ternation is observed in the melting points of the compounds containing sixteen, seventeen and eighteen carbon atoms in the chain.

The determination of mixed melting points between successive members of this series was undertaken to ascertain whether there would be significant depression in melting points. It was observed that the magnitude of depression decreases with an increase of chain length.[5]

Experimental[6]

The following experimental procedures are described below: the preparation of *n*-heptadecyl bromide and of *n*-heptadecyllithium and the reaction of triphenylsilane with *n*-hexadecyllithium and of tetradecene-1 with triphenylsilane. Similar procedures were used in the preparation and reactions of the homologs. The identity of the corresponding compounds prepared by the two methods was shown by infrared spectra.

***n*-Heptadecyl Bromide.**—To a solution of 56.8 g. (0.2 mole) of stearic acid, m.p. 69.5–70°, in 400 ml. of methanol (95%) in a 2-l. beaker or other appropriate container fitted with a mechanical stirrer was added a solution of 15 ml. (about 0.2 mole) of concd. ammonium hydroxide (C.P., sp. gr. 0.9) in 100 ml. of distilled water. The mixture developed a milky appearance and became clear when it was warmed to 45–50° with continuous stirring. A solution of 42 g. (0.25 mole) of silver nitrate in 200 ml. of distilled water was added over a 20-minute period. When the addition was complete, 300 ml. of distilled water was added and the mixture was allowed to cool to room temperature with continuous stirring.

The precipitated silver stearate was filtered on a 12-cm. büchner funnel, then placed in a 1-l. beaker and stirred with 500 ml. of distilled water for 20 minutes. The silver stearate was filtered again on a büchner funnel and washed with about 100 ml. of distilled water; a similar washing procedure was carried out with about 500 ml. of acetone. Finally the silver stearate was dried in a vacuum oven (1 mm. pressure) at 60–65° for 12 hours in the flask in which the degradation was to be performed. The yield was 62.0 g. (81%). A melting point taken in an evacuated sealed tube was 206° (lit. 208°).

Fifty-one grams (0.13 mole) of silver stearate was placed in a 1-l. four-necked flask. Dry carbon tetrachloride (400 ml.) was distilled from phosphorus pentoxide into this flask. A solution of 21 g. (about 0.13 mole) of bromine in 60 ml. of carbon tetrachloride was distilled from phosphorus pentoxide into a dropping funnel and then was added over a 40- to 50-minute period. When the addition was completed, the mixture was heated for one hour on a steam-bath with continuous stirring. It was then filtered hot and the silver bromide was washed thoroughly with 100 ml. of warm carbon tetrachloride. The reddish colored filtrate was concentrated under reduced pressure at steam-bath temperature. The residue was dissolved in 400 ml. of ether and washed once with 100 ml. of 10% sodium carbonate solution. The ether layer was separated and dried over anhydrous potassium carbonate. The solvent was removed and a white solid was obtained. The weight of this product melting between 27 and 29° was 35.3 g. The yield was 84% based on the weight of the silver stearate.

The improvement of the yield depended not only on the scrupulous exclusion of moisture but also to a great extent on the purity of the silver salt. The presence of free acid in the silver salt reduced the yield significantly.

***n*-Hexadecyllithium.**—In a 500-ml. four-necked flask equipped with a stirrer, a low-temperature thermometer, and a dropping funnel was placed 100 ml. of anhydrous ether. After the apparatus was swept with dry, oxygen-free nitrogen, 1.08 g. (0.15 gram atom) of lithium wire (30 cm.) was cut into small pieces which were allowed to fall directly into the reaction flask in a stream of nitrogen. With the stirrer operating, about 2 ml. of a solution of 18.3 g. (0.06 mole) of *n*-hexadecyl bromide in 100 ml. of anhydrous ether was added from the dropping funnel. After a period of about 30 minutes the mixture became slightly cloudy. The reaction mixture then was cooled to −10° by immersing the flask in a Dry Ice–acetone-bath. The remainder of the *n*-hexadecyl bromide solution was added over a period of two hours while the internal temperature was maintained at −10°. After addition was completed the reaction mixture was kept at the same temperature for one hour and then was allowed to warm up to 0–10° with stirring. The reaction mixture was filtered under an atmosphere of nitrogen by decantation through glass wool into a graduated dropping funnel. The yield, determined by double titration, was 60%.

***n*-Hexadecyltriphenylsilane.**—In a 1-l. three-necked flask equipped with a stirrer, a reflux condenser and a dropping funnel, 10.3 g. (0.035 mole) of triphenylchlorosilane was dissolved in 200 ml. of anhydrous ether. After the apparatus had been swept with dry, oxygen-free nitrogen, the stirrer was started; a colorless solution resulted. Over a period of 30 minutes a solution of 8.06 g. (0.035 mole) of *n*-hexadecyllithium in 295 ml. of anhydrous ether was added. Since Color Test I[7] was still positive after ten hours, another 1.4 g. of triphenylchlorosilane was added. Color Test I was negative after ten minutes. Water (200 ml.) was added and the mixture was stirred for about 20 minutes. The two layers were separated, the water layer was washed with 100 ml. of ether, and the ether extracts were collected and dried over anhydrous sodium sulfate. After about three hours the ether solution was filtered and the ether evaporated. The residue (25.1 g.) was dissolved in 350 ml. of anhydrous petroleum ether (b.p. 60–90°) and was passed through an alumina column (20 × 180 mm.). The column was eluted with 200 ml. of petroleum ether, and the colorless eluate was concentrated on a steam-bath. This material was distilled in a modified molecular still. The main fraction, between 225–235° (0.005 mm.) (air-bath temperature), was recrystallized twice from glacial acetic acid and twice from absolute ethanol, giving 4.4 g. (30%) of *n*-hexadecyltriphenylsilane, m.p. 68.5–69.5°.[8]

***n*-Tetradecyltriphenylsilane.**—A 250-ml. three-necked flask equipped with a stirrer and a reflux condenser was flushed with dry oxygen-free nitrogen; 26.0 g. (0.10 mole) of triphenylsilane, 0.32 g. (0.0013 mole) of benzoyl peroxide, 3.2 g. (0.016 mole) of tetradecene-1 and 25 ml. of *n*-hexane were added and stirring commenced. The flask was heated with an oil-bath (70–75°). The reaction mixture was refluxed gently for 14 hours in a dry nitrogen atmosphere. The reaction product was transferred to a modified molecular still and distilled. After an initial fraction between 110–160° (0.005 mm.) (air-bath temperature) a colorless product was obtained between 180–220° (0.005 mm.). It was redistilled to give 4.2 g. of white crystals, m.p. 54–56° (59%). After repeated recrystallizations of this crude material from absolute ethanol and treatment with charcoal, 3.9 g. (52%, based on tetradecene-1) of *n*-tetradecyltriphenylsilane, m.p. 66–67°, was isolated.

Acknowledgment.—The use of the Baird infrared instrument of the Institute of Atomic Research, Ames, Iowa, is herewith gratefully acknowledged.

(5) The triphenyllead salts of lauric, myristic, palmitic and stearic acids exhibit the same behavior: H. Gilman and G. M. Ford, *Iowa State Coll. J. Sci.*, **13**, 135 (1939).

(6) All reactions involving organometallic compounds were carried out in a dry, oxygen-free nitrogen atmosphere. Melting points are uncorrected.

(7) H. Gilman and F. Schulze, THIS JOURNAL, **47**, 2002 (1925).

(8) The qualitative test for silicon described by H. Gilman, R. K. Ingham and R. D. Gorsich, *ibid.*, **76**, 918 (1954), was used successfully to characterize solid fractions obtained from a chromatogram of the product of the reaction of *n*-heptadecyllithium and triphenylchlorosilane.

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Preparation and $NaBH_4$ Reduction of 7-Ketocholanic Acid

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RECEIVED MAY 29, 1954

Studies on the metabolism of 7-ketocholesterol[1] made it desirable to have 7-ketocholanic and the

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isomeric 7α- and 7β-hydroxycholanic acids available as reference compounds. Published methods for the preparation of these substances from free (unesterified) cholic acid[2,3] in our hands gave rise to troublesome chromium complexes during the oxidation steps, resulting in lowered yields.

In the present study 7-ketocholanic acid was prepared from methyl cholate 7-acetate[4] as suggested by Fieser. The 7-ketocholanic acid was then reduced with $NaBH_4$. In a previous investigation, reduction of 7-ketocholesterol in this way gave rise to a mixture of 7α- and 7β-hydroxycholesterol which could be resolved by column partition chromatography.[5] It was found, however, that the major reduction product of 7-ketocholanic acid with $NaBH_4$ was 7α-hydroxycholanic acid, and only traces of the β-isomer were produced. This result is in accord with a reaction mechanism proposed by Paddock[6] for the stereo-specific reduction of cyclic ketones with metal hydrides.

Experimental[7]

Methyl 7α-Acetoxy-3,12-diketocholanate.—To a solution of 14.5 g. of methyl cholate 7-acetate,[4] m.p. 175–176°, $[\alpha]^{26}D$ +15.5 ± 0.5°, dissolved in 160 ml. of glacial acetic acid was added 7.25 g. of CrO_3 which had been dissolved in 3.6 ml. of water and then diluted with 126 ml. of glacial acetic acid. The solution was heated on the steam-bath for 0.5 hour. At the end of this period the acetic acid was distilled *in vacuo* until solid began to separate, and 400 ml. of water was added to the residue in the distilling flask. A crystalline precipitate was obtained which was filtered off and washed thoroughly with water. The oxidation product was recrystallized by adding water to a solution of the crude methyl 7α-acetoxy-3,12-diketocholanate in hot methanol, giving 9.7 g. (67.8%) of colorless needles, m.p. 164–165°, $[\alpha]^{26}D$ +61.5 ± 1.0°.

Anal. Calcd. for $C_{27}H_{40}O_6$ (460.59): C, 70.40; H, 8.75. Found: C, 70.27; H, 8.80.

7α-Hydroxycholanic Acid.—Methyl 7α-acetoxy-3,12-diketocholanate (2.3 g.) was reduced by the Huang-Minlon modification of the Wolff–Kishner reduction[8] with 75 ml. of diethylene glycol, 6 g. of KOH and 15 ml. of an 85% solution of hydrazine hydrate. After the reduction the crude 7α-hydroxycholanic acid was recovered by acidification of the reaction mixture with HCl (congo red). This product was dried *in vacuo* and recrystallized from acetic acid by addition of water to the hot, saturated solution; yield 1.3 g. (69.2%) of stout prisms, m.p. 96–99°, $[\alpha]^{22}D$ +7 ± 4°. 7α-Hydroxycholanic acid has been reported to crystallize with one molecule of water of crystallization.[2] Determination of the neutral equivalent showed, however, that the crystals contained acetic acid which could not be removed by prolonged drying at 60° and 2 mm.

Anal. Calcd. for $C_{24}H_{40}O_3$: neut. equiv., 377. Found: neut. equiv., 294 ± 9.

7α-Formyloxycholanic Acid.—7α-Hydroxycholanic acid (200 mg.) was heated with 4 ml. of 88% formic acid at 60° for 4 hours. Excess formic acid was removed *in vacuo*, leaving a crystalline residue of crude 7α-formyloxycholanic acid. After three crystallizations from aqueous methanol there were obtained 90 mg. of colorless needles, m.p. 148–149°, $[\alpha]^{25}D$ +2 ± 1°.

Anal. Calcd. for $C_{25}H_{40}O_4$ (404.56): C, 74.22; H, 9.96. Found: C, 74.63; H, 9.81.

Methyl 7-Ketocholanate.—7α-Hydroxycholanic acid (5.8 g.) was dissolved in 60 ml. of absolute methanol containing 0.6 ml. of concentrated sulfuric acid. After standing for 18 hours the mineral acid was neutralized with a saturated aqueous solution of $NaHCO_3$. The methyl 7α-hydroxycholanate was extracted with ether and recovered from the dried ether solution after evaporation of the ether on the steam-bath. An oil (5.7 g.) was obtained which could not be crystallized and was oxidized directly: The methyl ester was dissolved in 162 ml. of glacial acetic acid, and to this solution there was added 3.3 g. of potassium chromate dissolved in 6 ml. of water.[4] After standing for two hours the acetic acid was distilled off *in vacuo* until solid began to separate. Dilution of the concentrated residue with ice-water yielded crystalline methyl 7-ketocholanate. This product was washed with water and recrystallized from aqueous methanol, yielding 3.84 g. (64.2%) of long colorless needles, m.p. 93–94°, $[\alpha]^{27}D$ −56.5 ± 1.7°.

Anal. Calcd. for $C_{25}H_{40}O_3$ (388.57): C, 77.27; H, 10.38. Found: C, 77.68; H, 10.45.

7-Ketocholanic Acid.—Methyl 7-ketocholanate (2 g.) was dissolved in 50 ml. of absolute methanol containing 3 g. of KOH. After refluxing for three hours the reaction mixture was cooled and diluted with 100 ml. of ice-water. This solution was acidified with HCl (congo red), and the crude keto acid was filtered off, washed with water and dried. Crystallization from aqueous methanol yielded 1.25 g. (65.2%) of colorless needles, m.p. 150–151°, $[\alpha]^{27}D$ −58.0 ± 1.9°.

Anal. $C_{24}H_{38}O_3$ (374.54): C, 76.96; H, 10.23; neut. equiv., 375. Found: C, 76.81; H, 10.10; neut. equiv., 380 ± 3.

$NaBH_4$ Reduction of 7-Ketocholanic Acid.—7-Ketocholanic acid (400 mg.) was dissolved in 20 ml. of methanol, and 4 ml. of a 5% solution of $NaBH_4$ in methanol was added dropwise with shaking. The reaction mixture was allowed to stand at room temperature (25°) overnight. At the end of this period an equal volume of water was added, and the solution was acidified with dilute HCl (congo red). After standing at room temperature for two hours, the reaction product was extracted with ether. The ether solution was washed with water, dried and the ether was evaporated. A non-crystalline material was obtained, weighing 375 mg. which was treated as follows: 34.4 mg. was esterified with methanol and sulfuric acid and oxidized with potassium chromate as described above. There were obtained 24.1 mg. of methyl 7-ketocholanate, m.p. 92–93°, not depressed by admixture with a sample of methyl 7-ketocholanate, prepared as described above. A second portion (200 mg.) was recrystallized from aqueous acetic acid, yielding 74 mg. of crystalline 7α-hydroxycholanic acid, m.p. 96–98°, not depressed by admixture with an authentic sample of 7α-hydroxycholanic acid prepared as described above. A third portion (56.7 mg.) was subjected to column partition chromatography as described below.

Column Partition Chromatography.—Details of this method have been described.[5,9,10] A known mixture of 7-ketocholanic acid and 7α-hydroxycholanic acid was separated with a 0.79 sq. cm. × 30 cm. partition column using propylene glycol (supported on Celite 545) as stationary phase, and petroleum ether (60–68°) containing increasing amounts of isopropyl ether as mobile phase. Elution was started with petroleum ether alone; 7-ketocholanic acid was eluted with petroleum ether containing 1% (by volume) of isopropyl ether; 7α-hydroxycholanic acid was eluted with 5% (by volume) isopropyl ether in petroleum ether. The acidic substances in the column effluent were determined by titration with 0.01 *N* NaOH after evaporation of the organic solvent on the steam-bath under a current of air. When 56.7 mg. of the reaction product from the 24-hour $NaBH_4$ reduction of 7-ketocholanic acid was analyzed by this procedure, it was found that 42.6 mg. (75%) of 7α-hydroxycholanic acid was obtained plus 3.8 mg. (7%) of an unidentified material, probably 7β-hydroxycholanic acid. No 7-ketocholanic acid could be recovered from the sample.

Acknowledgments.—This work was supported in part by grants from the National Heart In-

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The Preparation of 1,5,6-Trimethylbenzotriazole and 1-Methyl-v-triazole-4,5-dicarboxylic Acid

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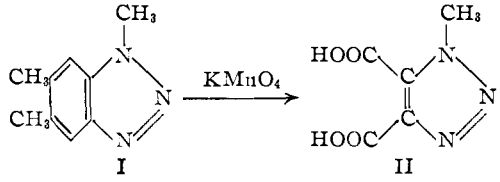
RECEIVED JUNE 9, 1954

During the course of an investigation of riboflavin biosynthesis[2] it was desired to convert one of the products of chemical degradation of the vitamin, 1,2-dimethyl-4-amino-5-methylaminobenzene, to 1,-5,6-trimethylbenzotriazole (I), and the latter to 1-methyl-v-triazole-4,5-dicarboxylic acid (II). These triazole derivatives have not been described previously in the literature.



I II

1,5,6-Trimethylbenzotriazole was prepared in two ways. In the first method 1,2-dimethyl-4-amino-5-methylaminobenzene was treated with nitrous acid by a modification of the procedure of Zincke.[3] The desired product was obtained in 30–40% yield. 1,5,6-Trimethylbenzotriazole also was synthesized by treatment of 5,6-dimethylbenzotriazole with dimethyl sulfate under conditions analogous to those described for the preparation of N-methylbenzotriazoles by Krollpfeiffer, *et al.*[4] The resulting 1,5,6-trimethylbenzotriazole and 2,5,6-trimethylbenzotriazole were separated by passage of hydrogen chloride gas through a dry ether solution of the mixture of these isomeric benzotriazoles. The more basic 1,5,6-trimethylbenzotriazole precipitates as a hydrochloride under these conditions.[4] The starting compound, 5,6-dimethylbenzotriazole, was prepared by the reaction of 1,2-dimethyl-4,5-diaminobenzene with nitrous acid under the conditions described for the synthesis of benzotriazole.[5] The melting points of 1,5,6-trimethylbenzotriazole prepared by either procedure were in agreement and no depression occurred upon mixing.

A second compound, presumably 2,5,6-trimethylbenzotriazole, was recovered from the ether mother liquor after the removal of 1,5,6-trimethylbenzotriazole as the hydrochloride. This compound had a melting point only 6° below that of 1,5,6-trimethylbenzotriazole but showed a marked depression of melting point when mixed with the latter.

It was shown by Bladin[6] that benzotriazole can be converted to v-triazole-4,5-dicarboxylic acid by oxidation with potassium permanganate. When either 1-methylbenzotriazole or 1,5,6-trimethylbenzotriazole was treated in this manner, 1-methyl-v-triazole-4,5-dicarboxylic acid was obtained. This dicarboxylic acid was identified further by thermal decarboxylation to 1-methyl-v-triazole which was characterized as the gold chloride double salt, m.p. 160°.[7]

Experimental

5,6-Dimethylbenzotriazole.—A sample of 11.5 g. of 1,2-dimethyl-4,5-diaminobenzene[8] was dissolved in a mixture of 9.8 ml. of glacial acetic acid and 25 ml. of water. The solution was cooled to 4°. A solution of 6.4 g. of sodium nitrite in 10 ml. of water was added. The temperature rose to 70° and the reaction was then kept at room temperature for 12 hours. The mixture was chilled to 0° for 1 hour and filtered. The residue was washed with 25 ml. of water, filtered and dried *in vacuo* at 50°. The compound was crystallized from 700 ml. of benzene. A total of 6.7 g. of product was obtained, m.p. 156–157°.

1,5,6-Trimethylbenzotriazole. Method A.—A solution of 6.7 g. of 5,6-dimethylbenzotriazole in 56 ml. of 2 *N* NaOH was prepared. To this stirred solution 6.7 ml. of dimethyl sulfate was added over a period of 15 minutes. The reaction was then placed in a boiling water-bath for 20 minutes. Upon cooling to 0° an oil formed which solidified. The mixture was filtered and the residue was taken up in 300 ml. of ether. The ether solution was dried with anhydrous $CaSO_4$. HCl gas was passed through the ether solution. A white precipitate was obtained which was washed with ether on the filter. This residue was placed in 125 ml. of boiling water; the hot aqueous solution was adjusted to *p*H 8–9 with concd. NH_4OH and left at room temperature for 12 hours. The resulting precipitate was recrystallized from 800 ml. of water, 4.1 g., m.p. 136–137°.

Anal.[9] Calcd. for $C_9H_{11}N_3$: C, 67.04; H, 6.88; N, 26.01. Found: C. 66.85; H, 6.84; N, 25.97.

The ether mother liquor obtained after the removal of the hydrochloride of 1,5,6-trimethylbenzotriazole was evaporated to dryness. The residue was taken up in 200 ml. of hot water. Sodium hydroxide was added to the hot solution till the reaction was definitely alkaline. The crystals which formed on cooling were washed with water and dried *in vacuo*, 1.2 g., m.p. 130–131°. This is presumably 2,5,6-trimethylbenzotriazole.

Anal. Calcd. for $C_9H_{11}N_3$: C, 67.04; H, 6.88; N, 26.07. Found: C, 67.39; H, 6.53; N, 25.63.

1,5,6-Trimethylbenzotriazole. Method B.—Amounts of 50 mg. of 1,2-dimethyl-4-amino-5-methylaminobenzene hydrochloride[8,10] and 24 mg. of sodium bisulfite were dissolved in 4 ml. of 0.223 *M* acetic acid at 0°. Sodium nitrite (77 mg.) in 3 ml. of water was added to the stirred solution over 1 hour. The reaction was left at 0° for 3 hours. The compound was recrystallized from water, 15 mg., m.p. 136–137°.

1-Methyl-v-triazole-4,5-dicarboxylic Acid. (a) Oxidation of 1,5,6-Trimethylbenzotriazole.—A sample of 0.5 g. of 1,5,6-trimethylbenzotriazole in 91 ml. of water was placed in a boiling water-bath. A dropwise addition of 5.5 g. of $KMnO_4$ in 25 ml. of water was made over a period of 30 minutes with stirring. The reaction was kept in the boiling water-bath for an additional 8 hours. At the end of this time enough methanol was added to discharge the purple color of permanganate. Manganese dioxide was removed and washed with 50 ml. of hot water. The combined fil-

(1) Department of Biochemistry, New York University College of Medicine, New York 16, N. Y. Supported in part by research grants from the Wisconsin Alumni Research Foundation and the National Heart Institute (No. H-1279), United States Public Health Service. This work was done during the tenure of an Established Investigatorship of the American Heart Association.

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