A thorough study of this reaction and its scope is continuing.

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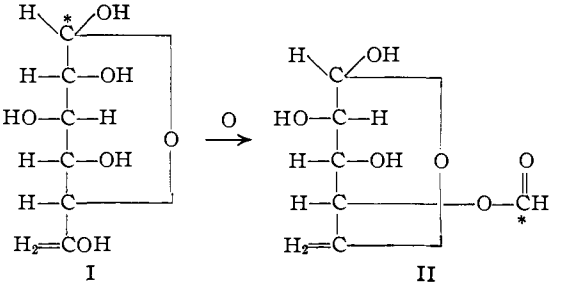
RECEIVED MARCH 29, 1954

SHORTENING THE CARBON CHAIN OF SUGARS[1]

Sir:

Several methods have been described for preparing sugars with fewer carbons than the starting compound by degradation of the appropriate higher-numbered family member. A critical evaluation of the various methods has been presented by Pigman and Goepp.[2] The author has now found that a reasonably selective degradation also may be achieved by direct oxidation of reducing sugars with lead tetraacetate or sodium bismuthate. The reaction appears to be controlled partly by the relative ease with which the oxidant cleaves hemiacetal-glycol groups,[3,4] *i.e.*, the diol of carbons 1 and 2 in the cyclic form of the reducing sugar (I), and also by the consequent formation of a stable formyl ester (II) which prevents attack of the lower portion of the molecule. Thus D-arabinose is easily prepared in at least 35% yield by oxidation of D-mannose in acetic acid with 1.5 moles of lead tetraacetate. The glycol cleavage is not, however, confined exclusively to carbons 1 and 2 for still lower members, such as D-erythrose, are also produced and indeed become the major products when a larger proportion of oxidant is used. The reaction has also been used to prepare D-arabinose from D-glucose, and D-lyxose from D-galactose, and appears to be applicable to reducing sugars generally.



I II

In addition to its use as a preparative method the reaction is well suited to the stepwise degradation of sugars containing radioactive carbon. For example, the possibility of radioactive-carbon transfer during an experiment with a sugar labeled in carbon atom 1 may be examined conveniently on the micro scale. Thus 0.5 mg. of glucose-1-C^{14} (I) (diluted with 1.5 mg. of glucose) was oxidized in 97% acetic acid with lead tetraacetate equivalent to 1–1.5 moles per mole and, after precipitation of lead, the formyl esters were gently hydrolyzed. The products were separated on the paper chromatogram and radioactive areas were located by radioautography and by scanning with an end-window counter. The oxidation products, arabinose and erythrose, were non-radioactive while the unoxidized glucose retained its activity. It was evident, therefore, that the radioactivity resided entirely in carbon atom 1 of the glucose, in agreement with the expected result. A chromatogram of the oxidation products prior to hydrolysis contained radioactive spots, one of which gave the characteristic pentose color. These compounds were undoubtedly the expected intermediate formates esterified with the radioactive formic acid derived from carbon atom 1 (*e.g.*, II) since after hydrolysis they were replaced on the chromatogram by the free, non-radioactive, pentose and tetrose.

It is seen also that the radioactivity of carbons 1 and 2, and possibly of other carbon atoms, in samples of glucose of unknown labeling may be assayed by difference, by determining the specific activities of the individual sugars in the oxidation mixture after elution from the chromatogram.

The work will be described in detail elsewhere.

The kind assistance of Dr. D. C. Mortimer, Dr. P. R. Gorham and of Mr. J. Giroux is gratefully acknowledged.

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RECEIVED MARCH 1, 1954

THE SYNTHESIS OF β-HYDROXY-β-METHYLGLUTARIC ACID IN RAT LIVER HOMOGENATES[1]

Sir:

β,β-Dimethylacrylic acid has been postulated as a precursor of the isoprene-like unit which is thought to polymerize to rubber[2] in plants and to cholesterol in animal tissues.[3] It has been found recently that the rat liver homogenate preparation of Bucher[4] could synthesize β-DMA from acetic acid.[5] Thus incubation with $C^{14}H_3COOH$ gave β-DMA labeled chiefly in carbons 2, 4 and 4′ and little or no labeling in carbons 1 and 3. These results show that β-DMA, a branched chain fatty acid, is synthesized in animal tissues from small units and suggest that this compound may be a precursor of cholesterol since the pattern of labeling is similar to that postulated for the isoprenoid precursors of cholesterol by Würsch, *et al.*[3]

The fact that carboxyl labeled acetoacetate appears to be incorporated into cholesterol[6] without prior cleavage into C_2 units, seems to rule out the

(1) Issued as N. R. C. No. 3277.

(2) W. W. Pigman and R. M. Goepp, Jr., "Chemistry of the Carbohydrates," Academic Press, Inc., New York, N. Y., 1948.

(3) R. Criegee, *Ber.*, **65**, 1770 (1932).

(4) R. C. Hockett and W. S. McClenahan, THIS JOURNAL, **61**, 1667 (1939).

(1) The following abbreviations are used: β-DMA = β,β-dimethylacrylic acid; β-HMG = β-hydroxy-β-methylglutaric acid; ATP = adenosine triphosphate; DPN = diphosphopyridine nucleotide; s.a. = specific activity; CoA = Coenzyme A. This investigation was supported by research grants from the Life Insurance Medical Research Fund and the Elisabeth Severance Prentiss Fund of Western Reserve University.

(2) B. Arreguin, J. Bonner and B. F. Wood, *Arch. Biochem.*, **21**, 104 (1949).

(3) J. Würsch, R. L. Huang and K. Bloch, *J. Biol. Chem.*, **195**, 439 (1952).

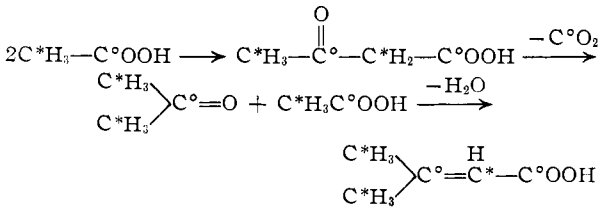
(4) N. L. R. Bucher, THIS JOURNAL, **75**, 498 (1953).

(5) H. Rudney, *Fed. Proc.*, **13**, 286 (1954).

(6) R. O. Brady and S. Gurin, *J. Biol. Chem.*, **189**, 371 (1951).

mechanism suggested by Würsch, *et al.*, in Scheme I for β-DMA synthesis.[7]

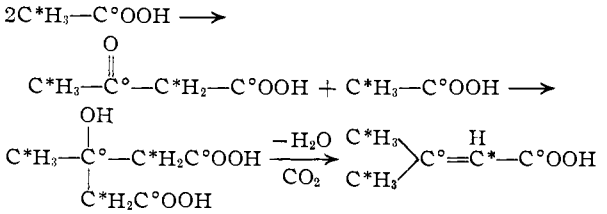
SCHEME I



° = Acetate-COOH; * = Acetate CH_3

However the reactions of Scheme II would explain the incorporation of carboxyl labeled acetoacetate.

SCHEME II

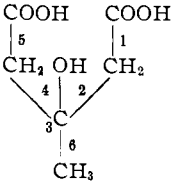


It is assumed that these acids are probably in the form of acyl CoA derivatives.[8,9]

β-Hydroxy-β-methylglutaric acid[10] (β-HMG) occurs naturally[11,12] and might be expected to decarboxylate asymmetrically.[13] Upon feeding to animals it appears to be incorporated directly into cholesterol.[14] To test the validity of Scheme II, 10 μM. of $C^{14}H_3$-COOH (s.a. = 5.0×10^5 c.p.m./μM. acetate)[15] + 50 μM. β-HMG were added to 21.4 ml. of incubation mixture containing rat liver homogenate and ATP and DPN prepared as previously described.[4] Incubation was for three hours at 38° with 100% O_2 in large Warburg cups. The mixture was made alkaline with KOH (final concentration 0.17 *N*) 0.3 mM. of β-HMG was added as carrier, and allowed to stand for half an hour at room temperature. It was acidified with H_2SO_4, then mixed with Celite and extracted with ether for four hours. The ether extract was steam distilled and β-HMG was isolated and purified from the non-volatile fraction by separation on Dowex-1 (formate form) with 0.1 *N* formic acid as eluant.

The β-HMG contained considerable activity. Its purity was established by the coincidence of the titration and radioactivity curves on Dowex-1 and on a Celite column with butanol and chloroform as solvent. Also, no depression of the melting point occurred on admixture with an authentic sample. β-HMG was degraded in the following manner. A Schmidt reaction gave carbons 1 and 5 as CO_2. Dehydration with H_2SO_4 followed by $KMnO_4$ oxidation gives acetoacetic acid which was degraded further to acetate (carbons 3 and 6) and formate (carbons 2 and 4).[16] Acetate and formate were isolated and identified by partition chromatography. Formate was oxidized by HgO. Acetate was degraded by pyrolysis of the barium salt. The distribution of isotope found is shown in Table I.

TABLE I

β-HMG	c.p.m./mM. C^{15}
	500
	11100
	250
	10500
Total oxidation calculated	5650
found	6050

It will be noted that it parallels that found in β-DMA.[5] These results offer support for Scheme II and indicate that β-DMA and β-HMG may be precursors in the synthesis of cholesterol. They suggest that acetoacetic acid or its CoA derivative may condense with acetyl CoA in a manner analogous to the condensation of oxalacetate and acetyl CoA to form citric acid. These results do not rule out Scheme I nor the possibility that β-HMG may also be formed by CO_2 fixation with β-DMA.[8]

The author wishes to acknowledge the technical assistance of Miss Lillian Brown.

(7) M. Blecher (*Fed. Proc.*, **13**, 184 (1954)), also R. W. Chen, *et al.* (*J. Biol. Chem.*, **205**, 383 (1953)) however, present evidence indicating that acetoacetic acid is equilibrated with two carbon units prior to incorporation into cholesterol.

(8) W. G. Robinson, B. K. Bachawat and M. J. Coon, *Fed. Proc.*, **13**, 281, 1954.

(9) H. Klein and F. Lipmann, *J. Biol. Chem.*, **203**, 101 (1953).

(10) While this work was in progress we found that a similar mechanism was suggested by Bloch in a Harvey Lecture delivered December 18, 1952, but published in February, 1954 (Academic Press, Inc., New York, N. Y., 1954).

(11) R. Adams and B. L. Van Duuren, THIS JOURNAL, **75**, 2377 (1953).

(12) H. J. Klosterman and F. Smith, *ibid.*, **76**, 1229 (1954).

(13) D. W. Racusen and S. Aranoff, *Arch. Biochem. Biophys.*, **34**, 218 (1951).

(14) L. C. Clark, I. Harary, O. Reiss and K. Bloch, *Fed. Proc.*, **13**, 192 (1954).

(15) β-HMG counted as carbon dioxide in gas phase counter.

(16) S. Weinhouse and R. Millington, *J. Biol. Chem.*, **181**, 645 (1949).

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RECEIVED MARCH 22, 1954

THE STEREOCHEMISTRY OF SQUALENE. A NEW METHOD FOR THE DETERMINATION OF *CIS-TRANS* ISOMERISM[1]

Sir:

When X-ray diffraction patterns are taken of single crystals of urea or thiourea adducts, continuous layer lines appear in addition to the sharp spots produced by the host. The distance between these lines is a function of the length of the adducted molecule. Such lines have been reported.[2,3,4]

This property was used to measure the length of aliphatic molecules and their olefinic derivatives to see what shortening effect a *cis* or *trans* double bond has on the length. Results for urea adducts of a series of C_{18} acids are summarized in Table I.

(1) This investigation was supported in part by the Medical Research and Development Division, Office of the Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-411.

(2) A. E. Smith, *Acta Crystallographica*, **5**, 224 (1952).

(3) W. Borchert, *Heidelberger Beiträge z. Min. u. Pet.*, **3**, 124 (1952).

(4) F. Laves and N. Nicolaides, Abstr. of Am. Cryst. Assoc. Meeting, pp. 16–17 (1952).