acid required was noticeable. When water was added to remove the expected amine salt an emulsion formed which precipitated later as finely divided, white crystals. Repeated recrystallization of this product from hot methanol gave well defined, colorless, orthorhombic crystals melting at 167.0–167.5°. An average of twelve molecular weight determinations made in absolute ethanol by the boiling point elevation method gave 210 ± 10 (calcd. 219).

Anal.[6] Calcd. for $C_{10}H_{15}NO_2F_2$: C, 54.79; H, 6.90; N, 6.39. Found: C, 54.95; H, 6.87; N, 6.59.

Independent Reaction with Hexafluorocyclobutene.—The reaction cylinder was charged at Dry Ice temperature with equimolar quantities of triethylamine (86 g.) and hexafluorocyclobutene (138 g.). After standing at room temperature for 6 hr. the cylinder was shaken at 40° for 16 hr. The liquid portion of the reaction mixture was removed leaving approximately 75 g. of yellow, crystalline solid which reacted vigorously with water with the evolution of heat and hydrogen fluoride to form a product precipitating as white crystals. Recrystallization from methanol gave a compound identical with the one analyzed above.

The reactive crystalline product obtained initially was soluble in benzene but separated, upon the addition of *n*-heptane, as a red oil from which crystals precipitated when cooled below 0°. After centrifugation, the liquid was decanted from the solid which was then washed twice with heptane and finally recovered by suction filtration upon fritted glass under an atmosphere of dry nitrogen in an effort to remove the occluded heptane. The solid was transferred to a drying tube and the residual heptane was removed by evacuation for 16 hr. at room temperature and 4 hr. at 50°. Retention of susceptibility to hydrolysis was established but the spongy consistency of the product prevented determination of a reliable melting point.

Anal. Calcd. for $C_{10}H_{15}NF_6$: C, 45.63; H, 5.74; N, 5.32. Found: C, 45.72; H, 6.05; N, 6.35.

Methanolysis of the Quaternary Salt.—Solution of approximately 1 g. of the reactive quaternary salt in 5 ml. of absolute methanol liberated small amounts of hydrogen fluoride and heat. The product did not precipitate at Dry Ice temperature nor upon standing overnight in a closed container. After aeration of the warmed solution with dry nitrogen until the volume was reduced by approximately one-half, the solution became quite viscous and precipitation occurred. The crystals, recovered by filtration under an atmosphere of nitrogen and washed twice with anhydrous ether, initially melted at 131–134°. Hydrogen fluoride slowly evolved upon continued exposure to air. Finally, a compound was obtained which melted at 156° and gave a mixed melting point of 163° with the recrystallized diketone.

(6) Organic fluoride reported (*cf.* ref. 4) was 17.20 av. (calcd. 17.33).

RESEARCH LABORATORIES, K-25 PLANT
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Organic Salts of Benzylpenicillin. II. Local Anesthetic Amines

BY H. W. RHODEHAMEL, JR.

A number of amines possessing local anesthetic activity have been tested for their ability to form salts with benzylpenicillin. Emphasis has been placed on finding relatively water-insoluble combinations.

For this study, the ability of various local anesthetic amines to form relatively water-insoluble salts was tested as follows: the amine hydrochloride was dissolved in approximately the minimum amount of water necessary for solution at room temperature. This solution was then added to a water solution of potassium or sodium penicillin at a concentration of about 50,000 Oxford units per ml., using a slight stoichiometric excess of penicillin. In several cases, relatively water-insoluble crystalline salts formed immediately; in other cases, amorphous combinations formed which could be caused to crystallize by scratching and chilling; in many cases, no insoluble product resulted or an amorphous material formed which could not readily be made to crystallize. Only relatively water-insoluble crystalline combinations are reported here.

In cases where water-insoluble products did not result, attempts were made to form water-soluble derivatives following the procedure as outlined for the preparation of certain aliphatic amine vasoconstrictor salts of penicillin.[1] Such water-soluble combinations will be reported elsewhere.

The β-Diethylaminoethyl-2-chloro-4-aminobenzoate Salt of Benzylpenicillin.—White, needle-like crystals with a theoretical penicillin potency of 953 Oxford units per mg. and a water solubility of approximately 0.3% (25°), $[\alpha]^{25}D$ +172° (*c* 0.1 in water). *Anal.* Calcd. for $C_{29}H_{37}O_6N_4ClS \cdot H_2O$: C, 55.89; H, 6.31; N, 8.99; Cl, 5.69. Found: C, 55.98; H, 6.56; N, 8.97; Cl, 6.17.

The β-Diethylaminoethyl-2-methyl-4-aminobenzoate Salt of Benzylpenicillin.—White, needle-like crystals with a theoretical penicillin potency of 985 Oxford units per mg. and a water solubility of approximately 0.3% (25°), $[\alpha]^{25}D$ +169° (*c* 0.1 in water). *Anal.* Calcd. for $C_{30}H_{40}O_6N_4S \cdot H_2O$: C, 59.77; H, 7.02; N, 9.30. Found: C, 59.06; H, 7.41; N, 9.48.

The N,N′-Bis-*p*-ethoxyphenylacetamidine[2] Salt of Benzylpenicillin.—White, irregularly shaped crystals tending to form a gum on exposure to air at room temperature with a theoretical penicillin potency of 915 Oxford units per mg. and a water solubility of about 0.37% (25°), $[\alpha]^{25}D$ +157° (*c* 0.1 in water). *Anal.* Calcd. for $C_{34}H_{40}O_6N_4S \cdot H_2O$: C, 62.74; H, 6.51; N, 8.61. Found: C, 62.89; H, 6.46; N, 8.91.

The β-Diethylaminoethyl Ester of 4-Amino-1-naphthoic Acid[3] Salt of Benzylpenicillin.—Yellow, long, thin needle-like crystals with a theoretical penicillin potency of 931 Oxford units per mg. and a water solubility of approximately 0.65% (25°), $[\alpha]^{25}D$ +154° (*c* 0.1 in water). *Anal.* Calcd. for $C_{33}H_{40}N_4O_6S \cdot H_2O$: C, 62.04; H, 6.63; N, 8.77. Found: C, 61.96; H, 6.77; N, 9.48.

The β-Diethylaminoethyl-1-cyclohexylcyclohexanecarboxylate[4] Salt of Benzylpenicillin.—White, needle-like crystals with a theoretical penicillin potency of 896 Oxford units per mg. and a water solubility of about 0.3% (25°), $[\alpha]^{25}D$ +159° (*c* 0.1 in water). *Anal.* Calcd. for $C_{35}H_{53}N_3O_6S \cdot H_2O$: C, 63.46; H, 8.38; N, 6.35. Found: C, 62.95; H, 8.35; N, 6.56.

The author is indebted to Mr. W. L. Brown for microanalyses and to Miss Mary Stieff for technical assistance.

(1) H. W. Rhodehamel, Jr., THIS JOURNAL, **72**, 3302 (1950).

(2) Available as the hydrochloride from Winthrop-Stearns, Inc., under the trade name of Holocaine Hydrochloride.

(3) Available as the hydrochloride from Parke, Davis and Company under the trade name of Naphthocaine Hydrochloride.

(4) Available as the hydrochloride from the Wm. S. Merrell Company under the trade name of Bentyl Hydrochloride.

THE LILLY RESEARCH LABORATORIES
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The Preparation of 5-Chloro-2-thenyl Chloride

BY NORMAN A. ROSENTHAL[1]

The value of 5-chloro-2-thenyl chloride in the synthesis of antihistamines,[2] as well as the recent interest displayed in chloromethylation studies of

(1) Nestle Le Mur Co., New York, N. Y.

(2) L. P. Kyrides, F. C. Meyer, F. B. Zienty, J. Harvey and L. W. Bannister, THIS JOURNAL, **72**, 745 (1950).

thiophene[3,4] and its derivatives, has prompted the author to publish the details of a method of chloromethylation reference to which has been made in a previous publication.[5] This procedure differs from most chloromethylations in that the reaction occurs in an anhydrous medium. The method was devised after earlier attempts to adapt the procedure of Blicke and Burckhalter to the chloromethylation of chlorothiophene had failed. Kyrides and Clapp,[6] on the other hand, have reported success in adapting the method of Blicke, although no yields have ever been reported.

Experimental

One hundred grams (3.3 moles) of trioxane (du Pont), 240 g. of chlorothiophene (2 moles) and 40 g. of fused zinc chloride sticks were introduced into a three-necked flask equipped with sealed stirrer, thermometer and gas delivery tube. The mixture was chilled and held at 0–5° throughout the reaction by means of an ice–salt-bath while vigorous stirring was maintained. The addition of hydrogen chloride gas was initiated and allowed to proceed for an hour and a quarter, after which the contents of the flask were poured into a separatory funnel, diluted with water and extracted with ether. The ethereal solution was washed with water, neutralized by washing with a sodium bicarbonate solution, allowed to dry over anhydrous potassium carbonate for a period of three days, and fractionated. After removal of ether the first fraction, unreacted chlorothiophene, came over at 42° (20 mm.). The main fraction, 5-chloro-2-thenyl chloride, distilled as a clear colorless liquid at 97° (15 mm.),[7] and was redistilled at the same temperature and pressure to give 148 g. (44.5%). The 5-chloro-2-thenyl chloride was characterized by condensing it with 2-aminolepidine to yield 5-chloro-2-thenyl-2-N-aminolepidine.[5]

Similar to 2-thenyl chloride, 5-chloro-2-thenyl chloride undergoes spontaneous decomposition often with explosive violence. It may be safely stored by placing the loosely stoppered vessel containing this liquid within a metal container in a refrigerator.

(3) F. F. Blicke and J. H. Burckhalter, *ibid.*, **64**, 477 (1942).
(4) L. Kyrides and D. Sheets, U. S. Patent 2,527,680, 1950.
(5) I. A. Kaye, THIS JOURNAL, **71**, 2322 (1949).
(6) R. C. Clapp, *et al.*, *ibid.*, **69**, 1549 (1947).
(7) R. C. Clapp, *et al.*, ref. 6, report b.p. 68° (1 mm.) for this compound. L. P. Kyrides, *et al.*, ref. 4, report b.p. 83–85° (8 mm.).

DEPARTMENT OF CHEMISTRY
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Use of Borate in the Paper Chromatography of Ribosides[1]

BY IRWIN A. ROSE[2] AND B. S. SCHWEIGERT

In the course of studies on the incorporation of isotopically labeled compounds into nucleic acids, it became necessary to rigorously separate ribosides from a mixture that contained free purine and pyrimidine bases and desoxyribosides. Existing methods of paper chromatography do not provide adequate resolution of such a mixture. Although periodate oxidation products of α-glycol containing compounds show much different mobilities in some solvent systems,[3] the compounds thus separated are no longer subject to enzymatic attack.

Cohen and Scott[4] have used boric acid to slow the migration of *cis*-diol sugar esters. It was considered possible that the borate esters of the ribosides might be immobile in a solvent system of low water content and could then be effectively separated from other compounds by chromatography. By the use of water saturated with boric acid rather than water alone in making up the solvent as described by Hotchkiss,[5] the ribosides did not move whereas the other compounds moved at their usual rate.

In practice, a crude mixture of nucleic acid constituents was chromatographed in a system, such as the butanol–water system, that would resolve the nucleosides from one another. The strip was then removed and allowed to dry. Control compounds were used for comparison, when available, and the areas that absorb in ultraviolet light (Mineralight SL 2537 lamp has been found useful) were outlined in pencil. The paper was then rerun in the butanol–borate system. The ribosides did not migrate and the area occupied by them was freed of contaminants. By using Hotchkiss' system as the first solvent, guanine and nucleotides will be found at the starting line, followed by the ribosides and then the free bases and desoxyribosides. For example, hypoxanthine and uridine both have R_f values of about 0.20 in butanol–water and cannot be separated in this system. The uridine does not migrate when the paper is rerun in butanol–borate–water, whereas hypoxanthine does with an R_f of 0.30.

The ribosides thus separated may be eluted by any of the usual methods. They are subject to the same limitation of concentration on the ascending chromatogram (Whatman No. 1 was used) as is encountered in other solvent systems, and they retain their natural form as judged by spectrum and lability toward the nucleosidase of *E. coli*.[6]

This technique has been used as an adjunct to differential spectrophotometry in studying enzymatic nucleoside synthesis and has proven useful in investigating the incorporation of precursors into desoxyribosides. Because of the usually slower turnover of desoxyribonucleic acid, it is necessary to completely remove any contaminating ribonucleic acid before any separation of constituent compounds can be considered. This is particularly difficult when working with small amounts as is often the case in tracer work. If the samples are degraded to the nucleoside state, the present method precludes cross contamination.

(1) Journal Paper No. 41 American Meat Institute Foundation.
(2) Predoctoral Fellow of the National Institutes of Health, U. S. Public Health Service, 1951.
(3) J. G. Buchanan, A. W. Johnson, J. A. Mills and A. R. Todd, *J. Chem. Soc.*, 2845 (1950).
(4) S. S. Cohen and D. B. M. Scott, *Science*, **111**, 543 (1950).
(5) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).
(6) L. M. Paege and F. Schlenk, *Arch. Biochem.*, **28**, 348 (1950).

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A Study of *n*-Octadecenoic Acids. IV. Further Confirmation of Structure of Octadecenoic Acids

BY C. B. STEWART, W. F. HUBER AND E. S. LUTTON

In a paper on the synthesis of octadecenoic acids,[1] structure was proved by degradation of the corresponding dihydroxystearic acids to dicarboxylic

(1) W. F. Huber, THIS JOURNAL, **73**, 2730 (1951).