bonation to the corresponding α-naphthoic acid. A mixture of 4.14 g. (0.02 mole) of α-bromonaphthalene and diethylstrontium, prepared from 0.02 g. atom of strontium metal and 0.02 mole of diethylzinc in 10 cc. of benzene, was refluxed two hours before carbonation. The small quantity of α-naphthoic acid obtained after working up according to the usual procedure melted at 157–159° after crystallization from dilute ethanol.

Reaction with Carbon Dioxide.—A mixture of 12 g. (0.097 mole) of diethylzinc, and 6.6 g. (0.075 g. atom) of finely rasped strontium metal in 20 cc. of benzene, was refluxed with stirring for four hours. The resulting suspension was carbonated by pouring onto a petroleum ether–crushed carbon dioxide slurry. The products were worked up in the customary manner, and the ensuing propionic acid isolated as the silver propionate. The yield was 2.2 g. (16.3%). The silver salt was converted to sodium propionate which was treated with *p*-phenylphenacyl bromide to give the corresponding ester (m. p. and mixed m. p. 98.5–100°).

Reaction with Benzonitrile.—The formation of a normal reaction product (propiophenone) from diethylstrontium and benzonitrile[8] indicates that organostrontium compounds will react readily with other functional groups.

Summary

Diethylstrontium has been prepared from strontium and diethylzinc.

In confirmation of an earlier prediction that organostrontium compounds would have the general high reactivity of organoalkali compounds, it has been shown that diethylstrontium adds to the olefinic linkage in 1,1-diphenylethylene.

Metalation of dibenzothiophene by diethylstrontium involves the 4-position. This result was unexpected in view of an earlier observation that the very closely related phenylcalcium iodide metalates dibenzothiophene in the 3-position.

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(8) Entemann and Johnson, This Journal, 55, 2900 (1933).

Ames, Iowa Received October 30, 1942

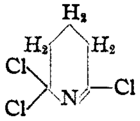
[Contribution No. 314 from the Department of Chemistry of the University of Texas]

Reaction of Glutarimides with Phosphorus Pentachloride. A New Pyridine Synthesis

By W. W. Crouch and H. L. Lochte

Recent studies in this Laboratory of the nitrogen bases from petroleum distillates have led to the isolation of certain compounds which appear to be β-substituted pyridines. Since no reliable method is known for the synthesis of these compounds, it seemed desirable to attempt to find a general procedure by which they could be made. A promising approach appeared to be the reaction of properly substituted glutarimides with phosphorus pentachloride followed by the elimination of the elements of hydrogen chloride to form chloropyridines from which the chlorine atoms could be removed by hydrogenation.

The corresponding reaction of phosphorus pentachloride with succinimide was first studied by Bernthsen,[1] who pointed out that the reaction with glutarimide should yield chloro compounds which could be reduced with zinc dust to form pyridine. A careful study of the reaction with succinimide and related compounds was made by Anschütz[2] and co-workers who likewise made a brief study of the reaction with glutarimide but reported that this reaction was not so simple as that of succinimide. The reaction of glutarimide with phosphorus pentachloride was studied by Bernheimer,[3] who isolated a crystalline compound to which he assigned structure I. While a compound of this structure would be expected to be hydrolyzed with ease, Bernheimer reported that his compound could be purified by steam distillation.



I

This reaction has now been repeated and a steam-distillable crystalline compound similar to that described by Bernheimer was isolated, but the formula of our compound was found to be $C_5H_2NCl_3$ instead of $C_5H_6NCl_3$. Its hydrogenation to form pyridine showed that it was a trichloropyridine, indicating that the elimination of hydrogen chloride had occurred spontaneously during the reaction. Since its formation from glutarimide requires that two of its chlorine atoms be bonded to carbon atoms 2 and 6 of the pyridine ring, only two molecular structures are possible for the trichloropyridine. Its properties

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(1) Bernthsen, *Ber.*, 13, 1047 (1880).

(2) Anschütz, *Ann.*, 295, 27 (1897).

(3) Bernheimer, *Gazz. chim. ital.*, 12, 283 (1882).

are not those of 2,4,6-trichloropyridine[4]; it must therefore be 2,3,6-trichloropyridine. Since the α-hydrogen atoms of open chain amides are readily replaced by chlorine on treatment with phosphorus pentachloride while the β-hydrogen atoms are not affected by this treatment, it was not surprising to find that the third chlorine atom was bonded at position 3 rather than 4 of the pyridine ring.

2,3,6-Trichloropyridine has not been identified previously, but a trichloropyridine having the properties of this compound was isolated by Sell and Dootson[5] as one of the products of the reaction of phosphorus pentachloride with pyridine.

The reactions of phosphorus pentachloride with α-methyl- and α,α′-dimethylglutarimide were also studied and were likewise found to proceed readily to form crystalline products closely resembling 2,3,6-trichloropyridine. Their molecular formulas of $C_6H_4NCl_3$ and $C_7H_7NCl_2$, respectively, indicated that the expected 2,5,6-trichloro-3-picoline and 2,6-dichloro-3,5-lutidine had been formed by these reactions.

The chloropyridines are low-melting solids having similar, agreeable odors. They are steam distilled and sublimed with remarkable ease. They are insoluble in cold dilute or concentrated hydrochloric acid but dissolve in concentrated nitric acid from which they are precipitated on diluting the solution.

Experimental

2,3,6-Trichloropyridine.—The following modification of Bernheimer's procedure was used in preparing this compound. Five and one-half grams of glutarimide was mixed with 30 g. of phosphorus pentachloride. A reaction began at once, hydrogen chloride was evolved and a viscous liquid formed. It was heated slowly to 50° and was kept at that temperature for fifteen minutes after which it was poured in shallow watch glasses and allowed to evaporate in a hood. After a few minutes a crystalline residue formed. It was washed into a beaker with ice-water and was purified to a constant melting point of 66–67° by recrystallization from dilute ethanol and dilute acetic acid.

Anal. Calcd. for $C_5H_2NCl_3$: C, 32.91; H, 1.10; Cl, 58.30. Found: C, 32.80; H, 1.15; Cl, 58.30.

The same compound was obtained when only two moles of phosphorus pentachloride per mole of glutarimide were used but one-third of the glutarimide was recovered unchanged at the end of the reaction by the addition of petroleum ether as described by Bernheimer.

Hydrogenation of 2,3,6-Trichloropyridine.—A methanol–hydrochloric acid solution containing 2 g. of the trichloropyridine was hydrogenated at atmospheric pressure and room temperature using a catalyst made from the reduction of 0.1 g. of palladium chloride in the presence of 1 g. of acid-washed charcoal.[6] After shaking for five hours, three moles of hydrogen had reacted. The catalyst was filtered off and the solution was concentrated, made basic with sodium hydroxide solution and steam distilled. The odor of pyridine was clearly perceptible in the distillate. On addition of an aqueous solution of picric acid, 2.2 g. of pyridine picrate was recovered and recrystallized once to have a melting point and mixed melting point of 167–168°. The chloroplatinate, m. p. 242°, was made in the same way by the addition of chloroplatinic acid to the aqueous solution of the base.

Anal. Calcd. for $C_{10}H_{12}N_2Cl_6Pt$: Pt, 34.36. Found: Pt, 34.39.

α-Methylglutarimide.—By a modification of the procedure of Howles, Thorpe and Udall[7] for the preparation of this compound, 56.5 g. of ethyl cyanoacetate was added to 11.5 g. of sodium dissolved in 400 cc. of absolute alcohol. Fifty grams of methyl methacrylate[8] was added to this product with vigorous shaking and the mixture was refluxed for one hour. The alcohol was evaporated under reduced pressure, 250 cc. of water was added and the basic solution was extracted twice with ether to remove unreacted neutral esters. The residue was acidified and the methyl hydrogen ester of 2-methyl-4-cyanoglutaric acid was extracted from the aqueous solution with ether. It was recovered and heated in an oil-bath to 220°, at which temperature a vigorous evolution of carbon dioxide occurred. The residue was distilled yielding 24 g. of methyl 2-methyl-4-cyanobutyrate boiling at 157–160° at 58 mm. pressure. It was refluxed for three hours in 180 g. of 50% sulfuric acid from which it was recovered by extracting with ether and hydrolyzed further by refluxing for two hours in 80 g. of 25% sodium hydroxide solution. Acidification of this solution followed by extraction with ether resulted in the recovery of 21 g. of crystalline 2-methylglutaric acid melting, after two recrystallizations from benzene, at 75–76°. A melting point of 76° is given for this compound in the literature.[7]

Seventeen grams of the acid was converted to the anhydride by refluxing for one hour with 40 g. of acetyl chloride, after which the excess acid chloride and acetic acid were removed by heating on a steam-bath under reduced pressure. Dry ammonia was passed into the anhydride at 120° for one-half hour and the product was heated to 200° to split out water and form the imide which was then distilled at 5 mm. pressure. Fourteen grams of crude α-methylglutarimide was obtained which crystallized in the receiver. It was purified by recrystallization from benzene–petroleum ether and melted at 91°.

Anal. Calcd. for $C_6H_9O_2N$: N, 11.02. Found: N, 11.21.

2,5,6-Trichloro-3-picoline.—This compound was obtained from the reaction of three moles of phosphorus pentachloride with α-methylglutarimide as described above. The purified product melted at 94–95°.

Anal. Calcd. for $C_6H_4NCl_3$: C, 36.68; H, 2.05; Cl, 54.14. Found: C, 36.41; H, 2.04; Cl, 53.60.

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[4] Graf, *J. prakt. Chem.*, **133**, 36 (1932).

[5] Sell and Dootson, *J. Chem. Soc.*, **73**, 439 (1898).

[6] Stevens, Beutel and Chamberlin, THIS JOURNAL, **64**, 1095 (1942).

[7] Howles, Thorpe and Udall, *J. Chem. Soc.*, **77**, 942 (1900).

[8] Kindly donated by E. I. du Pont de Nemours Co.

α,α′-Dimethylglutarimide.—Twenty-five grams of methyl methacrylate was condensed with ethyl sodiocyanoacetate as described above and the alcoholic solution of the product was refluxed for ten hours with 40 g. of methyl iodide.[7] Five grams of water was added, then 70 g. of solid potassium hydroxide was introduced and dissolved by refluxing the solution. After refluxing for six hours, the alcohol was completely removed by evaporating to dryness under reduced pressure followed by the addition of 50 cc. of water and another evaporation to dryness. An aqueous solution of the residue was extracted with ether, acidified and again extracted with ether. Evaporation of the second ether extract yielded a crude acid which solidified on standing and was pressed on a porous plate to yield 11.5 g. of impure crystals. No solvent was found from which this acid could be recrystallized readily, but it is believed to have been the dicarboxylic acid formed by decarboxylation of the tricarboxylic acid during the alkaline hydrolysis, since it apparently evolved no carbon dioxide when heated to 230°. Without further purification it was converted to the imide by treatment with acetyl chloride and ammonia as described above. The α,α′-dimethylglutarimide was purified by sublimation and recrystallization to the reported melting point of 172–174°; yield, 4 g.

Anal. Calcd. for $C_7H_{11}O_2N$: N, 9.92. Found: N, 9.90.

2,6-Dichloro-3,5-lutidine.—The reaction of α,α′-dimethylglutarimide with three moles of phosphorus pentachloride occurred readily at room temperature as with the other imides described above except that some unreacted phosphorus pentachloride was left in the mixture at the end of the reaction. The product was recrystallized from dilute acetic acid to a constant melting point of 97–98°. Neither this compound nor 2,5,6-trichloro-3-picoline gave a precipitate with cold or hot alcoholic silver nitrate solution, thus excluding the possibility of side chain halogenation.

Anal. Calcd. for $C_7H_7NCl_2$: Cl, 40.28; N, 7.96. Found: Cl, 40.12; N, 8.09.

Summary

The reaction of phosphorus pentachloride with glutarimide has been shown to occur with the spontaneous evolution of hydrogen chloride to form 2,3,6-trichloropyridine which was subsequently hydrogenated to pyridine.

Similar reactions with α-methyl- and α,α′-dimethylglutarimide with phosphorus pentachloride yielded the corresponding pyridine homologs containing three and two atoms of chlorine, respectively. Based on the earlier reaction they were assigned structural formulas of 2,5,6-trichloro-3-picoline and 2,6-dichloro-3,5-lutidine.

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[CONTRIBUTION FROM THE HENRY PHIPPS INSTITUTE OF THE UNIVERSITY OF PENNSYLVANIA]

Proteins of Tuberculin

BY FLORENCE B. SEIBERT[1,2] AND J. WALTER NELSON

Introduction

The purification and isolation of the tuberculin protein has been difficult for many reasons. In the first place, two colloidal impurities, neither of which has proved to be responsible for eliciting or even contributing to the tuberculin skin reaction, are present in considerable quantities. These are nucleic acid and polysaccharide. Methods have, however, been found[3] for removing them practically completely from the protein with no significant loss in the potency of the protein. The remaining protein is far from being a molecularly homogeneous substance, as can be demonstrated by means of the ultracentrifuge, diffusion and electrophoresis. It is the purpose of this paper to discuss the evidence for the presence of at least two proteins with different properties in this protein mixture.

Experimental Technique.—The electrophoretic technique of Tiselius[4] has been extremely useful in demonstrating the presence of these proteins and in guiding the procedures for isolating them. All mobilities were determined in phosphate buffer, *p*H 7.6 to 7.7, $\mu = 0.1$, and a potential gradient of 9 to 10 volts per cm. Mobilities are recorded in units of cm.^{2} volt^{-1} sec.$^{-1}$.

Evidence for Several Proteins in Tuberculin.—Two or more proteins can be demonstrated to be present in all tuberculins, whether or not the original culture has been heated. Their relative proportions, as can be demonstrated by the electrophoretic diagram, depend upon the source and manner of preparing the tuberculin.

For example, Fig. 1 shows three typical types

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(1) Aided by a grant from the Committee on Medical Research of the National Tuberculosis Association.

(2) The data in this paper were used in an address given before the American Chemical Society at Buffalo, New York, on September 10, 1942, on the occasion of the awarding of the Francis P. Garvan gold medal.

(3) F. B. Seibert and J. T. Glenn, *Am. Rev. Tuberc.*, **44**, 9 (1941).

(4) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).