specific hydroxylation of aromatic compounds.[1] When salicylic acid is hydroxylated by this system, 2,3-dihydroxybenzoic and gentisic acids are formed in equal amounts, together with smaller quantities of trihydroxybenzoic acids. We have carried out this reaction in an atmosphere of O^{18}_2, in H_2O solution free of heavy metal contaminants according to the method of Keilin and Hartree[2] and have separated the products on silica gel columns. Oxygen which was incorporated into salicylic acid as hydroxyl to form 2,3-dihydroxybenzoic and gentisic acids was found to arise entirely from the atmosphere. When the reaction was carried out in H_2O^{18} with an atmosphere of O_2, no excess of labelled oxygen was detected (Table I).

TABLE I

SOURCE OF OXYGEN INCORPORATED AS HYDROXYL INTO SALICYLIC ACID BY THE SYSTEM: HORSERADISH PEROXIDASE,[a] DIHYDROXYFUMARATE,[b] OXYGEN[c]

Experiment	Conditions[d]	Compd. isolated[d]	% Theor. O^{18} incorporation[e]
1, 2	$O^{18}_2 + H_2O^{16}$	2,3-Dihydroxybenzoic acid	106
		Gentisic acid	110
3	$O^{16}_2 + H_2O^{18}$	2,3-Dihydroxybenzoic acid	0
		Gentisic acid	0

[a] We gratefully acknowledge a gift of purified horseradish peroxidase (RZ = 1.9) from Professor D. Keilin. [b] Recrystallized from mixtures of acetone and deionized distilled water; $E_{mol.}$ = 8350, 308 mμ, in ether. [c] Prepared by electrolysis of H_2O containing 1.4 atom % O^{18} (Stewart Oxygen Company, San Francisco). [d] Hydroxylations were carried out in 20 ml. solutions containing phosphate (0.046 *M*), salicylic acid (300 μmoles), peroxidase (1.2 μmoles) and dihydroxyfumarate (0.81 mmole), brought to *p*H 6.0 with heavy-metal-free KOH. The mixture was acidified after two hours of reaction, and the products extracted with ethyl acetate. These were separated on silica gel columns, using chloroform and 4% ethanolic chloroform developers. Identities of the recovered gentisic acid (m.p. 202–203°) and 2,3-dihydroxybenzoic acid (m.p. 205–206°) were verified both spectroscopically and chromatographically. Under the conditions described, the combined yield (based upon a quantitative chromatographic procedure) was 16–21%. With heat-denatured peroxidase, a yield of 1.9% was observed. [e] We are grateful to Dr. C. C. Delwiche for performing the mass spectrometry upon samples which we obtained by Unterzaucher pyrolysis[3] of the hydroxylation products. An apparent isotope effect shown by our results is under study.

Since complex III of peroxidase predominates[4] in the dihydroxyfumarate–oxygen system[4] and the enzyme is inhibited by carbon monoxide, displaying the spectrum of carbon monoxide ferroperoxidase,[4,5] since dihydroxyfumarate (for which peroxidase is an oxidase) cannot be replaced by ascorbate in the hydroxylating system,[1] and since in this system molecular oxygen is activated toward non-specific hydroxylation of aromatic substances, oxyferroperoxidase appears to be involved.[6]

A complete description of these experiments will be published elsewhere.

(6) This study has been supported by a grant from the United States Public Health Service (A-971).

(7) Post-doctorate Fellow of the United States Public Health Service.

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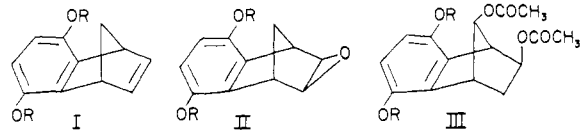
RECEIVED AUGUST 12, 1957

Ar_1-3 PARTICIPATION IN THE BICYCLO[2,2,1]HEPTANE SERIES

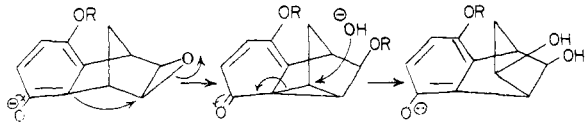
Sir:

The rearrangement of the bicyclo[2,2,1]heptane carbon skeleton, proceeding *via* carbonium ions, is a well known phenomenon.[1] We wish to report the observation of a novel, base-catalyzed analog of this type of transformation.



The key compound in this study is the epoxide IIA (m.p. 107.5–108.0°; calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.97; H, 5.16), prepared by treatment of IA[2] with peracetic or perfluoroperacetic acid. IIA reacted rapidly with hot 2 *N* sodium hydroxide to give an unstable phenolic product, the infrared spectrum of which lacked the strong 11.7 μ (epoxide) band of IIA. Acetylation of this material with pyridine–acetic anhydride gave a product from which the tetraacetate IIIA (m.p. 139–140°; calcd. for $C_{19}H_{20}O_8$; C, 60.63; H, 5.36; acetyl, 46.7. Found: C, 60.47; H, 5.27; acetyl, 45.64) could be isolated in 20% yield. An authentic sample of IIIA was readily obtained by *acid* hydrolysis of IIA, which on the basis of the behavior of norbornene epoxide[3] would be expected to give the rearranged product, followed by acetylation.

An attractive interpretation of this reaction involves nucleophilic opening of the epoxide ring by an internal phenoxide ion as shown below. Further attack by base on the dienone intermediate would regenerate the aromatic ring and give rise to a rearranged product.



A close analogy for this series of reactions is provided by the recent realization by Winstein and Baird of Ar_1-3 phenoxide participation in the

(1) H. S. Mason, I. Onoprienko and D. Buhler, *Biochim. Biophys. Acta*, **24**, 225 (1957).

(2) D. Keilin and E. F. Hartree, *Biochem. J.*, **60**, 310 (1955).

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(4) H. Theorell and B. Swedin, *Naturwiss.*, **27**, 95 (1939); B. Swedin and H. Theorell, *Nature*, **145**, 71 (1940).

(5) B. Chance, *J. Biol. Chem.*, **197**, 577 (1952).

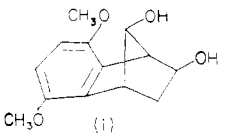
(1) See, for example, C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, pp. 482–494.

(2) W. R. Vaughan and M. Yoshimini, *J. Org. Chem.*, **22**, 7 (1957).

(3) H. M. Walborsky and D. F. Loncrini, THIS JOURNAL, **76**, 5396 (1954); H. Kwart and W. G. Vosburgh, *ibid.*, **76**, 5400 (1954).

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.

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RECEIVED AUGUST 28, 1957

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.

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

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.

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

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