same ketoacid exhibit essentially the same absorption spectrum in the range of 400 to 600 mμ. Furthermore, while the spectra of the single KAP's differ widely, those of KPP's are all very similar. KPP spectra, if compared to corresponding KAP spectra, show higher values of λ_{max} and of ϵ at λ_{max} and are characterized by the absence of the plateau between 480 and 540 mμ.

By paper chromatography, all KPP's give single yellow spots, which turn reddish after spraying with *n*-butanol saturated with 2.5 *N* NaOH (KAP main spots turn brown; faster spots, when present, do not change their yellow color).

The availability of synthetic PG allowed a comparison of its phenylhydrazone with the one isolated after transamination between AG and pyridoxal. The two compounds exhibited identical melting points (no depression by mixing), absorption spectra and R_f values.

MODENA, ITALY

[CONTRIBUTION FROM THE DIVISION OF CANCER RESEARCH, DEPARTMENT OF SURGERY, UNIVERSITY OF ROCHESTER MEDICAL CENTER]

A Method for the Synthesis of C_{21}-Labeled Cholesterol

BY P. KURATH AND MARGARET CAPEZZUTO

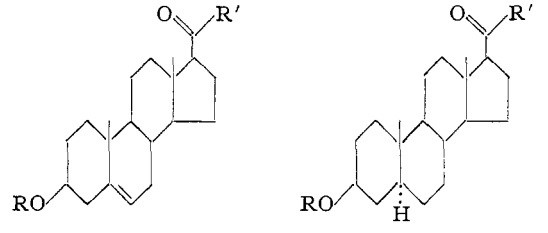
RECEIVED SEPTEMBER 29, 1955

A modification of Woodward's[3] and Robinson's[4] synthesis of the cholesterol side chain is described. This modification involves an alteration of the sequence of addition of carbon atoms to the C-20 acid. In this work carbon atom 21 is added after the addition of carbon atoms 22–27 to the C-20 acid. The synthesis was used to prepare cholestanol-21-^{14}C acetate which was then converted by known procedures to cholesterol-21-^{14}C.

The fact that cholesterol is a precursor of the adrenocortical hormones has been established.[1,2] What remains to be clarified, however, is the way in which the cholesterol side chain is degraded during the biosynthesis of the C-21 steroids. One approach to the study of the latter problem would be to utilize cholesterol labeled at C_{21}. Accordingly this report presents a method for the synthesis of such a labeled cholesterol.

Previous publications by Woodward[3] and Robinson[4] describing the total synthesis of cholesterol offered a method for the preparation of the C_{21}-labeled compound. For practical reasons it seemed more desirable to introduce the labeled carbon atom as late as possible. Therefore, the isohexyl side chain (carbon atoms 22–27) was added first to the C-20 acid followed by the introduction of the methyl group (carbon atom 21) as the last step to complete the carbon skeleton of cholesterol. A similar sequence of steps was followed in the syntheses of the 20α- and 20β-hydroxy compounds of the Δ^5-3β-hydroxy-24,24-dimethylcholen and of the corresponding saturated compound.[5]

As a convenient starting material for the synthesis, Δ^5-3β-acetoxyetiocholenic acid (I)[6] was chosen and converted into its acid chloride II.[5,7] The latter (II) was then allowed to react with diisohexylcadmium to yield 21-nor-20-ketocholesterol acetate (III).[8] Upon saponification of III, the free alcohol IV[8] was obtained. Hydrogenation of III yielded 21-nor-20-ketocholestanol acetate (VII).



I, R = Ac, R′ = OH
II, R = Ac, R′ = Cl
III, R = Ac, R′ = isohexyl
IV, R = H, R′ = isohexyl

V, R = Ac, R′ = OH
VI, R = Ac, R′ = Cl
VII, R = Ac, R′ = isohexyl
VIII, R = H, R′ = isohexyl

In another approach to the synthesis of VII, the acid acetate I was reduced to 3β-acetoxyetioallocholanic acid (V),[6] followed by the conversion to the corresponding acid chloride VI[3,5] which yielded with diisohexylcadmium the desired 21-nor-20-ketocholestanol acetate (VII). Upon mild saponification 21-nor-20-ketocholestanol (VIII) was obtained.

The acetate, VII, as well as the free alcohol, VIII, on reaction with methylmagnesium iodide yielded a mixture of the two possible stereoisomeric 20-hydroxycholestanols.[3,4] This mixture after treatment with acetic acid and acetic acid–acetic anhydride[3,4,9] yielded, upon hydrogenation over reduced platinum oxide,[3] cholestanol acetate (IX), which was identical with the natural product. Saponification of the acetate IX yielded cholestanol

(1) For reviews on the subject see, *e.g.*, O. Hechter in "Ciba Foundation Colloquia on Endocrinology, Volume VII, Synthesis and Metabolism of Adrenocortical Steroids," Little, Brown and Co., Boston, 1953, p. 161; S. Lieberman and S. Teich, *Pharmacol. Revs.*, **5**, 285 (1953).

(2) N. Saba, O. Hechter and D. Stone, THIS JOURNAL, **76**, 3862 (1954); W. S. Lynn, Jr., E. Staple and S. Gurin, *ibid.*, **76**, 4048 (1954); E. Reich and A. L. Lehninger, *Biochim. Biophys. Acta*, **17**, 136 (1955).

(3) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler and W. M. McLamore, THIS JOURNAL, **74**, 4223 (1952); R. B. Woodward, F. Sondheimer and D. Taub, *ibid.*, **73**, 3548 (1951).

(4) H. M. E. Cardwell, J. W. Cornforth, S. R. Duff, H. Holtermann and R. Robinson, *J. Chem. Soc.*, 361 (1953).

(5) J. R. Billeter and K. Miescher, *Helv. Chim. Acta*, **32**, 564 (1949).

(6) M. Steiger and T. Reichstein, *ibid.*, **20**, 1040 (1937).

(7) M. Steiger and T. Reichstein, *ibid.*, **20**, 1164 (1937).

(8) A. Wettstein (*ibid.*, **23**, 1371 (1940)) obtained the free alcohol IV by treating the acid chloride II with the diethyl ester of the sodium derivative of isoamylmalonic acid followed by the saponification and the decarboxylation of the intermediate β-ketoester. Upon acetylation of IV the acetate III was obtained.

(9) *Cf.* A. Butenandt and H. Cobler, *Z. physiol. Chem.*, **234**, 218 (1935); A. Butenandt and G. Müller, *Ber.*, **71**, 191 (1938); B. Koechlin and T. Reichstein, *Helv. Chim. Acta*, **27**, 549 (1944).

(X), which again was identical with the natural product. Cholestanol-21-^{14}C acetate was prepared and then converted to cholesterol-21-^{14}C by methods described in the literature.[10]

Experimental[11-13]

21-Nor-20-ketocholesterol Acetate (III).—The acid chloride II of Δ^5-3β-acetoxyetiocholenic acid (I) was prepared by refluxing 1 g. of the acid acetate I with 5 ml. of thionyl chloride and 5 ml. of benzene for 2 hr.[7] The solvents were evaporated under reduced pressure and the dry crude acid chloride II dissolved in 15 ml. of anhydrous benzene. This solution was added dropwise with stirring to a cold suspension of diisohexylcadmium prepared from 3.44 g. of isohexyl bromide,[4] 0.35 g. of magnesium turnings in 25 ml. of ether and 1.65 g. of cadmium chloride in an atmosphere of nitrogen as described in the literature for a similar reaction.[14] This reaction mixture was treated as described later for the 21-nor-20-ketocholestanol acetate (VII) to yield 1.165 g. of the crude acetate III. After chromatographic purification and recrystallization from methanol, 552 mg. (47% yield) of the desired compound III, m.p. 138–139°, was obtained. Upon concentration of the mother liquors an additional amount (103 mg., 9% yield) of a less pure material, m.p. 105–115°, was recovered. A portion from the first sample was recrystallized from methanol to a constant m.p. 140–142°, $[\alpha]^{25}D$ +11° (*c* 0.94).

21-Nor-20-ketocholesterol (IV).—The solution containing 150 mg. of the acetate III in 8 ml. of methanol was treated with 90 mg. of potassium carbonate in 1 ml. of water as described in the literature for a similar reaction,[15] to yield 138 mg. of the crude 21-nor-20-ketocholesterol (IV). This was recrystallized from aqueous methanol to give a product with a constant m.p. of 139–140°, $[\alpha]^{25}D$ +20° (*c* 1.22).

3β-Acetoxyetioallocholanic Acid (V) and the Acid Chloride VI.—The solution of 800 mg. of Δ^5-3β-acetoxyetiocholenic acid (I) in 56 ml. of acetic acid was reduced in the presence of 160 mg. of prereduced platinum oxide, according to the method described in the literature,[6] to yield after crystallization from methanol–water 640 mg. of 3β-acetoxyetioallocholanic acid (V), m.p. 245–247°. Concentration of the mother liquors yielded an additional 102 mg. of a less pure compound, m.p. 225–232°.

The acid chloride VI was prepared from 4.77 g. of 3β-acetoxyetioallocholanic acid (V) and 15 ml. of thionyl chloride, according to the literature,[3] dried and used for the conversion to the 21-nor-20-ketocholestanol acetate (VII).

21-Nor-20-ketocholestanol Acetate (VII). (a) From 3β-Acetoxyetioallocholanic Acid Chloride (VI).—The suspension of diisohexylcadmium was obtained from 16.5 g. of isohexyl bromide, 1.66 g. of magnesium turnings in 120 ml. of anhydrous ether and 7.9 g. of dry cadmium chloride as described before. The solution of the previously prepared acid chloride VI in 42 ml. of benzene was added dropwise to the cooled suspension of diisohexylcadmium in ether.[16] The reaction mixture was stirred in an atmosphere of nitrogen for 2 hr. in the cold and was then kept overnight at room temperature. The organic solvents, removed by the nitrogen stream, were replaced by benzene. The mixture was then steam distilled, the water suspension cooled and acidified with 2 *N* sulfuric acid, extracted with ether and the latter washed with 0.2 *N* sodium hydroxide and water. The organic extract was then dried over anhydrous sodium sulfate, filtered and concentrated to leave a dry residue of 5.776 g. of crude compound. To ensure complete acetylation this product was treated with 60 ml. of pyridine and 30 ml. of acetic anhydride overnight at room temperature. After working up as usual, 5.629 g. of crude 21-nor-20-ketocholestanol acetate (VII) was obtained. After chromatography of the latter on alumina (grade I/II), the eluates of petroleum ether–benzene 9:1, 8:2, 1:1 and benzene gave a total of 4.77 g. of the compound. This was recrystallized from methanol–water to yield 4.114 g. (72% yield) of the desired acetate VII, m.p. 83–86°. Concentration of the mother liquors yielded an additional amount of 0.25 g. (4% yield) of a less pure material, m.p. 78–82°. A portion of the first sample was recrystallized from aqueous methanol for analysis, m.p. 86–88°, $[\alpha]^{31}D$ +71° (*c* 1.36).

Anal. Calcd. for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 78.13; H, 10.61.

(b) From 21-Nor-20-ketocholesterol Acetate (III).—The acetate III (428 mg.) in 30 ml. of acetic acid was reduced over 80 mg. of prereduced platinum oxide and reoxidized with chromic acid, as described in the literature for pregnenolone acetate,[17] to yield 194 mg. of pure 21-nor-20-ketocholestanol acetate (VII), m.p. 87–88°. A mixture of this compound and an authentic sample showed no depression of the m.p. From the mother liquors an additional crop of 96 mg. of a less pure compound was obtained, m.p. 69–75°.

21-Nor-20-ketocholestanol (VIII).—A solution of 1.5 g. of 21-nor-20-ketocholestanol acetate (VII) in 80 ml. of methanol was saponified with 0.9 g. of potassium carbonate and 10 ml. of water by the same method as used for the corresponding unsaturated compound III.[15] After working up as usual, 1.38 g. of crude reaction product was obtained which after crystallization from methanol–water yielded 1.17 g. (87% yield) of pure 21-nor-20-ketocholestanol (VIII), m.p. 138–139°. After concentration the mother liquors yielded an additional amount of 150 mg. (11% yield) of a less pure compound, m.p. 116–127°. For analysis the first sample was recrystallized twice from methanol–water and showed a m.p. 138–139°, $[\alpha]^{31}D$ +83° (*c* 1.34).

Anal. Calcd. for $C_{26}H_{44}O_2$: C, 80.35; H, 11.41. Found: C, 80.66; H, 11.71.

Cholestanol Acetate (IX). (a) From 21-Nor-20-ketocholestanol Acetate (VII).—To the Grignard solution, prepared from 452 mg. (18.5 mmoles) of magnesium turnings and 3.29 g. (23.2 mmoles) of methyl iodide in 20 ml. of absolute ether in an atmosphere of nitrogen, the solution of 1 g. (2.32 mmoles) of 21-nor-20-ketocholestanol acetate (VII) in 15 ml. of dry benzene was added dropwise. The mixture was treated similarly as described below for the cholestanol-21-^{14}C acetate and 145 mg. (yield 14.5% based on steroid, 1.45% based on methyl iodide) of cholestanol acetate (IX), m.p. 103–105°, was obtained. An analytical sample, m.p. 108–109°, $[\alpha]^{32}D$ +13° (*c* 1.65), was prepared by further recrystallizations.

On admixture with the natural pure cholestanol acetate,[18] no depression in melting point was observed. The synthetic and the natural cholestanol acetate showed identical infrared spectra. Attempts to obtain more pure cholestanol acetate from the mother liquors of the first crop failed.

(b) From 21-Nor-20-ketocholestanol (VIII).—To the Grignard solution, prepared from one-half of the molecular equivalents used in the previous reaction, 1 g. of 21-nor-20-ketocholestanol (VIII) in 15 ml. of benzene was added and the mixture then treated as described below for the preparation and purification of the cholestanol-21-^{14}C acetate. After purification of the crude reaction product, 142 mg. of analytically pure cholestanol acetate (IX) was obtained, m.p. 108.5–109.5°, $[\alpha]^{30}D$ +12° (*c* 1.09). This compound was identical with the above obtained cholestanol acetate and the natural product, as shown by mixed melting point determination and infrared spectra. From the mother liquors a less pure sample of 70 mg. of this material, m.p. 99–101°, was obtained. The total yield of cholestanol acetate (IX)

(10) For references see papers of Woodward, *et al.*,[3] and Robinson, *et al.*[4]; L. Hellman, R. S. Rosenfeld, M. L. Eidinoff, D. K. Fukushima, T. F. Gallagher, C.-I. Wang and D. Adlersberg, *J. Clin. Invest.*, **34**, 48 (1955). For procedures used see Experimental part.

(11) Microanalyses by Mr. C. W. Beazley, Skokie, Illinois.

(12) The infrared spectra were determined by Mr. Carl A. Whiteman, Jr., Department of Chemistry, University of Rochester, Rochester, New York, on a Perkin–Elmer Infrared Spectrophotometer Model 12 AB in chloroform solution.

(13) The melting points are not corrected. All optical rotations were measured in chloroform solution. The analytical samples were dried under high vacuum over phosphorus pentoxide at 56° for one week.

(14) W. Cole and P. L. Julian, THIS JOURNAL, **67**, 1369 (1945); *cf.* L. F. Fieser and W.-Y. Huang, *ibid.*, **75**, 5356 (1953).

(15) A. Ruff and T. Reichstein, *Helv. Chim. Acta*, **34**, 70 (1951).

(16) In one experiment the ether was removed after the formation of the diisohexylcadmium and the reaction with the acid chloride VI was carried out in benzene (*cf.* J. Cason, *Chem. Revs.*, **40**, 15 (1947)). In this particular case the yield of the reaction was not improved by this method.

(17) Pl. A. Plattner, H. Heusser and E. Angliker, *Helv. Chim. Acta*, **29**, 468 (1946).

(18) Obtained from cholesterol acetate by hydrogenation; *cf.* W. F. Bruce and J. O. Ralls, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 191.

in this experiment was 19% based on steroid and 3.8% based on methyl iodide.

Cholestanol (X).—An amount of 110 mg. of the synthetic cholestanol acetate (IX) was hydrolyzed according to the method previously described[3] to yield 94 mg. of cholestanol (X), which after three recrystallizations from ethanol had a m.p. 141–142°, $[\alpha]^{30}D$ +24° (*c* 1.00). This compound was identical with the natural product, obtained by similar saponification of cholestanol acetate, as shown by mixed melting point determination.

Cholestanol-21-^{14}C Acetate.—The Grignard reagent was prepared by adding a solution of 3.684 g. (26 mmoles) of methyl iodide containing 1 mc. of methyl iodide-^{14}C in 40 ml. of ether to 505 mg. (20.8 mmoles) of magnesium turnings in an atmosphere of nitrogen. To this, the solution of 2 g. (5.15 mmoles) of 21-nor-20-ketocholestanol (VIII) in 30 ml. of dry benzene was added dropwise. A precipitate formed at once. This mixture was stirred in a nitrogen atmosphere for 1 hr. Following this the solvents were distilled until the b.p. reached 74°, and the volume of the solution was kept constant by the addition of benzene. The mixture was then refluxed with stirring for 3 hr. and after cooling, the Grignard complex was decomposed with 2 *N* sulfuric acid. The solution was then extracted with ether and the latter washed in the presence of ice-water, dried over anhydrous sodium sulfate, filtered and evaporated to leave a crude residue of 2.146 g. which probably represented a mixture of the two stereoisomeric 21-hydroxycholestanols. This mixture was refluxed with 30 ml. of acetic acid for 2 hr., then for an additional 40 minutes with 30 ml. of added acetic anhydride according to the procedures previously described.[3,4,9] The latter was then shaken with 530 mg. of prereduced platinum oxide in an atmosphere of hydrogen as previously described[3] and worked up similarly to yield 2.212 g. of a crude reaction product. To this, 1.2 g. of pure natural cholestanol acetate was added as a carrier. After chromatographic purification on 102 g. of alumina (grade I/II), a partly crystalline material amounting to 2.6 g. was obtained from the petroleum ether–benzene 9:1, 8:2, 1:1 and benzene eluates. This product yielded, after recrystallization from ethanol, 1.299 g. of pure cholestanol-21-^{14}C acetate, m.p. 108–110°. This sample was fully identical with the original sample. From the mother liquors an additional 292 mg. of cholestanol-21-^{14}C acetate, m.p. 101–104°, was obtained. This represented a net yield of 18% based on steroid and 3.6% based on methyl iodide. In terms of radioactivity, the recovery was 60 ± 6 μc. Further recrystallization yielded an additional 165 mg. of a less pure compound, m.p. 63–68°.

Cholestanone-3-21-^{14}C.—The saponification of cholestanol-21-^{14}C acetate (1582 mg.) was carried out as previously described,[3] and the crude cholestanol-21-^{14}C (1425 mg.) obtained was oxidized[19] to yield 1022 mg. of cholestanone-3-21-^{14}C, m.p. 125–127°, and a less pure sample of 114 mg., m.p. 124–126°. The total yield was 80%.

2,4-Dibromocholestanone-3-21-^{14}C.—The bromination was carried out using 1134 mg. of cholestanone-3-21-^{14}C with 940 mg. of bromine as previously described.[20] The dibromo compound was obtained in two crops, 754 mg., m.p. 188–190° and 509 mg., m.p. 187–189° (total yield 79%). An additional crop of 47 mg., m.p. 178–180°, was obtained but not used for further reactions.

Δ^4-Cholestenone-3-21-^{14}C.—The solution of 1263 mg. of 2,4-dibromocholestanone-3-21-^{14}C in 32 ml. of acetone was treated with 1516 mg. of sodium iodide as previously described,[21] and 1040 mg. of crude Δ^4-2-iodo-cholestenone-3-21-^{14}C was obtained. The latter was then treated with 25 ml. of chromous chloride solution[21] in 125 ml. of acetone and worked up as previously described.[21] After adding 400 mg. of Δ^4-cholestenone-3 as a carrier, 793 mg. of pure Δ^4-cholestenone-3-21-^{14}C, m.p. 79–80°, was obtained. From the mother liquors an additional 45 mg., m.p. 78–80°, was recovered. The net yield was 49%.

Δ^4-Cholestenone-3-21-^{14}C Enol Acetate.—This compound was prepared by treating 836 mg. of Δ^4-cholestenone-3-21-^{14}C with acetic anhydride and acetyl chloride.[22] A total of 816 mg. (88%) of the desired enol acetate, m.p. 79–81°, was obtained.

Cholesterol-21-^{14}C.—This compound was prepared from the enol acetate (816 mg.) by reduction with sodium borohydride as previously described.[23] From the crude reaction product (776 mg.), the digitonide was prepared[24] and split,[25] and 596 mg. of crude cholesterol-21-^{14}C was recovered. This was purified by chromatography and recrystallized from 95% ethanol to yield 487 mg. (66% yield) of pure cholesterol-21-^{14}C, m.p. 146–148°.

The activity of the cholesterol-21-^{14}C was determined with a thin window Geiger counter against a known standard to be 9.8 ± 0.98 μc./mmole.

All intermediates in the synthesis were identical with the natural products as shown by mixed melting point determinations.

Acknowledgment.—This investigation was supported by the John J. Morton Cancer Fund. The authors are indebted to Dr. J. J. Morton and Dr. M. Radakovich for constant encouragement during the course of this investigation. We thank Drs. M. Radakovich and Frank Ganis for their valuable help in the preparation of this manuscript. We wish to express our thanks to Mr. H. Mermagen and Mr. W. A. Kremer of the Atomic Energy Project of the University of Rochester for the determination of the radioactivity of the samples throughout the synthesis.

ROCHESTER, NEW YORK

(19) W. F. Bruce, ref. 18, p. 139.

(20) L. Ruzicka, W. Bosshard, W. H. Fischer and H. Wirz, *Helv Chim. Acta*, **19**, 1147 (1936); *cf.* A. L. Wilds and C. Djerassi, THIS JOURNAL, **68**, 1712 (1946).

(21) G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, *ibid.*, **72**, 4077 (1950).

(22) U. Westphal, *Ber.*, **70**, 2128 (1937); H. H. Inhoffen, *ibid.*, **69**, 2141 (1936).

(23) B. Belleau and T. F. Gallagher, THIS JOURNAL, **73**, 4458 (1951); *cf.* W. G. Dauben and J. F. Eastham, *ibid.*, **73**, 4463 (1951).

(24) A. Windaus, *Ber.*, **42**, 238 (1909).

(25) R. Schönheimer and H. Dam, *Z. physiol. Chem.*, **215**, 59 (1933).