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
BROOKLYN COLLEGE
BROOKLYN, NEW YORK
RECEIVED AUGUST 3, 1951

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).

DIVISION OF BIOCHEMISTRY AND NUTRITION
AMERICAN MEAT INSTITUTE FOUNDATION AND
DEPARTMENT OF BIOCHEMISTRY
UNIVERSITY OF CHICAGO
CHICAGO, ILLINOIS
RECEIVED JULY 18, 1951

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).

acids and aldehydes. The dicarboxylic acids were characterized by m.p., and the aldehydes by mixed m.p. of their 2,4-dinitrophenylhydrazones (DNPH) with known preparations. Since all the DNPHs from degradation aldehydes melted in the neighborhood of 106°, reference was made to the present publication in which the individual DNPHs, crystallized from 90–95% ethyl alcohol, are characterized by X-ray diffraction pattern. Diffraction patterns were obtained for rod-shaped pellets with a GE XRD unit, CuKα radiation, and 10-cm. sample-to-(flat)-film distance. The long spacings are shown in Table I for DNPHs from scission products of members of the series 7- through 12-octadecenoic acid, in comparison with values for authentic compounds prepared here and by Malkin and Tranter,[2] who have recently published data for the whole series of DNPHs from C_1- through C_{13}-aldehydes.

TABLE I

LONG SPACINGS (Å.) OF 2,4-DINITROPHENYLHYDRAZONES OF ALIPHATIC ALDEHYDES

Aliphatic chain	Samples from octadecenoic source[a]		Authentic samples	
	From cis	*From trans*	This study	M & T[2]
C_6	16.2	[b]	16.3	16.34
C_7	[c]	[c]	17.7	17.78
C_8	19.4	19.2	19.3	19.15
C_9	19.9	19.7	19.8	19.6
C_{10}	21.0	21.1	21.2	21.12
C_{11}	22.3	22.3	22.6	22.38

[a] C_6-aldehyde from 12-, C_7-aldehyde from 11-octadecenoic acid, etc. [b] Not prepared. [c] Original octadecenoic structure established by F. M. Bumpus, W. G. Taylor and F. M. Strong, THIS JOURNAL, 72, 2116 (1950).

Thus the structures of the synthetic acids are further confirmed, since, for instance, 10-octadecenoic acid (both *cis* and *trans*) gives the expected DNPH with normal C_8-carbon chain. The agreement with Malkin and Tranter[2] is excellent and confirms their reported break in properties between C_8 and C_9. A corresponding crystal habit difference was noted by both macro- and microscopic observation, the C_6, C_7 and C_8 giving in general larger, brighter, more plate-like and less tufted crystals as compared with the more needle-like and highly tufted C_9, C_{10} and C_{11}. No extensive study of possible polymorphism was attempted, but wide variations in alcohol–water proportions and crystallization temperatures effected no change in the form of the C_9-compound.

Some work was done on the DNPHs from C_1- through C_4-aldehydes which were crystallized from ethyl alcohol.

Each compound gave a single polymorphic form:

C_1, m.p. 165.5°, (166°[3]), (166°[2]); l.s. 10.5 Å., (10.3[3]), (7.33, 10.38[2])

C_2, m.p. 167.5°, (166°[3]), (167.5°[2]); l.s. 9.5 A., (9.35, 9.2[3]), (9.40[2])

C_3, m.p. 153°, (150°[3]), (155°[2]); l.s. 9.7 Å., (9.6, 11.03), (10.95, 9.64[2])

C_4, m.p. 121.5°, (123°[3]), (119°[2]); l.s. 14.0 A., (14.0, 11.7,[4] 12.6[3]), (13.5[2])

(2) T. Malkin and T. C. Tranter, *J. Chem. Soc.*, 1178 (1951).

(3) G. L. Clarke, W. I. Kaye and T. D. Parks, *Ind. Eng. Chem., Anal. Ed.*, **18**, 310 (1946).

(4) This form also reported[3] to have a weak spacing of 13.7 Å. The single long spacing value here obtained for a given DNPH is readily associated in each case with a value previously twice reported[2,3] except in the case of C_4, for which only one previous observation[3] (14.0 Å.) is checked.

CHEMICAL DIVISION
THE PROCTER & GAMBLE COMPANY
CINCINNATI 17, OHIO

RECEIVED AUGUST 13, 1951

Chromium Carbonyl Hydride

BY MARY G. ROHMBERG AND BENTON B. OWEN

Investigation in this Laboratory shows that the reactions of chromium hexacarbonyl are more extensive than anticipated, and run parallel to some of the reactions of iron and cobalt carbonyls. Of particular interest is the reaction with alkali which, contrary to previous reports,[1] leads to the formation of a carbonyl hydride. The reaction[2] of alcoholic potassium hydroxide and chromium hexacarbonyl produces a brilliant yellow derivative which, upon acidification, yields a white, volatile, unstable crystalline substance. Mass spectrographic data[3] indicate that this crystalline substance is a chromium carbonyl hydride, and its chemical reactions suggest that the formula is $Cr(CO)_5H_2$ rather than the $Cr(CO)_4H_4$ predicted by Blanchard.[4] The hexacarbonyls of tungsten and molybdenum also react with alkali under similar conditions. When either of these hexacarbonyls is dissolved in alcoholic potassium hydroxide and heated in the absence of oxygen, a yellow solution results which exhibits strong reducing properties.

The yellow product of the reaction of chromium hexacarbonyl with alcoholic potassium hydroxide readily undergoes a series of further reactions. Qualitative tests, color changes and general behavior indicate that the products of these reactions are the chromium counterparts of the derivatives of $Fe(CO)_5H_2$ and $Co(CO)_3H$. For example, an ammoniacal solution of cadmium acetate and an ammoniacal solution of ferroin[5] lead to two derivatives with properties similar to those of $Fe(CO)_5Cd(NH_3)_2$ and $(Fe(CO)_5H)_2Fe(C_{12}H_8N_2)_3$.[6] In the absence of oxygen, the cadmium ammonia derivative of chromium carbonyl hydride slowly changes from brilliant yellow to green with loss in weight: exposure to air produces further loss in weight. The yellow cadmium ammonia derivative is volatile, and passes through an interesting series of steps when decomposed by heat. When first

(1) W. Hieber and E. Romberg, *Z. anorg. allgem. Chem.*, **221**, 321 (1935).

(2) Temperature is not critical. A good yield is obtainable in one hour at 90°.

(3) F. J. Norton, private communication. Comparison of the mass spectra of a freshly prepared chromium carbonyl hydride sample and of the products of its decomposition, after standing for three days at room temperatures, showed the disappearance of peaks corresponding to possible chromium-hydrogen fragments of masses 51, 55, 56, 57 and 58, and return to the normal pattern of peaks for the chromium isotopes 50, 52, 53 and 54.

(4) A. A. Blanchard, *Chem. Revs.*, **21**, 3 (1939).

(5) 1,10-(ortho)Phenanthroline ferrous sulfate.

(6) For properties of these iron compounds consult J. S. Anderson, *Quart. Rev.*, **1**, 341 (1947), and W. Hieber, *Die Chemie (Angew. Chem.)*, **55**, 24 (1942).