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.).

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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).