ever it is, must be a single event phenomenon. Two possibilities exist for interpreting the decrease in infectivity. One is that the reaction is completely independent of any changes detected by chemical or electrophoretic means. The other possibility is that infectivity is lost when the first one of several particular amino groups on a virus particle has reacted irreversibly with formaldehyde. If several groups are capable of leading to inactivation, they must all be of the same sort with respect to rate of reaction with formaldehyde; otherwise, the first order law for inactivation could not be followed. If one assumes that m out of a total of ν groups of a particular sort on a virus particle can lead to loss in infectivity when irreversible reaction with formaldehyde takes place, then loss of infectivity would result when the first of these groups happened to react. Equation (9) shows that the reaction velocity constant for the loss of infectivity would be equal to m times the velocity constant for the reaction of the particular groups under study.

The reaction velocity constant for the destruction of infectivity at room temperature has been shown to be about 0.42 reciprocal hour. This is about ten times the rate constant for the change in mobility. Thus, if groups of the sort which result in shifts in mobility are responsible for the inactivation of the virus, there must be ten special groups of that sort on each virus particle. Similarly, since the rate of the reaction which leads to irreversible loss of free amino groups but not to change in charge is 0.14, there would have to be 0.42/0.14 or 3 special groups of this sort to account for the loss of infectivity.

Summary

The kinetics of the changes which take place when tobacco mosaic virus is treated with 2% formaldehyde at *p*H 7 and 30° were studied. Infectivity was found to decrease according to the law of a first order process with a rate constant of about 0.42 hour^{-1}. Electrophoretic mobility was found to increase and approach a maximum value as the time of treatment was extended indefinitely. The rate constant for this process was found to be 0.04 hour^{-1}. Free amino groups as determined by the ninhydrin color reaction were found to decrease according to a complex pattern. The results can be interpreted in terms of the assumption that 28% of the amino groups do not react irreversibly with formaldehyde; 42% react irreversibly at a rate of 0.14 reciprocal hour, and 30% react irreversibly at a rate of 0.04 reciprocal hour. These latter 30% can be assumed to be the same groups which cause shift in electrophoretic mobility. The loss of infectivity could be due either to some process entirely independent of the reactions indicated by the chemical and physical changes or to the first one of several particular amino groups of one sort reacting irreversibly with formaldehyde.

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[CONTRIBUTION FROM THE RICHARDSON CHEMICAL LABORATORY, TULANE UNIVERSITY]

A New Synthesis of Tuberculostearic Acid[1a]

BY GUSTAV A. SCHMIDT[1b] AND DAVID A. SHIRLEY

Tuberculostearic acid or 10-methyloctadecanoic acid (compound V in Fig. 1) was isolated by Anderson and Chargaff[2] from the fatty envelope surrounding the tubercle bacillus and its structure later proved by Spielman[3] who prepared a synthetic sample. Recently Prout, Cason and Ingersoll[4] have reported preparation of the *dl*-form and the *d*- and *l*-enantiomorphs of 10-methyloctadecanoic acid, establishing that the naturally occurring isomer is the levorotatory form. The *dl*- and active forms have also been prepared by Ställberg-Stenhagen[5] by a still different method.

As a part of a study of derivatives of modified branched-chain fatty acids as potential antitubercular chemotherapeutic agents, we have undertaken the preparation of moderate amounts of tuberculostearic acid to be used in further synthetic work. We have developed a new method of synthesis of *dl*-tuberculostearic acid which appears to be an improvement over the one used by Spielman.[3] It is much shorter than the synthesis used by Prout, Cason and Ingersoll[4] and Ställberg-Stenhagen,[5] since these authors prepared the *d*- and *l*-forms which involved working with optically active intermediates and avoiding racemization in the transformations employed.

The steps in this synthesis are outlined in Fig. 1. Azelaic acid was converted to its half ethyl ester acid chloride and this was allowed to react with 2-decylzinc chloride (II), to give ethyl 9-keto-10-methyloctadecanoate (III). Reduction of the keto ester (III) by the Clemmensen method gave ethyl 10-methyloctadecanoate (IV) which was hydrolyzed to the corresponding acid (V). Purification of 10-methyloctadecanoic acid was effected by converting it to the amide (VI) followed by recrystallization of the amide and hydrolysis to the acid.

A distinctive feature of this synthesis is the use

(1a) Presented before the Organic Division, Atlantic City A. C. S. meeting, Sept. 21, 1949.

(1b) Frederick G. Cottrell, Research Fellow, 1948–1949.

(2) Anderson and Chargaff, *J. Biol. Chem.*, **85**, 77 (1929).

(3) Spielman, *ibid.*, **106**, 87 (1934).

(4) Prout, Cason and Ingersoll, THIS JOURNAL, **70**, 298 (1948).

(5) Ställberg-Stenhagen, *Arkiv Kemi, Mineral. Geol.*, **26A**, No. 12 (1948), 28 pp.

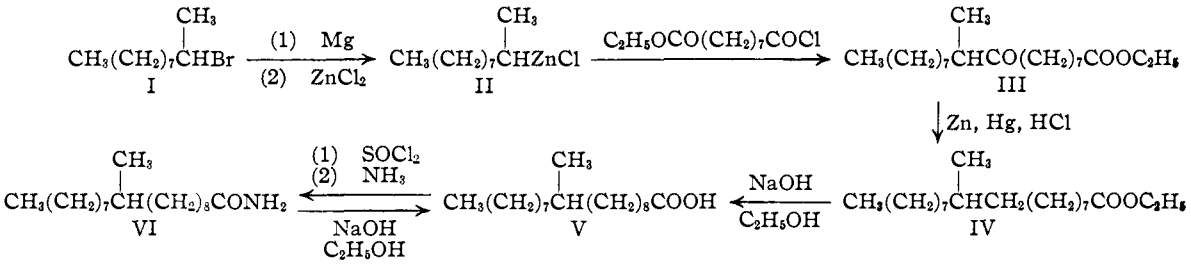

Fig. 1.—Diagram of method of synthesis of 10-methyloctadecanoic acid

of a secondary organometallic compound in its reaction with an acid chloride to form a ketone. The organocadmium compounds, usually found advantageous for ketone formation, are not satisfactory when secondary types are used.[6] The secondary organozinc compound used here allows formation of the ketone in 55% yield.

Experimental

2-Decanol.—Reaction of *n*-octylmagnesium bromide with acetaldehyde in customary fashion gave an 85% yield of 2-decanol, b. p. 123° (29 mm.), $n^{25}D$ 1.4326.

2-Bromodecane.—2-Decanol was converted to 2-bromodecane in essential accordance with the procedure of Hsueh and Marvel[7] except that the crude bromide was washed with cold concentrated sulfuric acid, then with water and dried and distilled. The yield of pure bromide was 88–92%. It boiled at 124–125° (30 mm.), $n^{25}D$ 1.4526.

Ethyl Hydrogen Azelate.—Pure azelaic acid and its diethyl ester were obtained from "Plastolein 9111," a commercial material reported to have the following composition of dicarboxylic acids; pimelic 4%, suberic 15%, azelaic 71% and undecandioic 10%.[8]

The mixed acids refluxed with excess ethanol, benzene and a small amount of sulfuric acid catalyst under conditions for continuous separation of the benzene, ethanol and water azeotrope allowed formation of the mixed diethyl esters in 96% yield. Diethyl azelate was separated from the mixed esters by two distillations through a fractionating column consisting of two 2 × 35-cm., helices packed, electrically heated sections on which was mounted a total condensation partial take-off head. Pure diethyl azelate obtained in 77% yield (based on the 71% azelaic acid present in the mixed starting acids) boiled at 140° (1 mm.),[9] $n^{25}D$ 1.4348. Saponification of diethyl azelate with alcoholic sodium hydroxide gave an essentially quantitative yield of azelaic acid, m. p. 105.5–106°, with no purification by recrystallization or other methods being necessary.

Diethyl azelate and azelaic acid were used for the preparation of the half ester, ethyl hydrogen azelate, in accordance with the procedure of Swann, Oehler and Buswell[10] for the preparation of ethyl hydrogen sebacate. The half ester was formed in 63% yield, b. p. 170° (1 mm.), m. p. 28–29°.[9]

ω-Carbethoxyoctanoyl Chloride.—Ethyl hydrogen azelate (224 g., 1.03 moles) was refluxed with excess thionyl chloride for one and one-half hours. Distillation gave the acid chloride in 84% yield, b. p. 155° (14 mm.). Ruzicka and Stoll[11] report this compound and give 155–158° at 15 mm. as its boiling point.

Anal. Calcd. for $C_{11}H_{19}ClO_3$: Cl, 15.1. Found: Cl, 15.0.

Ethyl 9-Keto-10-methyloctadecanoate (III).—The preparation of this ketone was carried out by reaction of 2-decylzinc chloride (II) with ω-carbethoxyoctanoyl chloride in essential accordance with the procedure used by Jones[12] for the synthesis of long-chain aliphatic ketoacids.

2-Decylmagnesium bromide (I) was prepared in customary manner from 280 g. (1.27 moles) of 2-bromodecane, 48 g. (2.0 g. atoms) of magnesium and 600 ml. of absolute ether. Titration of an aliquot with standard acid[13] showed an 80% yield of Grignard reagent. The solution of Grignard reagent was added to a solution of 136 g. (1.0 mole) of freshly fused anhydrous zinc chloride in 350 ml. of ether at such rate that gentle reflux was maintained. Reflux temperature was maintained for an additional one and one-half hours during which time the solvent was slowly distilled from the reaction mixture until the volume was approximately 600 ml. To the resulting mixture was added with stirring a solution of 170 g. (0.72 mole) of ω-carbethoxyoctanoyl chloride in 400 ml. of anhydrous benzene. After a three-hour reflux period, the mixture was treated with excess water containing a little hydrochloric acid and the organic layer separated and dried. The volatile organic material was removed by distillation until a head temperature of 150° (12 mm.) pressure was reached. It was found convenient at this point to treat the residue with ethanol under the esterification conditions described above until no more water was being removed from the system. In this way the ethyl hydrogen azelate (from unreacted acid chloride) present at this point was converted to its more volatile diethyl ester which increased the efficiency of its subsequent separation by distillation from the ethyl 9-keto-10-methyloctadecanoate. Distillation of the esterified material through the same column described above gave 78 g. of diethyl azelate boiling in the range 130–175° (95% boiled at 130°) at about 0.5 mm. pressure and 133 g. of ethyl 9-keto-10-methyloctadecanoate, b. p. 184–185° at about 0.5 mm., $n^{25}D$ 1.4470. The yield of ketoester was 55% based on the starting amount of ester acid chloride or 93% based on the acid chloride consumed.

9-Keto-10-methyloctadecanoic Acid.—Fifteen grams (0.044 mole) of ethyl 9-keto-10-methyloctadecanoate was refluxed for four hours with a solution of 20 g. of sodium hydroxide in 40 ml. of water and 60 ml. of ethanol. The alcohol was distilled off and the reaction mixture diluted to a volume of 500 ml. with water. The resulting solution was extracted with three portions of petroleum ether (b. p. 30–60°) and then acidified. The acid mixture was then extracted with petroleum ether and the combined extracts washed with dilute hydrochloric acid and then ten times with distilled water. The petroleum ether solution was dried and the solvent removed by distillation including a final heating period at 100° under a pressure of about 0.5 mm. to ensure complete removal of solvent. The residual light yellow oil (13 g. or 94% yield) was used for preparation of the semicarbazone described below. A small amount recrystallized three times from petroleum ether at −30° gave white platelets, m. p. 24–25°.

(6) Cason, *Chem. Revs.*, **40**, 15 (1947).

(7) Hsueh and Marvel, THIS JOURNAL, **50**, 855 (1928).

(8) We are indebted to Emery Industries, Inc., of Cincinnati for a generous supply of "Plastolein 9111" and for information on its composition.

(9) Fourneau and Sabetay, *Bull. soc. chim. France*, [4] **45**, 834 (1929).

(10) Swann, Oehler and Buswell, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 276.

(11) Ruzicka and Stoll, *Helv. Chim. Acta*, **10**, 691 (1927).

(12) Jones, THIS JOURNAL, **69**, 2350 (1947).

(13) Gilman, Wilkinson, Fishel and Meyers, *ibid.*, **45**, 150 (1923).

Anal. Calcd. for $C_{19}H_{36}O_3$: neut. equiv., 313. Found: neut. equiv., 313, 314.

A semicarbazone derivative[14] prepared in 90% yield melted at 86–86.4° after three recrystallizations from methanol, petroleum ether and acetone in that order.

Anal. Calcd. for $C_{20}H_{39}N_3O_3$: N, 11.37. Found: N, 11.42.

Ethyl 10-Methyloctadecanoate (IV).—Using the general method of Schneider and Spielman[15] for a Clemmensen reduction, 51 g. (0.15 mole) of ethyl 9-keto-10-methyloctadecanoate dissolved in 1 l. of absolute ethanol and mixed with 420 g. of amalgamated zinc[12] was saturated with dry hydrogen chloride. The mixture was refluxed for twenty-four hours, again saturated with hydrogen chloride, and then refluxed for a second twenty-four-hour period. After removal of the unreacted zinc, the volume of the solution was reduced to one-half and excess water added. The precipitated organic layer was removed by extraction with benzene and the benzene solution distilled to remove the volatile material. Distillation of the residue gave 40 g. (83%) of ester, b. p. 175–180° (2 mm.), $n^{25}D$ 1.4440. Prout, Cason and Ingersoll[4] report $n^{25}D$ 1.4447 for this compound.

Anal. Calcd. for $C_{21}H_{42}O_2$: sapon. equiv., 326. Found: sapon. equiv., 322.

10-Methyloctadecanoic Acid (V).—A mixture of 17.5 g. (0.054 mole) of ethyl 10-methyloctadecanoate, 25 ml. of 40% aqueous sodium hydroxide solution and 100 ml. of ethanol was refluxed for twelve hours and worked up as described above for the saponification of ethyl 9-keto-10-methyloctadecanoate. There was obtained 15 g. (94%) of the acid, $n^{25}D$ 1.4513 (the literature[3,4] records $n^{25}D$ 1.4512 for 10-methyloctadecanoic acid). In view of the fact that the product had a low melting point (around 5–10° compared with literature values of 20–21°[3] and 25.4–26.1°[4]), and it was difficult to recrystallize without large loss, it was decided to convert the acid to its more easily handled amide, purify the amide by recrystallization, and hydrolyze the pure amide to the acid.

10-Methyloctadecanamide (VI).—Twelve and five-tenths grams (0.042 mole) of the 10-methyloctadecanoic acid isolated above was converted to the acid chloride by refluxing with excess thionyl chloride and the excess thionyl chloride removed by distillation. The residual acid chloride dissolved in dioxane was added dropwise with rapid stirring to cold concentrated aqueous ammonia. The precipitated amide was recrystallized once from acetone and four times from petroleum ether (b. p. 30–60°) to give 8.8 g. (70%) of pure amide, m. p. 77–78° (the literature reports 76–77°[3] and 77.5–79.2°[4]).

Hydrolysis of 10-Methyloctadecanamide.—The 8.8 g. of amide prepared above was hydrolyzed by refluxing for twelve hours with 50 ml. of 10% alcoholic sodium hydroxide. The mixture was treated as described above under saponification of ethyl 9-keto-10-methyloctadecanoate to give an essentially quantitative yield of 10-methyl octadecanoic acid, m. p. 23.5–25.8° (cor.), b. p. 200–203° (1 mm.), $n^{25}D$ 1.4512.

Anal. Calcd. for $C_{19}H_{38}O_2$: neut. equiv., 299. Found: neut. equiv., 301, 303.

Table I summarizes the physical constants of 10-methyloctadecanoic acid and its derivatives found in this work in comparison with those obtained by Spielman[3] and by Prout, Cason and Ingersoll.[4]

TABLE I

PHYSICAL CONSTANTS OF 10-METHYLOCTADECANOIC ACID AND ITS DERIVATIVES

Physical constant	This work	Spielman[3]	Prout, Cason and Ingersoll[4]
Melting point of acid, °C.	23.5–25.8 (cor.)	20–21	25.4–26.1 (cor.)
Boiling point of acid, °C.	200–203 at 1 mm.	.....	.....
Index of refraction of acid $n^{25}D$	1.4512	1.4512	1.4512
Melting point of amide, °C.	77–78	76–77	77.5–79.2
Melting point of 2,4,6-tribromoanilide, °C.	93.5–94	93–94	93.4–93.9

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Summary

A new and improved method of synthesis of tuberculostearic acid (10-methyloctadecanoic acid) is reported.

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(14) Shriner and Fuson, "Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 170.

(15) Schneider and Spielman, *J. Biol. Chem.*, **142**, 345 (1942).

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, UNIVERSITY OF MINNESOTA]

The Soluble Complex of Ferric Iron and 8-Hydroxyquinoline

BY E. B. SANDELL AND D. C. SPINDLER

In dilute mineral acid medium 8-hydroxyquinoline reacts with ferric ion to give a soluble green complex. A number of workers have made use of the formation of this green substance in the indirect colorimetric determination of magnesium by treating an acid solution of the magnesium hydroxyquinolate precipitate with a ferric salt. The present work deals with the composition of the green complex and its dissociation constant. The value of the dissociation constant is needed in calculating the solubility product of ferric hydroxyquinolate as well as in treating the problem of the extractability of ferric hydroxyquinolate by chloroform from aqueous solutions at various acidities.

The composition of the complex was established by applying the familiar method of continuous variations.[1] Solutions of ferric perchlorate and 8-hydroxyquinoline in perchloric acid were mixed in various ratios, the sum of molar concentrations of the two reactants being kept constant at 1.19×10^{-3} *M*. The transmittancy of the mixtures was determined at 645 mμ, the approximate wave length of maximum absorption by the iron–hydroxyquinoline complex; at this wave length, absorption by hydroxyquinolinium ion (in which form hydroxyquinoline is chiefly present at the acidities used) and ferric ion is negligibly small, at least in the concentrations employed. Transmit-

(1) P. Job, *Ann. chim.*, **9**, 113 (1928); **11**, 97 (1936).