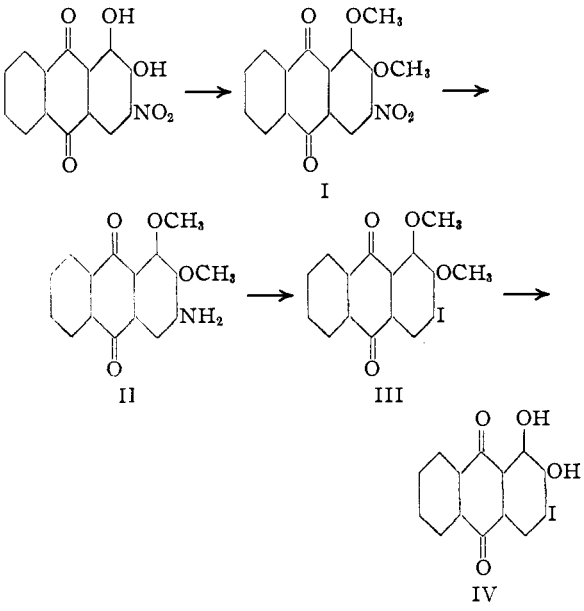

methylation of III gave the desired iodoalizarin which corresponded in properties to the compound prepared originally by Perkin.[1]

Experimental

1,2-Dimethoxy-3-nitroanthraquinone (II).—To a stirred suspension of 45 g. of 3-nitroalizarin in one liter of ethanol there was added a solution of 17.7 g. of potassium hydroxide in 400 ml. of ethanol. The dark precipitate was filtered off, washed with ethanol and dried. The salt was pulverized, leached with boiling ethanol and filtered. After drying at 100° the product weighed 57 g. It was suitable for the methylation step.

A suspension of 5.8 g. of the dry potassium salt and 32 g. of anhydrous sodium carbonate in 26 ml. of dimethyl sulfate was warmed to 140° in the course of forty-five minutes and held there for one-half hour. After cooling to room temperature the mixture was washed with water and the semi-solid residue taken up in boiling benzene. The extract was filtered from a small amount of insoluble material, concentrated and then treated with ligroin. On cooling, the ether separated. The crystals were removed by filtration and recrystallized from the same solvent pair to give 2.4 g. of yellow needles, m. p. 165–167° (lit. val. 168–171°). Runs five times the size gave comparable yields.

3-Amino-1,2-dimethoxyanthraquinone.—The reduction of the nitro compound was carried out according to the method of Perkin and Story.[3] From 10 g. of the nitro body we obtained 6.0 g. of the amine, m. p. 204°, after recrystallization from benzene.

1,2-Dimethoxy-3-iodoanthraquinone.—Six grams of the aminoanthraquinone was dissolved in 400 ml. of acetic acid with gentle warming. Then 27.6 ml. of 0.1 *N* hydrochloric acid and 140 ml. of water was added and the suspension cooled to 5°. Diazotization was carried out with the aid of 1.6 g. of sodium nitrite in 30 ml. of water. After two hours the solution was filtered and added to potassium iodide solution (8.5 g. in 100 ml. of water). The mixture was warmed to 60°, kept there for thirty minutes and filtered. After crystallization from ethanol the product melted at 165–167°; wt., 6.0 g.

Anal. Calcd. for $C_{16}H_{11}IO_4$: I, 32.2. Found: I, 31.3.

1,2-Dihydroxy-3-iodoanthraquinone (3-Iodoalizarin).—A quantity of 7.3 g. of the above ether was demethylated according to the method of Perkin and Story.[1] There was obtained 4.3 g. of pure 3-iodoalizarin which melted at 228.6–229.7° (cor.) after two crystallizations from xylene. Perkin[1] reported 227–229° as the melting point for the compound.

Anal. Calcd. for $C_{14}H_7IO_4$: I, 34.67. Found: I, 34.44.

STERLING-WINTHROP RESEARCH INSTITUTE
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Crystalline Naphthalene-β-sulfonates of Streptomycin and Dihydrostreptomycin

BY PETER P. REGNA AND R. A. CARBONI

Several crystalline derivatives of streptomycin have been reported, namely, the reineckate sulfate,[1] helianthate,[2] calcium chloride double salt,[3] and the trihydrochloride.[4] We have prepared crystalline naphthalene-β-sulfonates of streptomycin and dihydrostreptomycin which are sparingly soluble in water, but very soluble in methyl alcohol. Both salts crystallize from water in fine needles. Under similar conditions, the naphthalene α-sulfonate salt of streptomycin and dihydrostreptomycin showed no tendency to crystallize.

The streptomycin and dihydrostreptomycin naphthalene-β-sulfonates were prepared by dissolving 2.5 g. of the amorphous sulfate in 10 ml. of water. An equal amount of naphthalene-β-sulfonic acid was dissolved in 10 ml. of water. Both solutions were warmed to 40°, mixed, and the solution was neutralized to *p*H 6.5 with a saturated solution of barium hydroxide. The precipitate was filtered, and the filtrate was allowed to crystallize.

When the white streptomycin naphthalene-β-sulfonate was dried for three hours at 100° *in vacuo*, it gave the following analysis: Calcd. for $C_{21}H_{39}N_7O_{12}\cdot 3C_{10}H_7SO_3H$: C, 50.78; H, 5.26; N, 8.12; S, 7.97. Found: C, 50.69; H, 5.54; N, 8.45; S, 8.35. The anhydrous material gave $[\alpha]^{25}D$ −56.5° (*c*, 1% in water) and melted at 177–179°. Based on the Food and Drug Administration working standard, the crystalline salt should have a potency of 482 γ/mg. When assayed against *Escherichia coli* and *Bacillus subtilis* by methods of the Food and Drug Administration,[5] the material showed 475 γ/mg. and 505 γ/mg., respectively. In addition, chemical assays based on the maltol method[6] gave 490 γ/mg. and a modified guanidino assay[7] showed 490 γ/mg.

After drying the dihydrostreptomycin naphthalene-β-sulfonate for three hours at 100° *in vacuo*, the anhydrous salt had the following composition: *Anal.* Calcd. for $C_{21}H_{41}N_7O_{12}\cdot 3C_{10}H_7SO_3H$: C, 50.70; H, 5.42; N, 8.11; S, 7.96. Found: C, 50.95; H, 5.60; N, 8.33; S, 7.95. The anhydrous salt gave $[\alpha]^{25}D$ −52.1° (*c*, 1% in water); m. p. 184–186°. It produced a negative maltol test and assayed 530 γ/mg. by the streptidine analysis. Biological assays using *E. coli* gave 480 γ/mg. and using *B. subtilis* 505 γ/mg.

The acute mouse toxicity (LD_0) for the streptomycin salt is 4.5 mg. per 20 g. mouse (2100 γ) and for the dihydrostreptomycin salt 5.0 mg. per 20 g. mouse (2400 γ).

We wish to express our appreciation to Dr. B. Sobin

(1) Fried and Wintersteiner, *Science*, **104**, 273 (1946).
(2) Kuehl, Peck, Walti and Folkers, *ibid.*, **102**, 34 (1945).
(3) Peck, Brink, Kuehl, Flynn, Walti and Folkers, THIS JOURNAL, **67**, 1866 (1945).
(4) Heuser, Dolliver and Stiller, *ibid.*, **70**, 2833 (1948).
(5) Federal Register 12, 2224–2225 (April 4, 1947).
(6) Boxer, Jelinek and Leghorn, *J. Biol. Chem.*, **169**, 153 (1947).
(7) Monastero, unpublished results.

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

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Derivatives of N-Phosphorylated Amino Acids

BY LOUIS J. SCIARINI AND JOSEPH S. FRUTON

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 report[4] 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 benzoylglycine 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 chloride[5] 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 $C_{16}H_{18}O_5NP$: 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 $C_{23}H_{24}O_5NP$: 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 $C_{21}H_{26}O_7NP$: 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 $C_9H_{16}O_7NPNa_2$: 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.

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

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The Melting Point of 4-Aminosalicylic Acid

BY WILLIAM SEAMAN, WILLIAM ALLEN, R. LILLIAN PASTERNAK AND ALFRED POLLARA

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