and methyl isopropyl ketone were employed under the conditions of the acetaldehyde condensation (50 ml. of ethanolic potassium hydroxide). Products obtained were 2-methyl-2-pentenal, 16.7 g. (34.2%), b.p. 51–56° (50 mm.); propionaldol, 6.3 g. (11%), b.p. 91–93° (18 mm.) and residue, 16.7 g. The 2-methyl-2-pentenal gave a 2,4-dinitrophenylhydrazone derivative, m.p. 162–164°, which when mixed with an authentic sample showed no depression in melting point. The propionaldol on redistillation had b.p. 94° (20 mm.), $n^{25}D$ 1.4380 (lit.[16] b.p. 84–86° (11 mm.)). On standing 24 hours the aldol became characteristically very viscous, $n^{25}D$ 1.4500.[16] In another run in which 100 ml. of *N* ethanolic potassium hydroxide was employed the yield of 2-methyl-2-pentenal was 12% and propionaldol, 56%. No evidence for aldehyde–ketone condensation products could be detected.

Condensation of *n*-Butyric Anhydride with Methyl Isopropyl Ketone.—*n*-Butyric anhydride and methyl isopropyl ketone reacted by the procedure of Hauser and Adams[8] to form **3,3-dimethyl-2,4-heptanedione** in 15.8% yield, b.p. 91–94° (20 mm.), $n^{25}D$ 1.4322; it produced no color in the presence of ethanolic ferric chloride solution.

Anal. Calcd. for $C_9H_{16}O_2$: C, 69.19; H, 10.32. Found: C, 68.89; H, 10.47.

From the same reaction **2-methyl-3,5-octanedione** was also obtained (5.6% yield), b.p. 89° (20 mm.), $n^{25}D$ 1.4558; it produced a deep red color when treated with ethanolic ferric chloride solution. *Anal.* Found: C, 68.86; H, 10.40.

2,4,4-Trimethyl-3,5-octanedione.—5-Hydroxy-2,4,4-trimethyl-3-octanone,[10] 10 g. (0.054 mole), b.p. 112–113° (14 mm.), $n^{25}D$ 1.4437, and 28 g. of N-bromosuccinimide were dissolved in 150 ml. of *t*-butyl alcohol and 10 ml. of water.[17] After standing at room temperature for 24 hours, 400 ml. of water was added and then sodium bisulfite solution until the solution became colorless. The solution was extracted with four 100-ml. portions of ether; after drying the extracts and distilling the ether there was obtained 8.1 g. (81%) of 2,4,4-trimethyl-3,5-octanedione, b.p. 100–102° (14 mm.), $n^{25}D$ 1.4375 (lit.[10] b.p. 103–105° (14 mm.), $n^{25}D$ 1.4361). In a similar experiment using N-bromoacetamide the yield was 70%, b.p. 100–102° (14 mm.), $n^{25}D$ 1.4375.

In another experiment attempted oxidation of 5-hydroxy-2,4,4-trimethyl-3-octanone with chromium trioxide in acetic acid resulted in 76% recovery of the ketol. An attempted oxidation with aluminum *t*-butoxide and acetone resulted in quantitative deketolization to diisopropyl ketone and *n*-butyraldehyde.

(16) V. Grignard and P. Abelmann, *Bull. soc. chim. France*, [4] **7**, 638 (1910).

(17) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **72**, 5531 (1950).

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The Preparation of 17α-Hydroxy-20-ketosteroids

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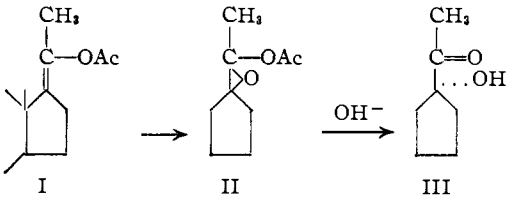
RECEIVED MAY 26, 1954

The elegant procedure of Gallagher[1] for the preparation of 17α-hydroxy-20-ketosteroids by the reaction of $\Delta^{17(20)}$-enol acetates with peracids unfortunately involves the preparation of perbenzoic or monoperphthalic acid, and this may become troublesome on a large scale. A procedure involving the substitution of the commercially available peracetic acid[2] would therefore be of value. This material has the following average composition: peracetic acid, 40% by wt.; hydrogen peroxide, 5% by wt.; acetic acid, 39% by wt.; sulfuric acid, 1% by wt.; water, 13% by wt.

(1) T. H. Kritchevsky and T. F. Gallagher, *J. Biol. Chem.*, **179**, 507 (1949); B. A. Koechlin, D. L. Garmaise, T. H. Kritchevsky and T. F. Gallagher, THIS JOURNAL, **71**, 3262 (1949); T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, *J. Biol. Chem.*, **74**, 483 (1952).

(2) Buffalo Electro-Chemical Co., Buffalo, N. Y.

Recently, the Upjohn research group[3] published a procedure using peracetic acid in which the sulfuric acid had been neutralized by the addition of sodium acetate, and under these conditions it behaves like perbenzoic and monoperphthalic acids



I II III

We have observed, however, that if the sulfuric acid is not neutralized, the intermediate epoxide II cannot be isolated, and the desired 17-hydroxy-20-ketone III can be obtained without resorting to alkaline hydrolysis. Acetate groups in other portions of the molecule are therefore preserved.

Allopregnan-3β-ol-20-one was converted to its enol acetate, then dissolved in benzene and reacted with peracetic acid. After destruction of the excess peracid, the organic layer was concentrated, and crystals of allopregnan-3β,17α-diol-20-one 3-acetate (Compound L 3-acetate) were deposited. Compound L was obtained by alkaline hydrolysis of the benzene mother liquor.

Similarly, pregnan-3α-ol-11,20-dione gave pregnan-3α,17α-diol-11,20-dione and its 3-acetate.

For preparative purposes, in order to obtain the highest possible yields, a complete hydrolysis is probably desirable, because it seems likely that the sulfuric acid, besides hydrolyzing the epoxide II, also may cause partial hydrolysis of the other esters in the molecule.

Experimental[4]

Allopregnan-3β,17α-diol-20-one (Compound L) and Its 3-Acetate.—A solution of 40.0 g. of pregnenolone acetate in 400 ml. of acetic acid was hydrogenated with 10 g. of 5% palladium-on-charcoal. The catalyst was removed by filtration and the filtrate concentrated to a residue under vacuum. This crude allopregnanolone acetate (m.p. 136–141°) was refluxed for 4 hr. in 500 ml. of acetic anhydride containing 6 g. of *p*-toluenesulfonic acid. During this time 400 ml. of distillate was collected, then the rest was removed *in vacuo*. The residue was dissolved in 300 ml. of benzene, the benzene washed with 200 ml. of 20% sodium acetate solution, and water, and then allowed to react at room temperature with 40 ml. of peracetic acid for 140 minutes. A solution of 40 g. of sodium sulfite in 150 ml. of water was then added with cooling, the benzene layer washed with water, dried and concentrated by distillation. When about half the benzene had been removed, crystals began to form. The distillation was stopped, the benzene cooled to *ca.* 20°, and a crop of 5 g. of allopregnan-3β,17α-diol-20-one 3-acetate was removed. Recrystallization from benzene gave 3.6 g., m.p. 184.8–186.8°, $[\alpha]D$ +17.0° (acetone). The benzene mother liquor was concentrated to a residue, and saponified by refluxing for 15 minutes with 20 g. of sodium hydroxide in 200 ml. of water and 1800 ml. of methanol. Upon concentration and cooling, there was obtained, in two crops, 19 g. of allopregnan-3β,17α-diol-20-one, m.p. 247–253°.

Its 3-acetate, prepared by the action of acetic anhydride in pyridine, was identical with the material obtained from the reaction mixture.

In a similar manner, pregnan-3α-ol-11,20-dione was converted to its enol acetate and reacted with peracetic acid to

(3) H. V. Anderson, E. R. Garrett, F. H. Lincoln, A. H. Nathan and J. A. Hogg, *J. Biol. Chem.*, **76**, 743 (1954).

(4) All m.p.'s are corrected. All rotations were taken in a 1-dcm. tube at a concentration of *ca.* 1%.

give pregnan-3α,17α-diol-11,20-dione 3-acetate, m.p. 195–198°, [α]D +50.3° (chloroform), and by hydrolysis, pregnan-3α,17α-diol-11,20-dione, m.p. 199.5–200.5°, [α]D + 33.9° (chloroform).

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The Biosynthesis of Radioactive Cholesterol, β-Methylglutaconic Acid and β-Methylcrotonic Acid by Aqueous Extracts of Liver[1]

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RECEIVED MAY 5, 1954

It has been previously reported that aqueous particle-free extracts of rat liver which possess the ability to incorporate C^{14}-labeled acetate or pyruvate into cholesterol[2] can also synthesize β-hydroxy-β-methylglutaric[3] and β-methylcrotonic acids.[4] Further investigation revealed the presence of another radioactive acid which has now been found to be *trans*-β-methylglutaconic acid (Table I). It is probable that this acid is derived from β-hydroxy-β-methylglutaric acid (HMG) by an enzymatically catalyzed dehydration. The formation of these two acids is consistent with the metabolic pathway

$$CH_3COCH_2COOH + CH_3COOH \longrightarrow CH_3C(OH)(CH_2COOH)_2 \longrightarrow CH_3C(CH_2COOH)=CHCOOH$$

Evidence for the mechanism of formation of the Coenzyme A derivative of HMG has recently been obtained by Robinson, Bachhawat and Coon.[5] The above postulated pathway undoubtedly also involves the participation of Coenzyme A.

C^{14}-Methyl-labeled HMG is incorporated into cholesterol by surviving liver slices, homogenates, or particle-free extracts of rat liver (Table II). When compared with 2-C^{14}-acetate it was found to be equally well incorporated. Similarly, the incorporation of HMG into β-methylglutaconic and β-methylcrotonic acids was equal to that observed with 2-C^{14}-acetate (Table III).

The role of these substances as precursors of cholesterol is under further investigation.

Experimental

Particle-free extracts (7 ml.) of rat liver,[3] homogenates (5 ml.), or tissue slices (1 g.) were incubated with 1 mg. each of adenosinetriphosphate, diphosphopyridine nucleotide and with 2-C^{14}-potassium acetate (1 mc./mmole) or C^{14}-methyl-HMG (0.08 mc./mmole). The labeled HMG was prepared from 2-C^{14}-acetate by the method of Klosterman.[6] The gas phase was 95% O_2–5% CO_2, and the time of incubation was 3 hours at 37°. The conditions used were those found to be optimum for cholesterol biosynthesis.[3] Following the incubation period, 34 mg. of carrier methylglutaconic acid, 20 mg. of β-methylcrotonic acid or 1 mg. of carrier cholesterol were added to separate incubation flasks. Each specimen was saturated with KCl, acidified to *p*H 2 with metaphosphoric acid, and extracted continuously with ether for 24–48 hours. The ether solution was evaporated to dryness and kept in a desiccator over KOH for 24 hours. The residue was extracted with 10% acetic acid and again extracted continuously with ether. The ether extract was afterward evaporated to dryness. For the separation of either β-methylcrotonic acid or β-methylglutaconic acid, the oily residue was transferred to a vacuum micro sublimation apparatus and heated with a small micro burner at 1 millimeter of mercury until sublimation ceased. A pale yellow crystalline sublimate was obtained. This material was dissolved in a few milliliters of absolute ether, decolorized by warming with charcoal and petroleum ether added to the filtrate to incipient turbidity. When β-methylglutaconic acid was isolated, the material obtained (4–5 mg.) melted at 114–115°. A mixed melting point with an authentic sample[7] showed no depression. The sublimed acid was plated and assayed for radioactivity; the counts were corrected to infinite thinness. The acid was diluted with carrier and subsequently converted to α,β-dibromo-β-methylglutaric acid[8] which was recrystallized (m.p. 145°). A mixed melting point with authentic dibromo-β-methylglutaric acid showed no change. The β-bromo-β-methylglutaric derivative[9] was also prepared (m.p. 129°); a mixed melting point with an authentic sample was unchanged. Subsequent radio-assay yielded the expected activity. Cholesterol was isolated as the digitonide and plated.[3] Incubations with inactive preparations of particle-free extracts of rat liver yielded no radioactive β-methylglutaconic acid. Variations in the activity of different derivatives of the same sample were within counting errors.

TABLE I

INCORPORATION OF 2-C^{14}-ACETATE INTO β-METHYLGLUTACONIC ACID BY AQUEOUS EXTRACTS OF RAT LIVER

Experiment	Radioactivity recovered in β-Methylglutaconic acid, c.p.m./mg. C	β-Bromo-β-methylglutaric acid, c.p.m./mg. C	α,β-Dibromo-β-methylglutaric acid, c.p.m./mg. C
1	354	302	..
2	791	..	..
3	269	..	234

TABLE II

INCORPORATION OF 2-C^{14}-ACETATE AND METHYL-C^{14}-HMG INTO CHOLESTEROL

Precursor	Radioactivity recovered[a] Aqueous extract	Homogenate	Slices
2-C^{14}-Acetate	0.0073	0.0081	0.0095
3′-C^{14}-β-Hydroxy-β-methylglutaric acid	.0086	.0093	.0071

[a] Recovered radioactivity expressed as μmoles of precursor incorporated = total recovered counts/counts per μmole of precursor. Aliquots of same extract, homogenate and slices obtained from same liver were employed.

TABLE III

INCORPORATION OF C^{14}-LABELED HMG AND 2-C^{14}-ACETATE INTO CHOLESTEROL, β-METHYLGLUTACONIC AND β-METHYLCROTONIC ACIDS BY PARTICLE-FREE EXTRACTS OF RAT LIVER

Precursor	Radioactivity recovered[a] Cholesterol	β-Methylglutaconic acid	β-Methylcrotonic acid
2-C^{14}-Acetate	0.003	0.183	0.095
3′-C^{14}-β-Hydroxy-β-methylglutaric acid	.005	.134	.094

[a] Radioactivity expressed as μmoles of precursor incorporated in recovered product (see Table II).

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(1) Supported by grants of the Heart Institute of the National Institutes of Health and the American Cancer Society administered by the Committee on Growth.

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(4) J. L. Rabinowitz, THIS JOURNAL, **76**, 3037 (1954).

(5) W. G. Robinson, B. K. Bachhawat and M. J. Coon, *Federation Proc.*, **13**, 281 (1954).

(6) H. J. Klosterman and F. Smith, THIS JOURNAL, **76**, 1229 (1954).

(7) N. Bland and J. F. Thorpe, *J. Chem. Soc.*, **101**, 856, 1557 (1912); **103**, 1569 (1913).

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(9) F. Fichter and J. Schwat, *Ann.*, **348**, 255 (1906).