were not purified but were used directly for the cyclization reaction.

Cyclization of Azo Compounds to Cinnoline Derivatives.—For the cyclization reaction the azo compounds were dissolved in about 10 parts of acetic anhydride containing 0.02 part of concentrated sulfuric acid, and the solutions were refluxed for thirty to sixty minutes. (The sulfuric acid was omitted in the case of the pyridine derivative derived from 3-aminopyridine.) The solutions were then concentrated *in vacuo* to a small volume, and ether was added. The products were filtered and washed thoroughly with ether. The other pertinent details are given in Table I.

Hydrolysis of 2-*m*-Acetylphenyl-3-acetoxy-6-keto-2,6-dihydrocinnoline (VI).—This acetyl compound (0.5 g.) was dissolved in 50 ml. of concentrated hydrochloric acid, and the solution was refluxed for fifteen minutes. The yellow product began to separate from the reaction mixture toward the end of the reflux period. The solvent was then distilled *in vacuo*, and water was added to the residue. The product was filtered and washed with water and dried. The yield was practically quantitative. Pure 2-*m*-acetylphenyl-3,6-diketo-2,3,4,6-tetrahydrocinnoline (VII) was obtained by precipitation from pyridine solution with ether, m. p. 290–300°, dec.

Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32. Found: C, 68.53; H, 4.45.

Acetylation of 2-*m*-Acetylphenyl-3,6-diketo-2,3,4,6-tetrahydrocinnoline (VII).—The diketo compound (VII), when treated with acetic anhydride in the manner described above for the azo compounds, gave a 70% yield of VI.

Reduction of 2-β-Naphthyl-3-acetoxy-6-keto-2,6-dihydrocinnoline.—The quinonoid compound (1.8 g.) was dissolved in 100 ml. of boiling glacial acetic acid, and 3.6 g. of zinc dust was added gradually. After a few minutes the mixture was filtered through sintered glass and the filtrate was concentrated *in vacuo* to a small volume. Addition of water precipitated the product, which was obtained in the theoretical yield. The 2-β-naphthyl-3-acetoxy-6-hydroxy-1,2-dihydrocinnoline was purified by recrystallization from dilute methanol, and was obtained as colorless plates, m. p. 131–133°.

Anal. Calcd. for $C_{20}H_{16}N_2O_3$: C, 72.27; H, 4.85. Found: C, 72.60; H, 4.98.

Two other dihydro derivatives were similarly prepared: 2-*m*-acetylphenyl-3-acetoxy-6-hydroxy-1,2-dihydrocinnoline, m. p. 164–166°, 70%.

Anal. Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.65; H, 4.97. Found: C, 66.15; H, 4.86. 2-*p*-Acetoxyphenyl-3-acetoxy-6-hydroxy-1,2-dihydrocinnoline (XIV), m. p. 164–165°, 83%. *Anal.* Calcd. for $C_{18}H_{16}N_2O_5$: C, 63.52; H, 4.74. Found: C, 63.32; H, 4.87.

Reductive Acetylation of 2-β-Naphthyl-3-acetoxy-6-keto-2,6-dihydrocinnoline.—This compound (2.0 g.) was dissolved in a mixture of 40 ml. of acetic anhydride and 20 ml. of glacial acetic acid, and 3 g. of zinc dust was added. The mixture was then refluxed for one-half hour, after which it was filtered and the filtrate concentrated *in vacuo* to a straw-colored oil. The oil was taken up in ether, washed with water, and the ether solution was dried over calcium chloride (acetone was added to prevent precipitation of the product at this point). Concentration of the ether solution *in vacuo* gave the product; yield, 32%. The 1-acetyl-2-β-naphthyl-3-acetoxy-6-hydroxy-1,2-dihydrocinnoline (IX) was recrystallized several times from benzene–petroleum ether for analysis, m. p. 141–143°.

Anal. Calcd. for $C_{22}H_{18}N_2O_4$: C, 70.58; H, 4.85; N, 7.48. Found: C, 70.57; H, 5.01; N, 7.66, 7.37.

Oxidation of 2-*m*-Acetylphenyl-3-acetoxy-6-hydroxy-1,2-dihydrocinnoline.—This colorless dihydro derivative (2.3 g.) was dissolved in 50 ml. of acetic acid at room temperature, and to it was added a solution of 4.0 g. of ferric chloride crystals in 5 ml. of water and 1.0 ml. of concentrated hydrochloric acid. Upon dilution of the solution with two volumes of water the yellow 2-*m*-acetylphenyl-3-acetoxy-6-keto-2-6-dihydrocinnoline separated. It was filtered and washed with water; yield, 84%; m. p. 236–238°, dec.

Summary

1. Azo dyes formed by coupling diazotized aromatic amines with *m*-hydroxyphenylacetic acid have been found to cyclize to 2-aryl-substituted cinnolines under acetylating conditions.

2. This new synthesis of cinnoline derivatives has been used to prepare heterocyclic analogs of the steroid hormones.

3. Several of the compounds show a slight estrogenic activity.

INDIANAPOLIS, INDIANA RECEIVED OCTOBER 20, 1947

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF THE UNIVERSITY OF CALIFORNIA, BERKELEY]

The Synthesis of Palmitic Acid and Tripalmitin Labeled with Carbon Fourteen

BY WILLIAM G. DAUBEN

Isotopic high molecular weight fatty acids, such as palmitic and stearic, have been prepared containing deuterium and radioactive bromine. In order to study the fate of the carbon chain itself, palmitic acid containing radioactive carbon was prepared.

The synthesis of palmitic acid labeled in the carboxyl group with C^{14} was readily accomplished, in a yield of 72%, by the carbonation of *n*-pentadecylmagnesium bromide with radioactive carbon dioxide.[1] Carboxyl-labeled tripalmitin was prepared from this acid in a yield of 75% by employing the acid chloride method of Stephenson.[2] The tripalmitin has a specific activity of 760 cts./min./mg. ester.

The preparation of *n*-hexadecanoic acid (palmitic acid) labeled at carbon atom six with C^{14} was carried out as shown in the scheme.

$$C_{10}H_{21}MgBr \xrightarrow{C^*O_2} C_{10}H_{21}C^*OOH \xrightarrow{CH_2N_2}$$

I II

$$C_{10}H_{21}C^*OOCH_3 \xrightarrow{CuCr_2O_4} C_{10}H_{12}C^*H_2OH \xrightarrow{HBr}$$

III IV

$$C_{10}H_{21}C^*H_2Br \xrightarrow[2.\ CdCl_2]{1.\ Mg.}$$

(1) Dauben, Reid and Yankwich, *Anal. Chem.*, **19**, 828 (1947).

(2) Stephenson, *Biochem. J.*, **7**, 429 (1913).

$$(C_{10}H_{21}C^*H_2)_2Cd \xrightarrow[2.\ KOH]{1.\ CH_3OOC(CH_2)_3COCl}$$

VI

$$C_{10}H_{21}C^*H_2CO(CH_2)_3COOH \xrightarrow[NH_2NH_2]{NaOH}$$

VII

$$C_{10}H_{21}C^*H_2(CH_2)_4COOH \quad \text{Yields: } C^*O_2 \longrightarrow$$

VIII

III, 83%; III ⟶ V, 79%; V ⟶ VIII, 54%

It was found necessary to use three times the normal amount of copper chromite catalyst in the hydrogenolysis of ester III to obtain high yields consistently.[3] This was probably due to the fact that the methanol produced in the reaction is a strong enough acid to destroy the activity of the catalyst. The over-all yield of palmitic acid, based on barium carbonate, was 30% and the palmitic acid has a specific activity of 40,000 cts./min./mg. acid.

After this work had been completed, Houston[4] reported the synthesis 4-keto-*n*-hexadecanoic acid by the same general procedure employing *n*-dodecyl bromide and β-carbomethoxypropionyl chloride. However, the yield of the keto-acid isolated, based on the bromide, was only 7.4%. In view of the great discrepancy in this yield and the yield obtained in the above synthesis, the reaction was investigated. It was found when the procedure outlined in this paper for the preparation of the 5-keto-acid was followed, the 4-keto-acid was obtained in 69% yield. The failure of Houston to obtain a high yield may have been due to the fact that insufficient time was allowed for the conversion of the Grignard reagent to the dialkylcadmium compound.

Acknowledgment.—The author wishes to express his appreciation to the Bio-Organic Group of the Radiation Laboratory for their kind assistance.

Experimental[5]

1-Pentadecanol. (a) From Paraformaldehyde.[6]—*n*-Tetradecylmagnesium bromide was prepared in an all-glass apparatus under nitrogen from 1.54 g. (0.0623 mole) of magnesium turnings and 15.5 g. (0.057 mole) of redistilled *n*-tetradecyl bromide in 60 cc. of anhydrous di-*n*-butyl ether. The bromide solution was added to the magnesium during one hour, and the temperature was kept at 35° or below. The temperature was raised to 105° and 2.84 g. (0.094 mole) of paraformaldehyde (dried in a vacuum desiccator) was added in small portions over a period of thirty minutes. After the addition was complete, the clear solution was heated for an additional period of forty-five minutes at 105° during which time the solution became cloudy. The heating was then continued for one hour at 110°, the reaction mixture was cooled, decomposed in the usual manner with dilute sulfuric acid, and the ether was removed by steam distillation. The cooled residue from the distillation was extracted with ether, the solvent was removed and the product was distilled. A small amount of 1-pentadecanol (2.2 g., 17.2%, b. p. 118° (0.3 mm.)) was obtained. The pot residue was heated for six hours on a steam-bath with 15 cc. of absolute ethanol and 3 cc. of concentrated hydrochloric acid but only *n*-octacosane was isolated.

When the Grignard reagent was prepared at a temperature above 35°, the yield was only slightly less, 15.7%.

(b) **From the Ester.**—Ethyl *n*-pentadecanoate (14.6 g., 0.054 mole) was hydrogenated over copper chromite catalyst (2.5 g.). The initial pressure was 3000 p. s. i. at room temperature and hydrogenolysis took place readily at 250° and was complete in five to six hours. The 1-pentadecanol distils at 112–114° (0.2 mm.), yield 10.5 g. (85.4%).

***n*-Pentadecyl Bromide.**—The bromide was prepared in the usual manner[7] employing anhydrous hydrogen bromide and 1-pentadecanol (27.3 g., 0.12 mole) except that the reaction mixture was diluted with 50 cc. of *n*-hexane before processing. *n*-Pentadecyl bromide boils at 127–128° (0.5 mm.), yield 28.4 g. (82.7%).

Carboxyl-Labeled Hexadecanoic Acid.—*n*-Pentadecylmagnesium bromide was prepared in an all-glass apparatus in a nitrogen atmosphere from 1.6 g. (0.066 mole) of magnesium turnings and 15.9 g. (0.0547 mole) of *n*-pentadecyl bromide in 110 cc. of anhydrous ether. An aliquot of the solution was titrated and the concentration was found to be 0.00044 mole of Grignard reagent per cc. of solution.

A volume of 110 cc. (0.0484 mole) of Grignard solution was carbonated with radioactive carbon dioxide generated from 9.1 g. (0.046 mole) of radioactive barium carbonate with a specific activity of 1060 cts./min./mg.[8] following the procedure described in detail in previous publications. The acid was isolated in the usual manner and converted directly to the methyl ester. The ester distils at 132–133° (0.3 mm.), yield 10.0 g. (80.4%), $n^{25}D$ 1.4386.

The ester (10.0 g., 0.037 mole) was saponified with a solution of 2.3 g. of potassium hydroxide, 35 cc. of methanol, and 2 cc. of water by heating on a steam-bath overnight. The crude acid was recrystallized from 40 cc. of ten per cent. aqueous acetone. The pure acid melts at 60–61°, yield 8.55 g. (90.1%). The over-all yield based on barium carbonate was 72.4%, specific activity × 16: 1050 cts./min./mg. barium carbonate,[9] activity of compound: 810 cts./min./mg. acid.

Carboxyl-labeled Tripalmitin.—Palmityl chloride was prepared as described by earlier workers[10] from 8.3 g. (0.0324 mole) of the carboxyl-labeled palmitic acid prepared above and 10 cc. of purified thionyl chloride.

Tripalmitin was prepared by slow addition, with stirring, of a chloroform solution of palmityl chloride to a cooled mixture of 0.975 g. (0.0106 mole) of redistilled glycerol, 8 cc. of dry pyridine, and 25 cc. of dry chloroform.[2] The light-yellow solution was allowed to stand three days at room temperature during which time it gradually darkened. The mixture was processed in the usual manner, and the residual light-tan solid was recrystallized twice from acetone (Norit) to give white tripalmitin, m. p. 61–62°, sinters 59°, yield 6.15 g. (75.5%). The over-all yield from barium carbonate was 54.7%, specific activity

(3) Adkins and Folkers, THIS JOURNAL, **53**, 1095 (1931).

(4) Houston, *ibid.*, **69**, 517 (1947).

(5) All melting points are corrected. Microcombustions by Mr. C. W. Koch and Mrs. W. B. Dandliker.

(6) Marvel, Blomquist and Vaughn, THIS JOURNAL, **50**, 2810 (1928).

(7) "Org. Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 246.

(8) All measurements of radioactivity were carried out with a thin mica-window Geiger–Müller tube on a scale of 64 circuit with a geometry of 17.6 ± 2.5 disintegrations per count. The activity was determined with thin uniform layers of barium carbonate according to the procedure described in earlier publications. The over-all counting error was ±2%.

(9) This value was obtained by the oxidation of a microsample with Van Slyke's oxidizing solution and precipitation of the carbon dioxide as barium carbonate, which was counted. To correct for the dilution of activity in the compound, the observed specific activity was multiplied by sixteen.

(10) (a) Hann and Jamieson, THIS JOURNAL, **50**, 1442 (1928); (b) Rose, *ibid.*, **69**, 1384 (1947).

× 17: 1040 cts./min./mg. barium carbonate, activity of compounds: 760 cts./min./mg. tripalmitin.

Carboxyl-labeled Methyl *n*-Hendecanoate (III).—*n*-Decylmagnesium bromide was prepared as described for the other Grignard reagents from 1.8 g. (0.074 mole) of magnesium turnings and 15 g. (0.068 mole) of *n*-decyl bromide dissolved in 80 cc. of anhydrous ether. An aliquot of the solution was titrated and the concentration was found to be 0.00075 mole of Grignard reagent per cc.

A volume of 15.7 cc. (0.0117 mole) of Grignard solution was carbonated with radioactive carbon dioxide generated from 1.860 g. (0.00942 mole) of radioactive barium carbonate with a specific activity of 54,400 cts./min./mg. following the procedure described previously.[1] The acid was isolated in the usual manner and was then methylated with an ethereal solution of diazomethane. The ester was distilled in a small sublimation-type still, block temperature 54–58°, pressure 0.3 mm., yield 1.560 g. (82.9%), $n^{23}D$ 1.4275, specific activity × 12: 53,500 cts./min./mg. barium carbonate, activity of compound: 52,800 cts./min./mg. ester.

Anal. Calcd. for $C_{13}H_{24}O_2$: C, 71.95; H, 12.07. Found: C, 71.97; H, 11.95.

***n*-Hendecyl Bromide Labeled at Carbon Atom One (V).**—Carboxyl-labeled methyl *n*-hendecanoate (1.538 g., 0.00767 mole) was hydrogenated over copper chromite catalyst (1.0 g.) at an initial pressure of hydrogen of 3000 p. s. i. at room temperature. The hydrogenolysis took place at 250° and was complete in six hours. The crude alcohol was converted directly to the bromide by passage of anhydrous hydrogen bromide through the alcohol at steam-bath temperature.[7] The *n*-hendecyl bromide was distilled in a small sublimation-type still, block temperature 70–75°, pressure 0.5 mm., yield 1.432 g. (79.3%), $n^{25}D$ 1.4548, specific activity × 11: 53,100 cts./min./mg. barium carbonate; activity of compound: 44,800 cts./min./mg. bromide.

Anal. Calcd. for $C_{11}H_{23}Br$: C, 56.17; H, 9.86. Found: C, 56.61; H, 10.28.

***n*-Hendecanoic Acid Labeled at Carbon Atom Six (VIII).**—Methyl 5-keto-*n*-hexadecanoate was prepared following the procedure outlined by Cason and Prout except for one modification.[11] The Grignard reagent was prepared from 0.15 g. (0.0062 mole) of magnesium turnings and 1.412 g. (0.006 mole) of *n*-hendecyl bromide labeled at carbon atom one in 50 cc. of anhydrous ether. The resulting Grignard reagent was converted to the dialkylcadmium compound with 0.71 g. (0.00389 mole) of anhydrous cadmium chloride. After the addition of the cadmium chloride, the mixture was heated under reflux until a negative Gilman test for a Grignard reagent was obtained. This required about two hours. The ether was replaced with benzene, and the resulting suspension was treated with 1.00 g. (0.0061 mole) of γ-carbomethoxybutyryl chloride.[12] The reaction mixture, after heating under reflux for one hour, had set to a solid mass and then was decomposed as usual. The crude reaction mixture was directly saponified with a solution of 0.4 g. of potassium hydroxide in 10 cc. of methanol. After dilution to 50 cc. with water, the mixture was extracted with ether to remove the neutral compounds. *n*-Docosane (100 mg.) marked at carbon atoms eleven and twelve was isolated. The alkaline layer was acidified and then extracted with ether.

The crude keto acid was reduced by the modified Wolff-Kishner method[13] using 6.4 cc. of diethylene glycol, 0.8 g. of sodium hydroxide, and 0.77 cc. of one-hundred per cent. hydrazine hydrate. The crude acid was distilled onto a cold-finger type condenser at a bath temperature of 110° and pressure of 1 mm. The distillate was recrystallized from 15 cc. of ten per cent. aqueous acetone (Norit), m. p. 61–62°, yield 700 mg. (45.7%), specific activity × 16: 52,700 cts./min./mg. barium carbonate, activity of compound: 40,500 cts./min./mg. acid.

Anal. Calcd. for $C_{16}H_{32}O_2$: C, 74.94; H, 12.58. Found: C, 74.55; H, 12.47.

In a practice experiment with non-radioactive *n*-hendecyl bromide (6.71 g., 0.00286 mole) and γ-carbomethoxybutyryl chloride (3.76 g., 0.0228 mole), the keto acid was isolated, and recrystallized from methanol, m. p. 84.5–85°, yield, 4.87 g. (64% based on bromide, 79% based on acid chloride). This acid, when subjected to the Wolff-Kishner reaction, was reduced to palmitic acid in a yield of 82%.

Summary

1. Palmitic acid and tripalmitin, labeled in the carboxyl carbon with carbon fourteen, have been prepared.

2. Palmitic acid, labeled at carbon atom six with carbon fourteen, has been synthesized.

3. A re-examination of the work of Houston on the synthesis of 4-ketohexadecanoic acid by the cadmium procedure, has shown that it is critical to allow sufficient time for the conversion of a Grignard reagent to a dialkylcadmium compound.

BERKELEY 4, CALIFORNIA RECEIVED AUGUST 4, 1947

(11) Cason, THIS JOURNAL, 2078 (1946), and earlier papers.

(12) The acid chloride was prepared from half ester that had been fractionally distilled through a column (b. p. 156° (10 mm.)) and purified thionyl chloride. The chloride boils at 84° (7 mm.); see Harris, Wolf, *et al.*, THIS JOURNAL, **67**, 2096 (1945), and "Org. Syntheses," **25**, 19 (1945).

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