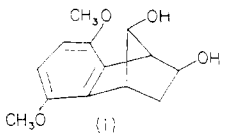
methanolysis of 2-*p*-hydroxyphenyl-1-ethyl bromide.[4] In this instance, the intermediate dienone has actually been characterized.[5]

In support of these suggestions, it should be noted that norbornene epoxide is extremely inert toward basic hydrolysis.[6] The special role of the internal anion is demonstrated in one further way. Lithium aluminum hydride reduction of IA gave the hydroquinone, IB (m.p. 144.0–144.5°; calcd. for $C_{11}H_{10}O_2$; C, 75.84; H, 5.79. Found: C, 75.82; H, 5.89), which upon treatment with dimethyl sulfate and base gave the dimethyl ether IC (m.p. 78.5–79.0°; calcd. for $C_{13}H_{14}O_2$: C, 77.20; H, 6.98. Found: C, 77.31; H, 6.71). Peracetic acid converted IC into the epoxide IIC (m.p. 119.5–120.0°; calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.56; H, 6.69). This epoxide was unreactive toward either sodium hydroxide or methanolic sodium methoxide under conditions which resulted in opening of the epoxide ring in IIA.[7]



(i)

The partial support of this research by a research grant from the National Institutes of Health is gratefully acknowledged.

(4) S. Winstein and R. Baird, THIS JOURNAL, **79**, 756 (1957).

(5) R. Baird and S. Winstein, *ibid.*, **79**, 4239 (1957).

(6) Unpublished observation of Norman Hudak.

(7) Acid-catalyzed opening of epoxide IIC, followed by hydrolysis, gave the rearranged diol *i* (m.p. 147.5–148.0°; calcd. for $C_{13}H_{16}O_4$: C, 66.08; H, 6.83. Found: C, 65.92; H, 6.92), whose structure is substantiated by its failure to reduce periodic acid in the standard vicinal diol test. This observation indirectly supports structure III for the analogous product derived from acetolysis of IIA.

(8) On leave from Kyôto University, Kyôto, Japan.

(9) Opportunity Fellow, John Hay Whitney Foundation, 1955–1956; Allied Chemical and Dye Corp. Fellow, 1956–1957.

DEPARTMENT OF CHEMISTRY
CORNELL UNIVERSITY
ITHACA, NEW YORK

JERROLD MEINWALD
HITOSI NOZAKI[8]
GEORGE A. WILEY[9]

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THE BIOSYNTHESIS OF β-HYDROXY-β-METHYLGLUTARYL COENZYME A[1]

Sir:

Previous work with rat liver and yeast preparations has established that AcCoA[2] and AcAc CoA are the reactants in the biosynthesis of HMG by the HMG condensing enzyme.[3,4] In this communication we wish to report the results of experiments with a purified preparation of condensing enzyme from baker's yeast, which demonstrated that the products of this condensation are HMG CoA and CoASH according to reaction 1.

$$CH_3CO{-}S{-}CoA^{I} + CH_3COCH_2CO{-}S{-}CoA^{II} + H_2O \longrightarrow CH_3COHCH_2CO{-}S{-}CoA^{II} + \underset{\displaystyle CH_2COOH}{|} \qquad CoA{-}SH^{I} \quad (1)$$

The enzyme was assayed by measuring the disappearance of the enolate ion absorption of AcAc CoA at 310 mμ[5] which occurs when Ac CoA is added in the presence of the enzyme. β-Keto-thiolase,[6] which interfered with this assay, was completely and irreversibly inhibited by treatment with concentrations of iodoacetamide which only partially inhibit the condensing enzyme.

During the course of the reaction, for each equivalent of AcAc CoA which disappeared one equivalent of free thiol appeared as measured by the nitroprusside reaction[7] (Table I). When acetyl-1-C^{14} CoA was incubated with AcAcCoA and the enzyme, one equivalent of AcAc CoA disappeared for each equivalent of Aa CoA which was incorporated into HMG CoA as determined by radioactivity in HMG (Table II).

TABLE I

The reaction was run in Beckman cuvettes, d = 1.0 cm. Each contained 15 mg. of bovine albumin, 400 μmoles of Tris-HCl buffer *p*H 7.75, 2.0 μmoles of AcCoA and 1.0 μmole of AcAc CoA. The final volume was 3.0 ml. Expt. 1 contained 1.8 mg. of enzyme protein, and Expt. 2 contained 0.6 mg. of enzyme protein. The incubation period was 20 minutes at room temperature.

	ΔAcAc CoA, μmoles	ΔSH, μmoles
Expt. 1	−0.54	+0.58
Expt. 2	−0.68	+0.69

TABLE II

Each Beckman cuvette contained 15 mg. of bovine albumin, 400 μmoles of Tris-HCl buffer *p*H 8.1, 2.4 mg. of enzyme protein, 0.4 μmole of AcAcCoA and 1.0 μmole of acetyl-1-C^{14} CoA with a specific activity of 975,000 counts/min./μmole. The incubation period was 20 min. at room temperature. Radioactivity of HMG was determined by the method of Rudney.[4]

	ΔAcAc CoA, μmoles	Total radioactivity found in HMG, counts/min.	Amount of Ac CoA incorporated, μmoles
Expt. 1	−0.18	180,000	0.185
Expt. 2	−0.19	170,000	0.175

The product of the reaction with acetyl-1-C^{14} CoA as reactant was treated with neutral hydroxylamine to convert the CoA esters to hydroxamates, and chromatographed[8] after the addition of mono HMG- and acetohydroxamates as carriers. Only two radioactive spots were observed, corresponding to acetohydroxamate and HMG-hydroxamate. AcAc CoA did not form a detectable hydroxamate under these conditions.[6] These results show that the HMG formed during the reaction is in the form of an acyl derivative of CoA. The above experiments demonstrate the stoichiometry of reaction 1.

(1) This investigation was supported in part by grants from the Life Insurance Medical Research Fund, The American Cancer Society, and the Elizabeth Severance Prentiss Fund of Western Reserve University. The C^{14} used was obtained on allocation from the Atomic Energy Commission.

(2) The following abbreviations are used: Ac CoA and AcAc CoA, acetyl and acetoacetyl coenzyme A; HMG, β-hydroxy-β-methylglutaric acid; HMG CoA, β-hydroxy-β-methylglutaryl coenzyme A; CoASH, reduced coenzyme A.

(3) H. Rudney, *Federation Proc.*, **15**, 342 (1956).

(4) H. Rudney, *J. Biol. Chem.*, **227**, in press (1957).

(5) J. R. Stern, *ibid.*, **221**, 33 (1956).

(6) J. R. Stern, M. J. Coon and A. del Campillo, *ibid.*, **221**, 1 (1956).

(7) R. R. Grunert and P. H. Phillips, *Arch. Biochem.*, **30**, 217 (1951).

(8) A. R. Thompson, *Aust. J. Sci. Res.*, **B4**, 180 (1951).

Since the product is a mono CoA ester of HMG, the problem arises whether the thioester bond of Ac CoA or of AcAc CoA is hydrolyzed during the condensation reaction. This problem was resolved in the following manner: HMG CoA enzymatically formed by the condensing enzyme from acetyl-1-C^{14} CoA and AcAcCoA was mixed with carrier HMG CoA[9] and cleaved by the HMG CoA cleavage enzyme of Bachhawat, *et al.*,[10] to acetoacetic acid and AcCoA according to reaction 2.

$$\underset{\displaystyle CH_2CO{-}S{-}CoA}{CH_3\overset{}{C}OHCH_2COOH} \longrightarrow CH_3COCH_2COOH + CH_3CO{-}S{-}CoA \quad (2)$$

Decarboxylation of the acetoacetic acid to acetone and CO_2 showed the radioactivity to reside solely in the CO_2. These results show that the free carboxyl group of HMGCoA contained C^{14}. Since the C^{14} was originally in the carboxyl position of acetyl-1-C^{14} CoA it may be concluded that the CoA set free during the condensation reaction came from AcCoA according to reaction 1.[11]

Since HMG CoA is the product of the condensation reaction, we would redesignate the enzyme as the HMG CoA condensing enzyme. This enzyme resembles the citrate condensing enzyme,[12] in that (1) Ac CoA condenses with the carbonyl group of the other substrate, and (2) a net hydrolysis of Ac CoA results. The enzyme appears to be specific for thioesters of CoA, since the corresponding thioesters of pantetheine and glutathione were inactive. Efforts to reverse the condensation have been unsuccessful thus far.

At the present time two ways are known in which HMG CoA can be formed, the first *via* the condensation reaction described above, and the other the CO_2 fixation reaction of Bachhawat *et al.*[13] The obvious relationship between HMG CoA and mevalonic acid, a compound efficiently converted to cholesterol,[14] is under investigation.

DEPT. OF BIOCHEMISTRY
SCHOOL OF MEDICINE HARRY RUDNEY[15]
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO JAMES J. FERGUSON, JR.[16]
RECEIVED AUGUST 5, 1957

(9) Prepared from HMG anhydride by the method of E. J. Simon and D. Shemin, THIS JOURNAL, **75**, 2520 (1953).

(10) B. K. Bachhawat, W. G. Robinson and M. J. Coon, *J. Biol. Chem.*, **219**, 539 (1956). We are grateful to Dr. M. J. Coon for a sample of the HMG CoA cleavage enzyme.

(11) This result would also be obtained if an intramolecular transfer of CoA occurred from one carboxyl group of HMG CoA to the other. This possibility appears to be eliminated by experiments (H. Rudney and T. G. Farkas, *Federation Proc.*, **14**, 757 (1955); M. J. Coon, *J. Biol. Chem.*, **187**, 71 (1950); G. W. E. Plaut and H. A. Lardy, *ibid.*, **186**, 705 (1950)) which demonstrated that when $C^{14}O_2$ was fixed into acetoacetate by liver homogenates, assuredly *via* HMG CoA formation and subsequent cleavage to acetoacetate and Ac CoA, isotope appeared almost exclusively in the carboxyl group of acetoacetate. If intramolecular CoA transfer occurred between the carboxyls of HMG CoA, then after cleavage the thiolase present in the homogenates would have randomized the label between the carboxyl and carbonyl carbons of acetoacetate.

(12) J. R. Stern, S. Ochoa and F. Lynen, *J. Biol. Chem.*, **198**, 313 (1952).

(13) B. K. Bachhawat, W. G. Robinson and M. J. Coon, *ibid.*, **216**, 727 (1955).

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

(15) Scholar in Cancer Research of the American Cancer Society.

(16) Post Doctoral Research Fellow, National Institute of Arthritis and Metabolic Diseases.

THE SCAVENGER EFFECT IN SOLID ETHYL BROMIDE

Sir:

During an investigation of the Szilard–Chalmers effect in solid ethyl bromide, it was found possible to produce a solid phase containing variable proportions of elementary bromine. This technique could prove useful in the study of the rather complicated reactions following (n,γ) processes in solid organic halides.[1]

The ethyl bromide and bromine used were purified as described elsewhere[2] and mixtures of these were frozen using liquid nitrogen. Twenty-five cc. of the mixture was contained in a narrow necked glass vessel into which a quartz tube was fitted. Two methods of freezing were used. In the first, liquid nitrogen was poured into the central quartz tube and complete freezing occurred in about one hour; using the second method, the freezing time was reduced to 5 minutes by also immersing the vessel in liquid nitrogen. In the latter case, care was taken to transform any transparent glass produced on the walls of the vessel into opaque crystals[1a] by leaving it at room temperature for a minute before irradiation. The sealed vessel containing the frozen mixture was placed in a Dewar fitting in a paraffin moderator castle. A 500 mc. polonium–beryllium source was lowered into the center of the quartz tube which was kept full of liquid nitrogen during irradiation (50 minutes). After irradiation, the solid was left to melt at room temperature and the liquid was extracted with an aqueous sodium sulfite solution. The activities due to Br^{80} (18 min. half-life) in the unextracted and extracted specimen were determined, the activity of the other bromine isotopes being negligible.[3]

The results obtained are shown in Fig. 1 curve (a), where the retention[4] has been plotted against the molar fraction of the elementary bromine present. The speed of freezing did not influence the retention of pure ethyl bromide. When bromine was present, an appreciable drop in retention was only observed when the faster freezing method was used (see Table I).

TABLE I

Mol. fract. of Br	Freezing time	Retention of Br^{80}, %
0	1 hour	83.0 + 2.6
0	5 min.	82.1 + 2.6
0	5 min.	82.0 + 2.5[a]
5,7.10^{-2}	1 hour	78.2 + 2.3
5,7.10^{-2}	5 min.	69.5 + 2.2

[a] This specimen was allowed to melt in the presence of 3.8 cc. of a solution of bromine (0.38 molar fraction) in ethyl bromide.

(1) (a) F. S. Rowland and W. F. Libby, *J. Chem. Phys.*, **21**, 1495 (1953); (b) L. Friedman and W. F. Libby, *ibid.*, **17**, 647 (1949); (c) S. Goldhaber, R. S. H. Chiang and J. E. Willard, THIS JOURNAL, **73**, 2271 (1951); (d) G. Levey and J. E. Willard, *ibid.*, **74**, 6161 (1952).

(2) Miriam Milman and P. F. D. Shaw, *J. Chem. Soc.*, 1303 (1957).

(3) Details of precautions taken, counting technique and corrections applied have been previously reported.[2]

(4) The retention is defined as the fraction of the total activity present in organic combinations.