

for the biological assays, Dr. G. Hobby for the toxicity tests and the Microanalytical Department for the microanalyses and the chemical assays.

RESEARCH LABORATORIES
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Derivatives of N-Phosphorylated Amino Acids

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The problem of the synthesis of N-phosphorylated amino acids has received renewed attention since the recognition of the importance of phosphorylated intermediates in metabolic processes. In previous reports,^{1,2} there has been described the phosphorylation of amino acids by means of phosphorus oxychloride. The reaction was conducted in the presence of magnesium oxide and gave magnesium salts of the N-phosphoryl amino acids of approximately the correct composition.

It appeared desirable to examine the possibility of preparing such N-phosphorylated amino acids by the use of diphenylphosphoryl chloride,³ which reacts readily with amines to give the corresponding aminophosphonates. It was hoped that the reaction of this reagent with esters of α -amino acids would yield products which, upon saponification of the ester linkage and hydrogenolysis of the phenyl groups, would be converted to the desired N-phosphorylated amino acids. The direct reaction of the chloride with free amino acids in alkaline solution was avoided in view of the report⁴ that the products so obtained are not aminophosphonates but are rather diphenylphosphoric acid salts of amino acids.

As will be noted in the experimental section of this note, the reaction of diphenylphosphoryl chloride with the esters of glycine, of DL-phenylalanine, and of L-glutamic acid leads to the expected diphenylphosphoryl amino acid esters without difficulty. The further steps in the proposed synthesis of N-phosphoryl amino acids were less successful, however. The attempted saponification of the diphenylphosphoryl amino acid esters by treatment with 1.1 equivalents of sodium hydroxide in acetone solution for one hour at 25° led to the recovery of the unchanged esters. Since such treatment readily causes the saponification of acyl amino acid esters such as benzoyl-glycine ethyl ester, it would appear that the phosphorylation of the α -amino group has greatly increased the stability of the carboxylic ester linkage. More vigorous treatment with 4 equivalents of alkali either at 25° for two hours, or at 70° for fifteen minutes, gave mixtures of products which could not be separated satisfactorily. Furthermore, hydrogenolysis of diphenylphosphoryl-L-glutamic acid diethyl ester with platinum oxide, followed by treatment with sodium

methylate in absolute methanol, gave a product whose elementary composition approximated the theory for the disodium salt of N-phosphoryl-L-glutamic acid diethyl ester, rather than for the expected tetrasodium salt of N-phosphoryl-L-glutamic acid. This result may also be attributed to the stabilizing effect of the N-phosphoryl group on the ester linkage.

In the course of these studies, the reaction of dibenzylphosphoryl chloride⁵ with amino acid esters (*e. g.*, glycine benzyl ester) was investigated, but thus far has not yielded crystalline products. This work is being continued.

Experimental

Reaction of Diphenylphosphoryl Chloride with Amino Acid Esters.—The ester hydrochloride of the appropriate amino acid was dissolved in a minimal quantity of water and the free ester was liberated into ice-cold ethyl acetate with the calculated amount of 10 *N* sodium hydroxide. To the ethyl acetate solution, there was added diphenylphosphoryl chloride (one mole per mole of amino acid ester), and the acid formed during the reaction was neutralized with aqueous bicarbonate. The ethyl acetate layer was then washed successively with water, dilute hydrochloric acid, water, aqueous bicarbonate solution and water. After being dried over sodium sulfate, the solution was concentrated to a small volume under reduced pressure, and the reaction product was precipitated by the addition of petroleum ether (30–60°). Recrystallization was effected from ethyl acetate–petroleum ether.

Diphenylphosphorylglycine Ethyl Ester.—From 5 g. of glycine ethyl ester hydrochloride there was obtained 3.7 g. of the product; m. p. 77–78°.

Anal. Calcd. for C₁₆H₁₈O₆NP: C, 57.3; H, 5.4; N, 4.2; P, 9.2. Found: C, 57.4; H, 5.5; N, 4.1; P, 9.1.

Diphenylphosphoryl-DL-phenylalanine Ethyl Ester.—From 5 g. of DL-phenylalanine ethyl ester hydrochloride there was obtained 4.2 g. of the product, m. p. 78–79°.

Anal. Calcd. for C₂₃H₂₄O₆NP: C, 64.9; H, 5.7; N, 3.3; P, 7.3. Found: C, 64.9; H, 5.7; N, 3.4; P, 7.2.

Diphenylphosphoryl-L-glutamic Acid Diethyl Ester.—From 5 g. of L-glutamic acid diethyl ester hydrochloride there was obtained 2.4 g. of the product; m. p. 73.5–74°.

Anal. Calcd. for C₂₁H₂₆O₇NP: C, 57.9; H, 6.0; N, 3.2; P, 7.1. Found: C, 57.8; H, 6.0; N, 3.3; P, 7.0.

One gram of this substance was subjected to hydrogenolysis in dry methanol in the presence of platinum oxide until 8 moles of hydrogen had been taken up. The reaction required six hours, after which time the catalyst was removed by filtration. To the filtrate was added the calculated quantity of freshly prepared sodium methylate in dry methanol, and the solution was kept at 4° for four hours. Anhydrous ether was then added and the resulting precipitate (0.25 g.) was washed with methanol–ether and with ether.

Anal. Calcd. for C₉H₁₀O₇NPNa₂: C, 33.1; H, 4.9; N, 4.3; P, 9.5; Na, 14.1. Found: C, 34.8; H, 4.5; N, 4.4; P, 9.8; Na, 13.3.

(5) Atherton, Openshaw and Todd, *J. Chem. Soc.*, 382 (1945).

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
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The Melting Point of 4-Aminosalicylic Acid

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The melting point of 4-aminosalicylic acid (4-amino-2-hydroxybenzoic acid) is uncertain: 148°

- (1) Neuberger and Oertel, *Biochem. Z.*, **60**, 491 (1914).
- (2) Winnick and Scott, *Arch. Biochem.*, **12**, 201 (1947).
- (3) Brigl and Müller, *Ber.*, **72**, 2121 (1939).
- (4) Bernton, *ibid.*, **55**, 3361 (1922).

(dec.),¹ 149–151° (dec.),² 150–151° (dec.),³ 139–141° (dec.),⁴ and 220° (dec.).^{5,6} have been reported. The capillary melting point is influenced by the rate of heating⁷ and the temperature of the bath. To avoid these effects the melting point apparatus of Dennis and Shelton⁸ was used in this work. With this apparatus, the melting point is the lowest temperature of instantaneous melting. The grand average of five melting point values on each of five samples was 240°, with a standard error of approximately $\pm 1^\circ$. (Capillary melting points of about 147° (dec.) were obtained by immersion in a bath at 145° with a rate of heating of 3° per minute.) The poor precision is caused by inadequate contact with the surface of the bar and by convection currents which disperse fine particles into the air. With samples of optimum particle size range, the lowest temperature of instantaneous melting obtained by testing at progressively increasing temperatures was the same as the lowest temperature of instantaneous melting obtained by testing at progressively decreasing temperatures.

These conclusions may be reached: (1) The most nearly correct melting point is about 240°. (2) The melting point is not a good criterion of purity. (3) The melting point reported by Seidel and Bittner of 220° (dec.) was probably obtained with aminosalicic acid, rather than with the hydrochloride, as some have assumed,⁹ and must have been taken by some technique involving the observation of instantaneous melting.

- (1) Kolbe, U. S. Patent 427,564; German Patent 50,835.
- (2) Erlenmeyer, *et al.*, *Helv. Chim. Acta*, **31**, 988 (1948).
- (3) O'Connor, *Lancet*, **254**, 191 (1948).
- (4) Whittet, *ibid.*, **254**, 268 (1948).
- (5) Seidel, *Ber.*, **34**, 4351 (1901).
- (6) Seidel and Bittner, *Monatsh.*, **23**, 415 (1902).
- (7) McAnnally and Seymour, *Lancet*, **254**, 303 (1948).
- (8) Dennis and Shelton, *THIS JOURNAL*, **52**, 3128 (1930).
- (9) Sheehan, *ibid.*, **70**, 1665 (1948).

CALCO CHEMICAL DIVISION
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A New Synthesis of Diethyl 1,1-Cyclobutanedicarboxylate

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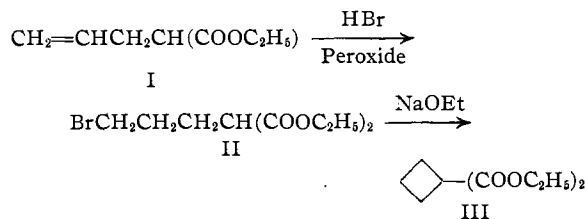
The standard method for preparation of diethyl 1,1-cyclobutanedicarboxylate (III), the reaction between trimethylene bromide and diethyl malonate, gives a yield of approximately 25%.² The major product is tetraethyl 1,1,5,5-pentanetetracarboxylate formed by further reaction of the intermediate II with diethyl malonate. To eliminate this side reaction, II was independently prepared by the addition of hydrogen bromide to diethyl allylmalonate (I)³ and then cyclized to

(1) Present address: Chemistry Department, Ohio State University.

(2) Heisig and Stodola, "Organic Syntheses," **23**, 16 (1943).

(3) Linstead and Rydon, *J. Chem. Soc.*, 582 (1933), report the preparation of I in 80% yield from allyl bromide and diethyl malonate.

III by treatment with sodium ethylate. The over-all yield from I is about 50%.



Experimental

Diethyl γ -Bromopropylmalonate (II).—A solution of 67 g. of I in an equal volume of toluene containing a small amount of dibenzoyl peroxide was saturated at 0° with gaseous hydrogen bromide. After removal of excess hydrogen bromide and toluene, the residue was distilled *in vacuo*. A fraction, 75 g. (79%), boiling over a 6° range was collected, b. p. ca. 140° at 5 mm., $n_{25}^{25}D$ 1.455. A portion of this material was allowed to stand for twelve hours with an excess of trimethylamine in benzene and the resulting quaternary salt was converted⁴ to the picrate, m. p. 102.5–103.5° from ethanol.

Anal. Calcd. for $\text{C}_{10}\text{H}_{20}\text{N}_4\text{O}_{11}$: C, 46.7; H, 5.8. Found: C, 47.0; H, 5.9.

An authentic sample⁵ of II, b. p. 139–141° at 5 mm., $n_{25}^{25}D$ 1.455, gave the same quaternary picrate, m. p. and mixed m. p. 102.5–103.5°.

Diethyl 1,1-Cyclobutanedicarboxylate (III).—To the refluxing solution of 5.8 g. of sodium dissolved in 600 ml. of absolute ethanol was added slowly with stirring 72 g. of II. After refluxing for two and one-half hours the solvent was removed *in vacuo* and the residue was treated with water; the product was taken up in ether and distilled at 23 mm., b. p. 119–126°, $n_{25}^{25}D$ 1.433,⁶ yield 38 g. (74%).

This work was performed under a contract between the Office of Naval Research and the California Institute of Technology.

(4) Cf. Howton, *THIS JOURNAL*, **69**, 2555 (1947), footnote 5.

(5) Willstätter and Etilinger, *Ann.*, **326**, 99 (1903).

(6) This is the value given in the literature for III, Gladstone cited by Perkin, *J. Chem. Soc.*, **51**, 4 (1887).

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The Synthesis of Some 4-Alkoxy-7-chloroquinaldic Acid Derivatives

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Although the 2-alkoxycinchoninic acid derivatives¹ are known to possess strong local anesthetic activity, the corresponding quinoline derivatives in which substituents in the 2- and 4-positions are interchanged have not been reported in the literature. Inasmuch as a suitable starting material, ethyl 7-chloro-4-hydroxyquinaldate, was available in this Laboratory, it seemed desirable to prepare the 4-alkoxy derivatives in order to compare their physiological activity with the corresponding 2-alkoxy compounds.

Accordingly, 7-chloro-4-hydroxyquinaldic acid and its ethyl ester were treated with phosphorous oxychloride to give the corresponding 4-chloro compounds, I and II (Table I), respectively.

(1) Miescher, *Helv. Chim. Acta*, **15**, 163 (1932).