–11 g. (*ca.* 30%).

Anal. Calcd. for $C_8H_{11}O_4N_3$: C, 45.06; H, 5.20; N, 19.71. Found: C, 44.96; H, 5.21; N, 19.64.

(e) 4-Methoxy-1,6-dimethyl-2-pyrimidone.—To a mixture of 3 g. of 2,4-dimethoxy-6-methylpyrimidine[6] and 2.1 ml. of methyl iodide a few drops of pyridine were added; after 24 hr. at room temperature a solid deposited. The solid was recrystallized from hot alcohol by the addition of absolute ether, yield 95%, m.p. 112.5°.

Anal. Calcd. for $C_7H_{10}O_2N_2$: C, 54.52; H, 6.54; N, 18.18. Found: C, 54.70; H, 6.55; N, 17.96. Upon hydrolysis with HCl 1,6-dimethyluracil was obtained.

(f) 1,3-Diethyl-6-methyluracil.[7]—A more convenient method of preparation involved the addition of 45 ml. of

(1) Supported by a grant of the Cancer Institute of the National Institutes of Health.

(2) All melting points are uncorrected. We wish to thank the Organic Research Laboratory of Sharp and Dohme, Inc., for most of the analyses reported in this paper.

(3) H. L. Wheeler and L. M. Liddle, THIS JOURNAL, **30**, 1152 (1908).

(4) P. A. Levene, L. W. Bass and H. S. Simms, *J. Biol. Chem.*, **70**, 229 (1926).

(5) G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 2004 (1930).

(6) S. Gabriel and J. Colman, *Ber.*, **32**, 2921 (1899).

(7) J. Hoffmann, *Ann.*, **253**, 68 (1889); M. Hagen, *ibid.*, **244**, 8 (1888); O. Heobel and R. Behrend, *ibid.*, **353**, 246 (1907); O. Buchendorff, *ibid.*, **385**, 314 (1911).