0.35) from $Cr(H_2O)_6^{+++}$ (mainly) and $Cr(H_2O)_5Cl^{++}$ (partition coefficient 0.13). Separation was also effected by ion-exchange chromatography on Dowex 50-X4 resin and elution with 1 *M* perchloric acid. The ion $Cr(H_2O)_5Cl^{++}$ (green) was eluted first, followed by $Cr(H_2O)_5CH_2Ph^{++}$ (yellow to orange-yellow) whilst $Cr(H_2O)_6^{+++}$ remained on the column. All the above operations were carried out in an atmosphere of oxygen-free nitrogen and as rapidly as possible.

Solutions of the benzylchromium compound were yellow to brownish-red depending on the concentration, and the spectrum of the solution had a low intensity maximum at 540 mμ and a high intensity maximum at 358 mμ. Decomposition of the complex in the absence of oxygen by heating or by keeping for several days gave bibenzyl; in the presence of oxygen benzaldehyde was the main product. The compound was decomposed very rapidly by sulfur dioxide but was stable to carbon dioxide. Hydrogenation in presence of a palladium catalyst gave toluene. Solutions of the compound reacted very quickly with aqueous mercuric chloride with *no change in pH of the solution* to give benzylmercuric chloride and $Cr(H_2O)_6^{+++}$ (not $Cr(H_2O)_5Cl^{++}$) in equimolecular amounts. Therefore the compound must contain one benzyl group per chromium atom and two positive charges. Hence it is a Cr(III) complex and its stability is best rationalized by the normal octahedral complex structure mentioned before. It differs, as might be expected, from the chloride complex in having a tendency to dissociate in a homolytic fashion to give benzyl radicals and chromous ion. An alternate structure which can be considered for the compound is a complex of the tropylium ion and Cr(I) in the manner[2] of dibenzene–Cr(I) but with only one ring involved per chromium atom, although this is somewhat unlikely on the above evidence. However, such a structure can be excluded because all the carbon atoms would then become equivalent and it was found that α-methyl, *o*-methyl- and *p*-methylbenzyl halides gave different compounds on reduction with chromous perchlorate.

Reduction of benzyl chloride with chromous chloride in hydrochloric acid gave toluene rather than an organo-metallic compound. This can be understood from the work of Taube[4] as the initially formed complex, $Cr(H_2O)_4Cl{\cdot}CH_2Ph^+$, will have chloride ion as well as water molecules in the coördination sphere and such a chlorine atom favors reduction by bridging[4] with the reducing agent. The resultant Cr(II) complex is then no longer substitution-inert and dissociates to benzyl anions which react with the solvent to give toluene. Therefore, the isolation of $Cr(H_2O)_4CH_2Ph^{++}$ in the chromous perchlorate reduction is due to the fact that this compound (like $Cr(H_2O)_6^{+++}$ but unlike $Cr(H_2O)_5Cl^{++}$ and $Cr(H_2O)_4Cl_2^+$) is reduced by Cr^{++} extremely slowly, as well as to the substitution-inertness of dipositive complexes of Cr(III).

Allyl chloride and phenacyl chloride were readily reduced by chromous perchlorate, but without observable formation of organo-metallic compounds. Work on the reduction of halogen compounds by chromous salts is continuing.[5]

(4) H. Taube and E. L. King, THIS JOURNAL, **76**, 4053 (1954); H. Taube and H. Myers, *ibid.*, **76**, 2103 (1954).

(5) This work was supported by a grant from the National Research Council of Canada.

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RECEIVED APRIL 15, 1957

THE BIOSYNTHESIS OF SQUALENE FROM MEVALONIC ACID

Sir:

Tavormina, *et al.*,[1] have reported the incorporation of 2-C^{14}-labeled mevalonic acid (3,5-dihydroxy-3-methylvaleric acid) into cholesterol by homogenates of rat liver. These investigators have also provided evidence that the carboxyl carbon of the substrate is almost completely converted to carbon dioxide, and therefore not incorporated into cholesterol.[2]

We have confirmed the finding that 2-C^{14}-mevalonic acid[3] is converted to cholesterol, and in addition have demonstrated that it is rapidly converted to squalene by homogenates as well as by the supernatant fluid obtained from such preparations following high speed centrifugation.[4]

Employing homogenates of rat liver and 2-C^{14}-mevalonic acid, we have repeatedly isolated C^{14}-squalene into which has been incorporated 10 to 20% of the substrate. Squalene recovered from several of the experiments was combined and purified by chromatography on alumina.[4] It was subsequently chromatographed on silicic acid, eluting with an increasing gradient of benzene in petroleum ether. The latter process yields squalene as a single radioactive peak.[5]

In contrast to cruder preparations of squalene, this product is completely degraded by ozonolysis to acetone, levulinic acid and succinic acid. These substances were separated and further degraded. Acetone was converted to iodoform and acetic acid, and the acetic acid degraded by the Schmidt method.[6] The levulinic acid was converted to iodoform and succinic acid. The succinic acid was degraded by the Schmidt technique to carbon dioxide and ethylenediamine. All of the degradation products were converted to carbon dioxide and counted as barium carbonate.

The radio-analyses (Table I) demonstrated that approximately 80% of the isotopic carbon of the squalene is present in the methyl carbons of acetone and in carbon 3 of levulinic acid. The succinic acid sample, representing the central four carbons of squalene, contained only a small amount of isotope. It is clear that only one of the methylene groups of the levulinic acid was appreciably labeled,

(1) P. A. Tavormina, M. H. Gibbs and J. W. Huff, THIS JOURNAL, **78**, 4498 (1956).

(2) P. A. Tavormina and M. H. Gibbs, *ibid.*, **78**, 6210 (1956).

(3) The authors express their appreciation to Merck Sharp and Dohme for a generous supply of labeled mevalonic acid used in these experiments.

(4) F. Dituri, F. A. Cobey, J. V. B. Warms and S. Gurin, *J. Biol. Chem.*, **221**, 181 (1956).

(5) F. Dituri, J. L. Rabinowitz and S. Gurin, in preparation.

(6) E. F. Phares and M. V. Long, THIS JOURNAL, **77**, 2556 (1955).

TABLE I

SPECIFIC ACTIVITY OF THE DEGRADATION PRODUCTS OF C^{14}-SQUALENE

Degradation product	Specific activity, c.p.m. per mg. C
Acetone	306
Carbonyl carbon	10
CHI_3	360
Succinic acid	70
-COOH	30
$-CH_2-$	80
Levulinic acid	150
CHI_3 (carbon 5)	17
-COOH (carbons 1 and 4)	28
$-CH_2-$ (carbons 2 and 3)	310

and it is probable from the data that only one of the methyl carbons of the acetone fragment contained C^{14} (Fig. 1).

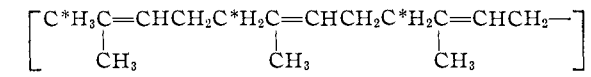

Fig. 1.—Principal distribution of isotopic carbon in biosynthetic squalene.

The distribution of isotope in the squalene suggests that the mevalonic acid was not decarboxylated to yield a five carbon intermediate prior to condensation. If a five carbon intermediate is first produced, the compound would have to react asymmetrically to give the observed isotope distribution. This possibility has not been excluded by the data presented here. Whether mevalonic acid is or is not the biological precursor of squalene and cholesterol, there appears to be little breakdown and re-condensation prior to polymerization. It is likely that decarboxylation occurs during or after polymerization.

Complete details will be presented in a subsequent publication.

Acknowledgment.—This work was supported in part by grants from the National Heart Institute of the National Institutes of Health, the American Heart Association, and the American Cancer Society.

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RECEIVED MARCH 16, 1957

SOME ENZYMATIC REACTIONS OF 6,8-DITHIOLOCTANOIC (DIHYDROLIPOIC) ACID AND ITS ACETOACETIC THIOESTER[1]

Sir:

Evidence has been presented that AcAc[2] synthesis in soluble liver fractions from a source of Ac-S-CoA involves the intermediate formation of AcAc-S-CoA by thiolase, which catalyzes reaction 1, followed by deacylation of AcAc-S-CoA to AcAc.[3-5] However, a direct deacylation of synthetic AcAc-S-CoA is not catalyzed by these enzyme fractions.

$$2\text{Ac-S-CoA} \rightleftharpoons \text{AcAc-S-CoA} + \text{HS-CoA} \quad (1)$$

We find that AcAc synthesis by partly purified ox or chicken liver enzyme preparations from Ac-S-CoA (generated by phosphotransacetylase from acetyl phosphate and CoA-SH[3]) requires the addition of a mono- or dithiol compound. Among active thiols, (±)-DTO[6] has the greatest activity, half maximum activation of AcAc synthesis occurring with 1×10^{-4} *M* (±)-DTO compared to 3.5×10^{-4} *M* BAL and 2×10^{-3} *M* GSH. These liver fractions also catalyze the reactions (2), (3)

$$(\pm)\text{-S-AcAc-DTO} + H_2O \longrightarrow \text{AcAc} + (\pm)\text{-DTO} \quad (2)$$

$$\text{AcAc-S-Pn (or CoA)} + (\pm)\text{-DTO} \rightleftharpoons \text{Ac-S-Pn (or CoA)} + (\pm)\text{-S-Ac-DTO} \quad (3)$$

Mono-(±)-S-AcAc-DTO was synthesized by reacting one equivalent of diketene with (±)-DTO. Presumably it is the 8-ester, since acetic anhydride and DTO have been shown to give 8-S-Ac-DTO.[7] While DTO does not give a positive nitroprusside assay for sulfhydryl,[8,9] we find the monothiol, monothioester form does. Thus, monoacylation of DTO results in the appearance of one sulfhydryl equivalent (by nitroprusside assay), as well as one thioester equivalent (measured with hydroxylamine or optically at 240 mμ). The synthesis and breakdown of mono-S-acyl-DTO compounds can therefore be measured by nitroprusside assay.

The thioesterase catalyzing reaction 2 is assayed optically by following the decrease in light absorption at λ 310 mμ or λ 240 mμ, since (±)-S-AcAc-DTO has an absorption spectrum characteristic of acetoacetyl thioesters.[10,11] It is present in liver but apparently not in other tissues. The enzyme has been purified 40-fold from chicken liver and shown to be different from AcAc-SG thioesterase.[12] It hydrolyzes S-AcAc and S-Ac esters of DTO, MTO and BAL (Table I).

As measured optically at λ 310 mμ, liver and heart enzyme fractions catalyze a disappearance of AcAc-S-Pn and AcAc-S-CoA, provided DTO (or certain mono- or dithiols) is added. With DTO, the decrease in E_{310} due to disappearance of AcAc-SR is accompanied by a simultaneous increase in E_{240}, signifying an increase in total thioester concentration. Balance studies (Table II) show that for each mole of AcAc-S-Pn disappearing, *two* moles of

(1) Supported by grants from the U. S. Public Health Service (No. A-739) and the Williams-Waterman Fund of the Research Corporation.

(2) Abbreviations: 6,8-dithioloctanoic (dihydrolipoic) acid, DTO; monothioloctanoic acid, MTO; coenzyme A (reduced), CoA-SH; pantetheine, Pn-SH; glutathione, GSH; dimercaptopropanol, BAL; thioesters, acyl-S-R; acids: acetic, Ac; acetoacetic, AcAc; *tris*-(hydroxymethyl)-aminomethane, Tris; E, optical density; all reference is to sign of rotation (*cf.* footnote 7).

(3) E. R. Stadtman, M. Doudoroff and F. Lipmann, *J. Biol. Chem.*, **191**, 377 (1951).

(4) J. R. Stern, M. J. Coon and A. del Campillo, *Nature*, **171**, 28 (1953).

(5) J. R. Stern, M. J. Coon and A. del Campillo, *J. Biol. Chem.*, **221**, 1 (1956).

(6) DTO, MTO and 6-thioldecanoate were kindly supplied by Dr. J. A. Brockman of Lederle Laboratories.

(7) I. C. Gunsalus, L. S. Barton and W. Gruber, THIS JOURNAL, **78**, 1763 (1956).

(8) I. C. Gunsalus in "The Mechanism of Enzyme Action," the Johns Hopkins Press, Baltimore, Md., 1954, p. 545.

(9) I. Fridovitch and P. Handler, *J. Biol. Chem.*, **223**, 321 (1956).

(10) F. Lynen, *Federation Proc.*, **12**, 683 (1953).

(11) J. R. Stern, *J. Biol. Chem.*, **221**, 33 (1956).

(12) J. R. Stern and G. I. Drummond, *Federation Proc.*, **15**, 363 (1956).