the simpler technique of the *in vitro* assay for the antitoxin should make the system a favorable one for further kinetic investigations. It is possible that the mechanism proposed for the antitoxin inactivation may prove to be applicable to other denaturations which deviate in a similar way from first-order behavior.

This investigation was carried out with the aid of a grant from The Rockefeller Foundation. The authors are indebted to Professor Linus Pauling for suggestions and discussion, to J. Y. Chien for carrying out preliminary experiments on the effect of temperature, and to Sharp and Dohme, Glenolden, Pennsylvania, for supplying the antitoxin and toxin.

Summary

A study has been made of the influence of *p*H, urea concentration, temperature and certain other factors on the course of the inactivation of diphtheria antitoxin in urea solutions. The reaction is first-order with respect to initial antibody concentration but departs from typical first-order behavior in that the specific rate decreases as the reaction proceeds. The degree of this effect was found to vary greatly with the *p*H of the solution, and somewhat with the urea concentration. No negative temperature coefficient was observed in the temperature studies, although it was clear that the effect of change in temperature is not uniform over the region studied and possibly tends toward a minimum rate of inactivation at or below 0°. The interpretation of the experimental results has been discussed, and a simple kinetic mechanism which accounts for the observed behavior within the error of the measurements has been proposed. The proposed mechanism involves two competing reactions of the protein, one of which leads irreversibly to inactivation of the antibody and the other of which leads reversibly to an active product, more stable in urea solution than the original antibody and subject to inactivation only by way of slow reconversion to the original antibody or its kinetic equivalent.

PASADENA, CALIFORNIA RECEIVED JULY 2, 1947

[CONTRIBUTION FROM THE CHEMISTRY DEPARTMENT OF THE UNIVERSITY OF MARYLAND]

Tetratriacontanoic Acid and Related Compounds[1]

BY NATHAN L. DRAKE AND SIDNEY MELAMED[1a]

The present research was undertaken with the object of preparing certain aliphatic carboxylic acids and ethers in the C_{20}–C_{40} range. Francis, King and Willis[2] have prepared tetratriacontanoic acid with a yield of 14% from docosanoyl chloride and ethyl sodio-α-acetylbrassylate. More recently dotriacontanoic acid was obtained with a yield of 26% by the reaction of docosanyl zinc iodide and 9-carbethoxynonanoyl chloride.[3] The procedure employed in the present work gave greatly improved yields and is applicable to the preparation of relatively large amounts of material.

The reaction of a dialkyl cadmium with an ω-ester-acid chloride has been extensively studied by Cason.[4] The method has been applied in this work to the preparation of 10-ketohexacosanoic acid from 9-carbethoxynonanoyl chloride and dihexadecyl cadmium and of 18-ketotetratriacontanoic acid from 17-carbethoxyheptadecanoyl chloride and dihexadecyl cadmium; the yields obtained were of 32 and 79%, respectively. The reaction has been further applied to the condensation of an acid chloride and an ω-alkoxyalkyl cadmium compound. In this manner 1-cyclohexoxy-11-ketodotriacontane was obtained from di-(10-cyclohexoxydecyl)-cadmium and docosanoyl chloride and 1-cyclohexoxy-11-ketohexatriacontane was similarly prepared from hexacosanoyl chloride and the same dialkyl cadmium compound. The requisite 1-bromo-10-cyclohexoxydecane was obtained from 1,10-dibromodecane and sodium cyclohexoxide.[5]

The conversion of the keto-acids and keto-ethers to the desoxy acids and ethers, respectively, was accomplished in excellent yield by the application of a recently described modification of the Wolff–Kishner reaction.[6] The only adaptation required was the use of larger amounts of solvent. It is of interest that the method of Clemmensen proved less satisfactory.[7]

The use of the cadmium alkyl reaction for the preparation of these keto-acids is dependent upon the availability of long-chain acid esters. Ethyl hydrogen sebacate[8] is readily obtained by the partial esterification of sebacic acid and can be isolated by fractional distillation. In the preparation of ethyl hydrogen hexadecanedicarboxylate, distillation is not a practicable method of isolation. The partial saponification of the diester[9] leads to

---

(1) The work described in this paper was done under a contract between the University of Maryland and the Bureau of Aeronautics, Navy Department.

(1a) Present address: Rohm and Haas Company, Philadelphia, Pennsylvania.

(2) Francis, King and Willis, *J. Chem. Soc.*, 999 (1937).

(3) Schuette, *Oil & Soap*, **22**, 107 (1945).

(4) Cason, THIS JOURNAL, **68**, 2078 (1946), and previous papers.

(5) Drake, Anspon, Draper, Haywood, VanHook, Melamed, Peck, Sterling, Walton and Whiton, THIS JOURNAL, **68**, 1540 (1946).

(6) Huang-Minlon, *ibid.*, **68**, 2487 (1946).

(7) "Organic Reactions," Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1942, Chap. 7.

(8) "Organic Syntheses," Coll. Vol. II, 276 (1943).

(9) The diethyl hexadecanedicarboxylate was prepared electrolytically from ethyl potassium sebacate by the procedure of Swann, Oehler and Pinkney, "Organic Syntheses," **21**, 48 (1941).

a mixture of the diacid, diester and half ester.[10,11] The diester was removed by extraction from the *dry* sodium salts by hot petroleum ether. The separation of the half-ester from the diacid was accomplished by extracting the mixture of free acids with petroleum ether (b. p. 30–60°), which selectively dissolved the half-ester.

The keto-esters obtained from the cadmium alkyl reaction were found difficult to purify and were therefore isolated as the keto-acids. Two main by-products were formed: the related dibasic acid and dotriacontane. Recrystallization of the crude product from petroleum ether readily removed the hydrocarbon which remained in solution. The separation of the keto-acid from the dibasic acid was accomplished by fractional extraction with sodium hydroxide dissolved in 60% aqueous ethanol.[12] The separation is dependent upon the greater acidity of the dibasic acid and the greater solubility of the sodium salt of the keto-acid in the organic phase.

Similarly in the preparation of the keto-ethers two by-products were formed: the related fatty acid and the diether, 1,20-dicyclohexoxycosane. Docosanoic acid was separated from the corresponding keto-ether by extraction with hot, alkaline 60% aqueous ethanol. In the case of hexacosanoic acid it was found that the sodium salt was completely soluble in hot, wet petroleum ether. The petroleum ether solution was therefore dried and the sodium hexacosanoate, which precipitated as a gel, was removed by filtration.

The dialkyl cadmium reaction is described in detail for the preparation of 18-ketotetratriacontanoic acid. The data for the other condensations are given in Table I. Similarly the modified Wolff–Kishner procedure employed is described for the preparation of tetratriacontanoic acid and the data for the other reductions are given in Table II.

Experimental

Ethyl Hydrogen Hexadecane-1,16-dicarboxylate.—A well-stirred solution of 370 g. (1 mole) of diethyl hexadecane-1,16-dicarboxylate[9] in 500 ml. of dry ethanol was treated dropwise with 400 ml. of dry ethanol containing one mole of sodium ethoxide and one mole of water and allowed to stand overnight. The solvent was removed by distillation and replaced with several liters of petroleum ether (90–100°). The mixture was heated and stirred to suspend the solid and extract the residual diester and finally cooled and filtered. The filtration may be facilitated by the use of a larger volume of petroleum ether or by the addition of ethyl methyl ketone or toluene to the petroleum ether. The sodium salts were extracted with several portions of petroleum ether or mixed solvent as described above and finally washed with ether and dried at 90° to give 271 g. of yellow, granular product; 98 g. of diester resulted from the evaporation of the petroleum ether filtrates.

The dried sodium salts were suspended in 4 liters of warm water (80–90°) and were converted to the free acids by the slow addition of 120 ml. of concentrated hydrochloric acid to the well-stirred suspension. The acids were liquid under these conditions and solidified to a cake when the solution was cooled. The solid cake could be readily separated from the water[13]; further separation of solution was effected by remelting and cooling. The product (252 g.) was powdered and employed in the next step.

The dry mixed acids were extracted in a Soxhlet extractor; 3–4 liters of petroleum ether (30–60°) was used as solvent.[14] The petroleum ether solution deposited 114 g. of ethyl hydrogen hexadecane-1,16-dicarboxylate, m. p. 67–70° (33%). *Anal.* Calcd. for $C_{20}H_{38}O_4$: C, 70.13; H, 11.18; mol. wt., 342. Found: C, 70.45, 70.59; H, 11.32, 11.20; mol. wt. (saponification equivalent), 328.

The material insoluble in the petroleum ether (30–60°) amounted to 85 g.; it melted at 113–119° (27%), and was largely the diacid. A pure sample which melted at 124.5–125.3°, was obtained by recrystallization from methanol. *Anal.* Calcd. for $C_{18}H_{34}O_4$: C, 68.75; H, 10.90. Found: C, 68.82, 68.56; H, 11.13, 10.94.

The petroleum ether filtrate from the crystallization of the half ester was concentrated to give an additional 35 g. of diester. The total quantity of diester recovered was 133 g. (36%); the total of products accounted for 96% of the starting material.

18-Ketotetratriacontanoic Acid.—A Grignard reagent was prepared from 14.9 g. (0.62 gram atom) of magnesium and 214 g. (0.7 mole) of cetyl bromide dissolved in 500 ml. of dry ether. The solution was cooled to 5° and 67.7 g. (0.37 mole) of dry cadmium chloride was added in one portion. The mixture was stirred and heated under reflux until a test for Grignard reagent was negative.[15] The bulk of the ether was removed by distillation and the solid was suspended in 1500 ml. of a mixture of benzene and toluene (2:1).

The acid chloride, 17-carbethoxyheptadecanoyl chloride, was prepared from 141 g. (0.412 mole) of ethyl hydrogen hexadecane-1,16-dicarboxylate by the addition of 85.8 g. (0.412 mole) of phosphorus pentachloride to the molten ester over a period of three to five minutes; the mixture was warmed on a steam-bath for ten to fifteen minutes to complete the reaction. The bulk of the phosphorus oxychloride formed was removed by distillation under diminished pressure; any traces remaining were removed by distilling 100 ml. of dry petroleum ether (60–80°) from the residue. After removal of the last of the petroleum ether, the acid chloride was dissolved in 400 ml. of hot toluene and used without further purification.

The solution of acid chloride in toluene was added over a period of thirty minutes to the well-stirred suspension of the dialkyl cadmium, and the mixture was heated under reflux for six hours and then allowed to stand overnight. A solution of 50 ml. of concentrated sulfuric acid in 1 liter of water was next added, and the mixture was heated to 70° and stirred for a time whereupon the layers were separated while hot. The toluene layer was washed with two 500-ml. portions of hot water and then concentrated to about 400 ml. by distillation. The residue was dissolved in 90% aqueous ethanol, 60 g. of potassium hydroxide was added, and the solution was heated under reflux for two hours to complete saponification of the ester. The well-stirred solution was diluted with 300 ml. of water and acidified with 150 ml. of concentrated hydrochloric acid while being heated under reflux. The hot slurry so formed was diluted with 500 ml. of hot water, cooled and filtered. The solid was melted, cooled and when solid again was removed as a cake from the water

---

(10) Ruzicka and Stoll, *Helv. Chim. Acta*, **10**, 692 (1927); **16**, 493 (1933).

(11) Drake, Carhart and Mozingo, THIS JOURNAL, **63**, 617 (1941).

(12) Weitkamp, *ibid.*, **67**, 447 (1945), notes the value of 60% aqueous ethanol as a solvent for the sodium salts of fatty acids.

(13) Precipitation of the acids from a cold solution resulted in incomplete neutralization and the separation of a product which contained much solution after filtration.

(14) It was found important to use petroleum ether of the boiling range specified. Higher-boiling petroleum ether extracted hexadecane-1,16-dicarboxylic acid as well as the half ester.

(15) Gilman, "Organic Chemistry." Vol. I. John Wiley and Sons. Inc., New York, N. Y., 1943, p. 496.

TABLE I

COMPOUNDS PREPARED BY REACTIONS OF ALKYL CADMIUMS

Compound	M. p., °C.	Solvent for recrystallization	Analytical data Calcd. C	H	Found C	H	Yield, %
10-Ketohexacosanoic acid[a]	94–96.5	Pet. ether	76.04	12.27	76.33	12.44	32
1-Cyclohexoxy-11-ketodotriacontane	96–97	Pet. ether	81.07	13.25	80.91 80.79	13.57 13.55	12[b]
1-Cyclohexoxy-11-ketohexatriacontane	93–95.5	Pet. ether or ethyl methyl ketone	81.48	13.35	80.65 80.31	13.45 13.47	15[c]

[a] Neutral equivalent: calcd. 410. Found: 414. [b] The yield based on the weight of recovered docosanoic acid is 32% [c] The yield based on the weight of hexacosanoic acid recovered is 58%.

TABLE II

COMPOUNDS PREPARED BY MODIFIED WOLFF–KISHNER METHOD

Compound	M. p., °C.	Solvent for recrystn.	Analytical data, % Calcd. C	H	Found C	H	Yield, %
Hexacosanoic acid (cerotic acid)	85.5–86.5[a]	Acetone	78.72	13.22	78.60 78.81	12.95 13.11	
1-Cyclohexoxydotriacontane	88–89	Pet. ether or acetone	83.13	13.95	83.11	14,43	76
1-Cyclohexoxyhexatriacontane	86.5–87.5	Ethyl methyl ketone	83.37	13.99	82.97 82.88	14.04 14.41	94

[a] Francis and Piper (ref. 17) have recorded the melting point as 87.7°. The cerotic acid obtained by the hydrolysis and oxidation of Chinese Insect Wax had a melting point of 78–80° (ref. 19).

which had separated. The keto-acid was recrystallized from 1 liter of petroleum ether (90–100°). After the acid had been dried at 90–100°, it melted at 98–103°; the yield was 170 g. (79%).

A 10-g. sample of the crude keto-acid was dissolved in 300 ml. of hot petroleum ether (90–100°) and heated and stirred with 200 ml. of 60% alcohol containing 0.004 mole of sodium hydroxide. The layers were separated hot, and the petroleum ether solution was concentrated and cooled; the crystals, after separation by filtration and drying, melted at 103–104°. A small sample, recrystallized twice from acetone for analysis, melted at 104–105°. *Anal.* Calcd. for $C_{34}H_{66}O_3$: C, 78.09; H, 12.72; mol. wt., 522.9. Found: C, 78.30; H, 12.75; mol. wt., (neut. equiv.) 508.

The petroleum ether filtrate from which the crude keto-acid had been isolated was heated and stirred with 700 ml. of 60% alcohol containing 30 g. of potassium hydroxide, the layers were separated, and the petroleum ether solution was washed with two 600-ml. portions of hot 60% alcohol. Acidification of the alkaline solution, filtration, and recrystallization of the solid from petroleum ether (90–100°) yielded 19 g. of impure hexadecane-1,16-dicarboxylic acid which melted at 110–120°. The petroleum ether solution was concentrated to small volume, and the solid which separated was recrystallized twice from absolute ethanol; 13 g. of white needles which melted at 68–70° was obtained. This product is probably dotriacontane. It can also be crystallized from ethyl methyl ketone. *Anal.* Calcd. for $C_{32}H_{66}$: C, 85.24; H, 14.76. Found: C, 84.06; H, 14.43.

1,20-Dicyclohexoxycosane.—The petroleum ether filtrate obtained from the recrystallization of the crude 1-cyclohexoxy-11-ketodotriacontane (see Table I) was concentrated to a small volume and cooled. The precipitate, 40 g. (from 0.2 mole of 1-bromo-10-cyclohexoxydecane), was recrystallized from 250 ml. of ethyl methyl ketone, and the solid so obtained was recrystallized twice from absolute ethanol; 5 g. of 1,20-dicyclohexoxycosane which melted at 54–56° was obtained. *Anal.* Calcd. for $C_{32}H_{62}O_2$: C, 80.26; H, 13.05. Found: C, 80.23; H, 13.28.

Docosanoic Acid (Behenic Acid).—Erucic acid[16] was hydrogenated in alcoholic solution in the presence of Raney nickel catalyst. Under 2000 p. s. i. of hydrogen and at a temperature of 75°, the hydrogenation proceeded rapidly and smoothly. The product, when recrystallized from petroleum ether (b. p. 90–100°), separated as white, shiny plates which melted at 79.5–80.5°. Francis and Piper[17] have recorded a melting point of 79.95° for this acid. *Anal.* Calcd. for $C_{22}H_{44}O_2$: mol. wt., 340. Found: mol. wt. (neut. equiv.), 355.

Tetratriacontanoic Acid.—A mixture of 85 g. of 18-ketotetratriacontanoic acid, 85 ml. of 85% hydrazine hydrate, 85 g. of sodium hydroxide and 3 liters of diethylene glycol was heated in a flask equipped with a stirrer, a thermometer, and an arrangement for the removal or return of refluxing liquid. When the temperature had reached 140–150°, the mixture began to foam, and it was necessary to apply heat less rapidly and to stir vigorously. Foaming stopped when the temperature passed 150°; solvent began to reflux at 175°, and the temperature was held at that point for one hour. Volatile material was then removed over a period of two hours, and the mixture was finally heated under reflux at 215° for an additional hour and a half. When the mixture had cooled to 160–180°, it was poured slowly into a warm (60°), well-stirred solution of 300 ml. of concentrated hydrochloric acid in 3 liters of water. The hot solution was extracted with 3 liters of hot toluene, and the toluene layer washed with hot water and finally with a dilute ethanolic solution of sodium hydroxide (60% aqueous ethanol). The toluene solution was then concentrated and cooled. Tetratriacontanoic acid, which melted at 92–93.5°, was obtained in 82% yield. The time of reaction was insufficient for the large quantities employed; the product contained unreduced keto-acid. Further treatment of the product by the procedure described gave purer material which melted at 95–96°. An analytical sample prepared by recrystallization from acetone melted at 95.5–96.4°.[18]

Anal. Calcd. for $C_{34}H_{68}O_2$: mol. wt., 508.9. Found: mol. wt. (neutral equivalent), 511.

Ethyl Tetratriacontanoate.—A solution of 5.0 g. of tetratriacontanoic acid in 150 ml. of dry alcohol, 250 ml. of benzene and 0.2 g. of *p*-toluenesulfonic acid were heated for two hours under an 18-inch helix-packed column. Distillate was then taken off slowly until 300 ml. had been collected. The residual solution was cooled, and the

---

(16) "Organic Syntheses," Coll. Vol. II, p. 258.

(17) Francis and Piper, THIS JOURNAL, **61**, 577 (1939).

(18) Francis, King and Willis, ref. 2, report a melting point of 98.2° for this acid obtained from distilled ethyl ester.

(19) Coover, Thesis, Cornell University, 1943.

precipitate was collected and recrystallized from petroleum ether (90–100°). The white, waxy crystals melted at 76–76.5°.[20] The ester also crystallized well from ethyl methyl ketone.

Anilide of Tetratriacontanoic Acid.—One gram of acid was mixed with 10 ml. of thionyl chloride, allowed to stand for thirty minutes and then heated under reflux for several minutes. Excess thionyl chloride was removed by distillation and the residue, dissolved in 60 ml. of petroleum ether, was added to 5 ml. of aniline. The mixture was stirred thoroughly and evaporated to dryness; the residue was triturated with cold, dilute hydrochloric acid and the crystals were separated by filtration. The product was recrystallized once from a small volume of ethyl methyl ketone and then twice from 100-ml. portions of petroleum ether (90–100°). The anilide, 1.05 g., melted at 110–110.5°. Francis[2] reported a melting point of 113–114°.

Acknowledgment.—We should like to acknowledge the continued interest and suggestions of Dr. Howard Gilbert of the Bureau of Aeronautics, Navy Department. Miss Eleanor Werble performed the microanalyses, and Mr. Herman Sanders aided in the preparation of certain intermediates.

Summary

1. The preparation of hexacosanoic acid and tetratriacontanoic acid is described.

2. The same method has been applied to the synthesis of 1-cyclohexoxydotriacontane and 1-cyclohexoxyhexatriacontane.

3. A convenient synthesis of ethyl hydrogen hexadecanedicarboxylate has been developed. The method depends upon the selective solvent action of petroleum ether which has been shown to be a solvent of useful specificity.

COLLEGE PARK, MD. RECEIVED JUNE 28, 1947

[20] Francis, King and Willis, ref. 2, have recorded the melting point of this ester as 75.4°.

[CONTRIBUTION FROM THE WOOD CONVERSION LABORATORY OF THE UNIVERSITY OF IDAHO]

The Constitution of Mesquite Gum. IV. 4-Methoxy-D-glucuronic Acid[1]

BY E. V. WHITE

A large number of the polysaccharides occurring in nature are known to contain uronic acid residues as an integral part of their molecular structure. The carboxyl group in such instances usually does not occur in the free state but rather is combined as a salt with metallic ions such as calcium, magnesium, and the like or as an ester with methyl alcohol. In general, the uronic acid possesses the glucose, mannose or galactose configuration and it has been considered to play an important role in the mechanism of plant synthesis. In a few instances polysaccharides have been reported to contain small percentages of ether type methoxyl and this grouping appears to be associated invariably with the uronic acid component. The observation is particularly interesting since it records the natural synthesis of an aliphatic ether linkage as contrasted with the usual aromatic, ester and acetal types. Unfortunately, however, the presence of this particular grouping[2] has been indicated hitherto only by analyses and no definite crystalline compounds or derivatives have been prepared to prove the actual existence of the group or to establish its exact location in any given polysaccharide.

The presence of ether methoxyl in mesquite gum was reported first by Anderson and co-workers[3a,b,c,d] who traced the group to the uronic acid component and, largely by a process of elimination, suggested the three position thereof as the most likely point of attachment. It has now been shown[4] that the uronic acid residues occupy terminal positions in the mesquite gum macromolecule and the present study was designed to locate definitely the position of the methyl ether linkage.

The well known reaction of polysaccharides with anhydrous acidic methyl alcohol solutions results in simultaneous esterification of the uronides and formation of the corresponding methyl glycosides and uronosides. In such reactions, whereas a single methyl group is substituted in the saccharides, two such groups are introduced into the uronic acid component. One of these is uronosidic or acetal in character while the second is of the ester type. In the present instance these two methyl groups together with the aliphatic ether group originally present furnish a trimethyl substituted uronic acid fission product which can be separated readily from the glycosidic fragments. Accordingly, mesquite gum which had been partially hydrolyzed to remove the labile arabinose fraction[5] was treated with methanolic hydrogen chloride and the resulting sirup extracted with anhydrous acetone. The extract was concentrated and then diluted with anhydrous ether to precipitate residual methyl galactoside. Evaporation of the mixed solvent and fractional

[1] Presented in part at the Fall Meeting of the Technical Association of the Pulp and Paper Industry, Appleton, Wisconsin, September 3–5, 1947.

[2] It should be noted that the aliphatic methyl ether grouping has been found in the carbohydrate component of certain cardiac glycosides, notably, cymarose and digitalose. A review of these compounds is given by Elderfield, "Recent Advances in Carbohydrate Chemistry," Vol. I, Academic Press, Inc., New York, N. Y., 1945, p. 147.

[3] (a) Anderson, Sands and Sturgis, *Am. J. Pharm.*, **97**, 589 (1925); (b) Anderson and Sands, *Ind. Eng. Chem.*, **17**, 1257 (1925); (c) Anderson and Sands, THIS JOURNAL, **48**, 3172 (1926); (d) Anderson and Otis, *ibid.*, **52**, 4461 (1930).

[4] White, *ibid.*, **68**, 272 (1946).

[5] White, *ibid*, **69**, 622 (1947).