derivatives of 1,2,3- and 1,2,4-trimethylbenzene a mixed melting point showed a slight depression; however, the degree of depression was not sufficient to permit a definite conclusion as to whether rearrangement of the hydrocarbon had or had not occurred.

In this series as in the previously reported one, several derivatives were observed which had at least two crystalline modifications. This phenomenon was exhibited by the tetrachlorophthalic anhydride derivatives of benzene, 1,2,4-trimethylbenzene, 1,3-diethylbenzene and 1,3,5-triethylbenzene.

### Experimental

The starting materials and procedures were the same as in the previous work with the following exceptions: (1) The tetrachlorophthalic anhydride used was Eastman Kodak Co. practical grade. (2) The *o*-aroyltetrachlorobenzoic acids were recrystallized by dissolving them in a small volume of hot glacial acetic acid and adding sufficient water to cause precipitation as the solution cooled. (3) The percentage chlorine was determined by burning the acid in a Parr oxygen bomb and then titrating with silver nitrate and potassium thiocyanate using ferrous ammonium sulfate as an indicator.

**Acknowledgment.**—The authors would like to thank Mr. A. E. Spakowski for his valuable assistance in the chlorine determinations.

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## The Preparation of N-Dialkylphosphorylated Glycine

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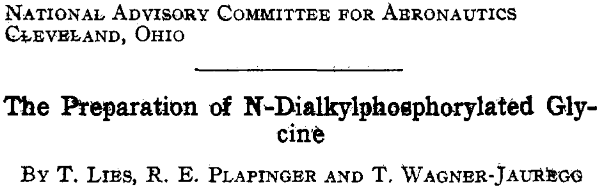
Various N-dialkylphosphorylated amino acid esters have been described recently.[1-3] Attempts to saponify the carboxylic ester group in compounds of this type have been unsuccessful.[1,3] Therefore it has been claimed that the phosphorylation of the α-amino group greatly increases the stability of the carboxylic ester linkage.[1]

By saponification of the esters of N-dialkylphosphorylglycine with one equivalent of barium hydroxide at room temperature we have been able to obtain the corresponding monobarium salts. These were transformed for further characterization into the crystalline guanidine salts.

The question arises as to whether the barium salts formed have the structure I or Ia.

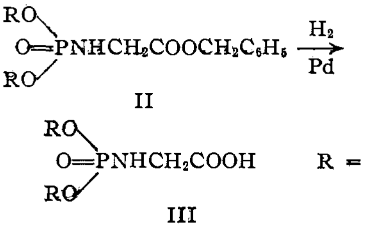

$$(RO)_2P(=O)NHCH_2COOR' \xrightarrow{{}^1/_2Ba(OH)_2} (RO)_2P(=O)NHCH_2COOBa^1/_2 \text{ or } (RO)(Ba^1/_2O)P(=O)NHCH_2COOR'$$

(I) (Ia)

When R and R′ are different alkyl groups (for instance R = butyl or isopropyl and R′ = methyl or ethyl) it was possible to exclude structure Ia as a possible product by elementary analysis of the guanidine salts. In the case where both R and R′ are ethyl groups the isolation of a product corresponding to structure I could be established by the absence of a positive hydroxamate test, a reaction characteristic of carboxylic acid esters.

By treatment of an aqueous solution of the guanidine salt of N-diethylphosphorylglycine with an ion-exchange resin the corresponding free acid can be liberated. Its titration curve indicates a $pK_a$ of 3.8, which is in agreement with the expected value. The free acid corresponding to the barium salt of the structure Ia should have a lower $pK_a$. The aforementioned results demonstrate that the assumption of a greatly increased stability of the carboxylic ester linkage in N-dialkylphosphorylated α-amino acid esters is unjustified.[4]

N-Diethyl- and N-diisopropylphosphorylglycines (III) also were prepared from the corresponding benzyl esters II by hydrogenolysis.


$$(RO)_2P(=O)NHCH_2COOCH_2C_6H_5 \xrightarrow[Pd]{H_2} (RO)_2P(=O)NHCH_2COOH$$

II

III

R = ethyl or isopropyl

No well-defined product could be obtained in the attempted hydrolysis of N-diphenylphosphorylglycine ethyl ester with barium hydroxide. Since the odor of phenol can be detected after addition of 0.5 mole of $Ba(OH)_2$ a hydrolytic attack on the O–P linkage has to be assumed.

The N-dialkylphosphorylglycine esters used for this investigation were prepared by the reaction of glycine esters with dialkylphosphoryl chlorides. N-Diethylphosphorylglycine ethyl ester (IV) is formed also during the reaction of glycine ester with tetraethyl pyrophosphate (TEPP). Previously[2] the interaction of these substances had been tentatively formulated as yielding N,N-bis-diethylphosphorylglycine ester, $H_5C_2O_2CCH_2N[PO(OC_2H_5)_2]_2$ (V). However, material presented in the Experimental Part of this paper makes it evident that a mixture of approximately two moles of IV with one mole of TEPP is obtained. This correction eliminates an apparent exception to the reaction of TEPP with amino compounds. It demonstrates that in the case of glycine ester also, the ordinary monophosphorylation takes place with a tetralkyl pyrophosphate, as originally shown by Atherton and Todd.[5]

### Experimental

**N-Diethylphosphorylglycine Ethyl Ester (IV).**—This substance was obtained by treating glycine ester with diethylphosphoryl chloride in a manner analogous to that of the reaction of diisopropylphosphoryl chloride with amino acid esters.[2] The product obtained had a boiling point of 123–128° (0.3 mm.), $n^{30}D$ 1.4338.

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[1] L. J. Sciarini and J. S. Fruton, THIS JOURNAL, 71, 2940 (1949).

[2] T. Wagner-Jauregg, J. J. O'Neill and W. H. Summerson, *ibid.*, 73, 5202 (1951).

[3] Si-Oh Li, *ibid.*, 74, 5959 (1952).

[4] N-Diisopropylphosphorylglycine methyl ester produced one equivalent of acid with a commercial horse serum at *p*H 7.6 and 38°, while glycine ethyl ester remained unattacked. We did not investigate whether this hydrolysis was due to the presence of an esterase or a phosphatase.

[5] F. R. Atherton and A. R. Todd, *J. Chem. Soc.*, 674 (1947).

*Anal.* Calcd. for $C_8H_{18}O_5NP$ (239.3): N, 5.9; P, 13.0. Found: N, 5.43; P, 13.3.

**Barium Salt of N-Diethylphosphorylglycine** (I, R = $C_2H_5$).—To 2.2 g. (0.009 mole) of N-diethylphosphorylglycine ethyl ester was added a solution of 0.77 g. (0.0045 mole) of barium hydroxide in 18 ml. of water. The *p*H of the resulting solution fell to 5.5 after about one-half hour at room temperature. The solvent was evaporated off under vacuum. The resulting sirup was dissolved in a small quantity of absolute ethanol. Fractional precipitation with dry ether yielded the salt as a finely divided white solid, which was collected by centrifugation and dried at 100° in a vacuum over phosphorus pentoxide.

*Anal.* Calcd. for $C_{12}H_{26}O_{10}N_2P_2Ba$: $OC_2H_5$, 32.3; N, 5.0; P, 11.1; Ba, 24.6. Found: $OC_2H_5$, 30.6; N, 4.8; P, 11.1; Ba, 25.8.

**Guanidine Salt of N-Diethylphosphorylglycine.**—To 2.4 g. (0.01 mole) of N-diethylphosphorylglycine ethyl ester was added a filtered solution of 0.85 g. (0.005 mole) of barium hydroxide in 20 ml. of water. The *p*H of the resulting solution dropped instantly to 8, and after five minutes the solution was approximately neutral. Addition of 0.9 g. (0.005 mole) of guanidine carbonate, $(CH_5N_3)_2H_2CO_3$, in a small amount of water precipitated barium carbonate, which was filtered off. Evaporation of the filtrate under vacuum to dryness yielded a solid, which was washed with acetone and recrystallized 3 times from absolute ethanol. Dried over phosphorus pentoxide in a vacuum the colorless crystals melted at 159–160°, yield 51%.

*Anal.* Calcd. for $C_7H_{19}O_5N_4P$ (270.2): P, 11.2; N, 20.7; C, 31.1; H, 7.1; $OC_2H_5$, 33.3. Found[6]: P, 11.8; N, 20.9; C, 31.7; H, 7.0; $OC_2H_5$, 31.8.

**Guanidine Salt of N-Diisopropylphosphorylglycine.**—(a) Prepared in the same manner as above from N-diisopropylphosphorylglycine ethyl ester[2] the salt melted at 167–168.5° after one recrystallization from anhydrous ethanol and vacuum drying over phosphorus pentoxide, yield about 50%.

*Anal.* Calcd. for $C_9H_{23}O_5N_4P$ (298.3): P, 10.4; N, 18.8; C, 36.2; H, 7.8; $OC_3H_7$, 39.5. Found: P, 10.9; N, 19.2; C, 36.2; H, 7.8; $OC_3H_7$, 38.6.

(b) The guanidine salt of N-diisopropylphosphorylglycine prepared from N-diisopropylphosphorylglycine methyl ester (b.p. 114–120° (0.1–0.2 mm.), $n^{27}$D 1.4314) melted at 166–167°.

*Anal.* Calcd. for $C_9H_{23}O_5N_4P$ (298.3): P, 10.4; N, 18.8; C, 36.2; H, 7.8; $OC_3H_7$, 39.5. Found: P, 10.3; N, 19.3; C, 35.2; H, 7.3; $OC_3H_7$, 38.5.[7]

**Guanidine Salt of N-Dibutylphosphorylglycine.**—The hydrolysis of N-dibutylphosphorylglycine methyl ester (b.p. 145–147° (0.15 mm.), $n^{26}$D 1.4392) with 0.5 mole of $Ba(OH)_2$ was best accomplished in the cold. The guanidine salt melted at 156.5–157° after recrystallization from ethanol and ether.

*Anal.* Calcd. for $C_{11}H_{27}O_5N_4P$ (326.3): C, 40.4; H, 8.33; N, 17.15; P, 9.48; $OC_4H_9$, 45.7. Found: C, 39.8; H, 8.1; N, 17.2; P, 9.6; $OC_4H_9$, 44.1.

**N-Diisopropylphosphorylglycine Benzyl Ester** (II, R = (II, R = Isopropyl).—Glycine benzyl ester was liberated from 5.0 g. of its hydrochloride[8] by neutralization with triethylamine. To a cooled solution of the free base in 10 cc. of dry ether was added a solution of 5.0 g. of diisopropyl chlorophosphate in 5 cc. of dry ether with stirring. An immediate reaction took place in the course of which triethylamine hydrochloride was precipitated. After stirring for 1 hour the triethylamine hydrochloride (3.0 g.) was removed by filtration and the ether solution concentrated. A yellow oil (7.6 g.) remained which did not solidify when kept at 20° overnight. This material gave a faint test for halogen. Extraction of an ethereal solution of this material with ice-water removed the halogen.

*Anal.* Calcd. for $C_{15}H_{24}O_5NP$: N, 4.24; P, 9.38. Found: H, 4.30; P, 9.40.

**Debenzylation of N-Diisopropylphosphorylglycine Benzyl Ester.**—Diisopropylphosphorylglycine benzyl ester (3.0 g.) in 100 ml. of absolute alcohol containing 3.0 g. of 5% Pd–C catalyst was catalytically hydrogenated at a pressure of 60 lb./in.[2] for 36 hours at room temperature. Removal of the catalyst by filtration and concentration of the alcohol yielded a sirup. This material was dissolved in cold aqueous sodium bicarbonate and the water solution extracted with ether. Acidification of the aqueous solution in the cold, followed by extraction with ether and drying, yielded the product, a colorless viscous oil which was strongly acidic (III, R = isopropyl).

*Anal.* Calcd. for $C_8H_{18}O_5NP$: C, 40.17; H, 7.58; N, 5.85; P, 12.95; equiv. wt., 239.2. Found: C, 40.3; H, 7.4; N, 5.3; P, 13.2; equiv. wt. (by titration with NaOH), 239.7.

**Diethylphosphorylglycine Benzyl Ester** (II, R = Ethyl).—*Anal.* Calcd. for $C_{13}H_{20}O_5NP$: N, 4.64; P, 10.28. Found: N, 4.75; P, 10.18.

**Diethylphosphorylglycine** (III, R = Ethyl).—*Anal.* Calcd. for $C_6H_{14}O_5NP$: N, 6.63; P, 14.67; equiv. wt., 211.2. Found: N, 6.75; P, 14.80; equiv. wt. (by titration with NaOH), 211.5, 212.2.

The titration curve of an aqueous solution of this acid gave a $pK_a$ 3.85, identical with that of a sample obtained from the guanidine salt by treatment with Dowex-50. On standing of the solution a change in the titration curve could be observed, first noticeable after 4 hours, and complete within 22 hours. This demonstrates that N-diethylphosphorylglycine slowly hydrolyses.

**Interaction of Glycine Ethyl Ester with Tetraethyl Pyrophosphate (TEPP).**—A substance with the formula V and a 2:1 mixture of N-diethylphosphorylglycine ethyl ester (IV) and TEPP have almost the same analytical composition so that no exact distinction is possible by elementary analysis. Since the boiling points of IV and TEPP are almost the same at reduced pressure, a separation by distillation is not possible. Differentiation between V and a 2:1 mixture of IV and TEPP could be made in favor of the latter mainly by a determination of the molecular weight. The comparison of the reaction product (R) of glycine ethyl ester with TEPP described previously[2] with a mixture (M) prepared from two moles of N-diethylphosphorylglycine ethyl ester (IV) and one mole of TEPP gave the values

| Reaction product | Mixture |
|---|---|
| Boiling point 120–125° (0.5 mm.) | 124–125° (0.5 mm.) |
| Refractive index $n^{28}$D 1.4275–1.4290[2] | $n^{28}$D 1.4281 |
| Molecular weight[9] 270.4 | 267.8; calcd. for M, 256 (calcd. for V, 375) |
| $LD_{50}$ for rabbits[10] 0.17 mm.$^3$/kg. (i. v.), in propylene glycol | 0.2 mm.$^3$/kg. (i. v.), in propylene glycol |

For an aqueous solution of R an $LD_{50}$ = 1 mg./kg. rabbit has been reported.[2] Since R is a mixture of IV and TEPP, the low toxicity found previously was due to the rapid hydrolysis of TEPP when water, rather than propylene glycol, was used as the solvent.

**Acknowledgment.**—The diethyl and dibutyl chlorophosphates used were kindly furnished by Victor Chemical Works, Chicago 4. The analytical determinations were performed by Cpl. Patrick Tetta of this Branch and by the Analytical Branch, Cml C Chemical and Radiological Laboratories. We wish to thank Mr. B. E. Hackley, Jr., for the manometric determination of the enzymatic hydrolysis of N-diisopropylphosphorylglycine methyl ester.

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[6] All CH determinations were obtained by combustion in the presence of $V_2O_5$; compare T. Wagner-Jauregg and H. Griesshaber, *Ber.*, **70**, 1458 (1937).

[7] The low analytical values found for carbon and alkoxy probably indicate that hydrolysis of the phosphate ester linkage took place to a certain extent.

[8] H. K. Miller and H. Waelsch, THIS JOURNAL, **74**, 1092 (1952).

[9] Determined by a modified Signer method; see C. A. Rush, J. J. Schrock and D. H. Rosenblatt, Abstracts 123rd Meeting Am. Chem. Soc., Los Angeles, Calif., March, 1953, page 5B.

[10] Determined by the Field Toxicology and the Pharmacology Branches, Cml C Med Labs.