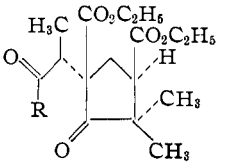
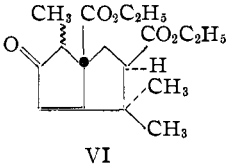


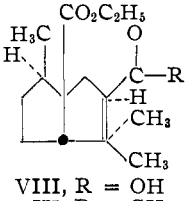
IV, R = OH
V, R = CH_3

The dry sodium salt of IV was transformed by oxalyl chloride into the corresponding acid chloride which reacted readily with diazomethane to give a crystalline diazoketone. The diazoketone was converted into the chloromethyl ketone, m.p. 72–73° (Found: C, 56.41; H, 7.08), which was readily reduced to the crystalline methyl ketone (V) (Found: C, 62.34; H, 7.92. Semicarbazone, m.p. 191–193°; Found: C, 56.41; H, 7.91).

Short treatment with potassium *t*-butoxide changed the diketone (V) into the bicyclic aldol which was readily dehydrated by heating with *p*-toluene sulfonic acid in benzene. The cyclopentenone so obtained (VI) is a mixture of C_7 epimers, one of which had m.p. 65–67°. (Found: C, 66.21; H, 7.87). Epimerism at C_7 at this stage of the synthesis is irrelevant, and the 65° isomer was reduced with lithium and liquid ammonia, or



VI



VIII, R = OH
IX, R = CH_3

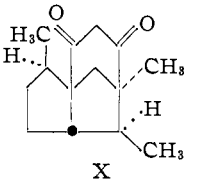
with palladium on charcoal, to the *cis* bicyclic ketone (VII) m.p. 33.5–35.0°; dinitrophenylhydrazone, m.p. 160–161° (Found: C, 56.26; H, 6.04). The stereochemistry at C_7 in this ketone corresponds to that of the more stable epimer since base hydrolysis, followed by reesterification gave back unchanged VII. Transformation of VII into the thioketal, m.p. 75–76° (Found: C, 59.23; H, 7.77) and Raney nickel desulfurization of the latter gave *dl*-diethyl norcedrenedicarboxylate, hydrolyzed to *dl*-norcedrenedicarboxylic acid VIII, m.p. 221–223°. (Found: C, 65.02; H, 8.36). The infrared spectrum of the anhydride of VIII was indistinguishable from that of *l*-norcedrenedicarboxylic anhydride obtained by the degradation of natural cedrene.[3]

Resolution of the *dl*-acid was readily effected by means of the quinine salt, m.p. 209–210°; $(\alpha)^{27}D$ −122° (*c* 1.00 in chloroform). (Found C: 70.13; H, 8.18), which was decomposed to produce *l*-norcedrenedicarboxylic acid, m.p. 212–213°, undepressed on mixture with the natural isomer; the rotation, $(\alpha)^{27}D$ −38.9° ± (*c* 1.08 in acetone), was identical with that of the acid from natural sources.

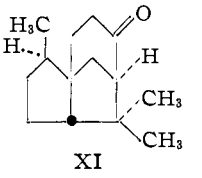
Conversion of the *l*-acid by established paths[4] into the methyl ketone (VIII), followed by treatment of the ester with potassium *t*-butoxide led to the cyclohexane dione (X), m.p. 202–204°. (Found: C, 76.66; H, 9.07). Reduction of X

(3) We thank Dr. T. F. Gallagher for carrying out this comparison.

(4) G. Stork and R. Breslow, THIS JOURNAL, **75**, 3292 (1953).



X



XI

with lithium aluminum hydride gave a mixture of saturated and unsaturated alcohols which, on oxidation with chromic acid, followed by catalytic hydrogenation, gave rise to the saturated ketone (XI); 2,4-dinitrophenylhydrazone, m.p. 146–147°. Reaction of the ketone (XI) with methyl lithium gave *l*-cedrol (III), identical in all respects with the natural product (m.p. and mixed m.p. 86.5–87.5°, identical infrared absorption curves).

The stereochemically simpler cedrene is obtainable by dehydration of cedrol with formic acid, and the synthesis of cedrol is also a synthesis of cedrene.

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RECEIVED JANUARY 28, 1955

ENZYMATIC RACEMIZATION OF β-HYDROXYBUTYRYL-S-CoA AND THE STEREOSPECIFICITY OF ENZYMES OF THE FATTY ACID CYCLE[1]

Sir:

Some evidence has been presented that the enzymes crotonase[2,3] and β-hydroxybutyryl-S-CoA dehydrogenase (β-keto reductase)[4,5,6] which catalyze reactions (1) and (2) are specific for *d*-BOH-S-CoA.[5,6]

$$\text{Crotonyl-S-CoA} + H_2O \rightleftharpoons d\text{-BOH-S-CoA} \quad (1)$$

$$d\text{-BOH-S-CoA} + DPN^+ \rightleftharpoons \text{acetoacetyl-S-CoA} + DPNH + H^+ \quad (2)$$

However, an "activating" enzyme of liver forms both the *d*- and *l*-isomers of BOH-S-CoA from the corresponding free acids,[5] probably according to reaction (3).

$$BOH + \text{CoA-SH} + ATP \rightleftharpoons \text{BOH-S-CoA} + AMP + PP \quad (3)$$

By use of synthetically prepared *l*-BOH-S-CoA and *d*-BOH-S-CoA[7] we have been able to establish directly the stereospecificity of enzymes catalyzing reactions (1) and (2), and have found that liver and other organs contain an enzyme or enzyme system

(1) Supported by grants from the U. S. Public Health Service, the American Cancer Society (recommended by the Committee on Growth National Research Council), the Nutrition Foundation, Inc., and by a contract (N6onr279, T.O. 6) between the Office of Naval Research and New York University, College of Medicine. Abbreviations: Coenzyme A (reduced), CoA-SH; acylcoenzyme A derivatives, acyl-S-CoA; β-hydroxybutyric acid, BOH; β-hydroxybutyryl-S-CoA, BOH-S-CoA; *d* and *l* refer to direction of rotation; DPN^+ and DPNH, oxidized and reduced diphosphopyridine nucleotide; 2-amino-2-methyl-1,3-propanediol, Diol.

(2) J. R. Stern and A. del Campillo, THIS JOURNAL, **75**, 2277 (1953).

(3) S. J. Wakil and H. R. Mahler, *J. Biol. Chem.*, **207**, 125 (1954).

(4) F. Lynen, L. Wessely, O. Wieland and L. Rueff, *Angew. Chem.*, **64**, 687 (1952).

(5) A. L. Lehninger and G. D. Greville, *Biochim. Biophys. Acta*, **12**, 188 (1953).

(6) S. J. Wakil, D. E. Green, S. Mii and H. R. Mahler, *J. Biol. Chem.*, **207**, 631 (1954).

(7) Prepared from *l*-BOH and *d*-BOH (donated by Dr. G. D. Greville) by the general method of T. Wieland and L. Rueff, *Angew. Chem.*, **65**, 186 (1952).

catalyzing the reversible interconversion of the two stereoisomers

$$l\text{-BOH-S-CoA} \rightleftharpoons d\text{-BOH-S-CoA} \quad (4)$$

Preparations of pig heart[8] or rat liver BOH-S-CoA dehydrogenase oxidize synthetic *d*-BOH-S-CoA with concomitant reduction of DPN as determined spectrophotometrically. *l*-BOH-S-CoA is not oxidized by the dehydrogenase, nor does it inhibit the oxidation of *d*-BOH-S-CoA (Fig. 1).

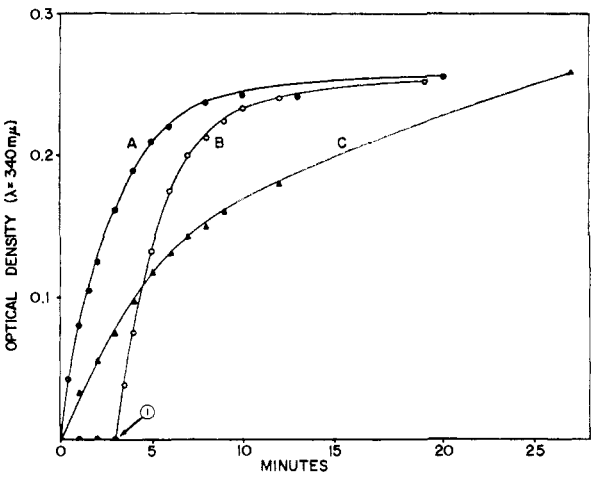

Fig. 1.—The experimental cell (d = 0.5 cm.) contained initially 100 μM. Diol buffer *p*H 9.5, 0.27 μM. DPN+ and 0.14 μM. *d*-BOH-S-CoA (A), or 0.13 μM. *l*-BOH-S-CoA (B), or 0.21 μM. *l*-BOH-S-CoA (C). The reaction was started at zero time by addition of 20 γ of pig heart fraction (A and B) or 600 γ of ox liver fraction (C). At Arrow 1, 0.14 μM. *d*-BOH-S-CoA was added. A concomitant increase in O.D. at λ310 mμ (not shown) due to acetoacetyl-S-CoA formation was recorded in Experiments A and B, but was partly obscured in Experiment C by contamination of liver fraction with thiolase; volume, 1.5 ml.; temp., 25°.

Hence the dehydrogenase is stereospecific for the *d*-isomer. However, certain enzyme fractions from ox and rat liver which oxidize *d*-BOH-S-CoA were also found to oxidize *l*-BOH-S-CoA (*cf.* Figure 1), but at much slower rates. This suggested the presence either of a racemase or of a DPN+-linked dehydrogenase specific for *l*-BOH-S-CoA which in concert with the *d*-BOH-S-CoA dehydrogenase could effect racemization. As shown in Table I, the dialyzed ox liver fraction catalyzes the conversion of *l*-BOH-S-CoA to *d*-BOH-S-CoA, measured with *d*-BOH-S-CoA dehydrogenase, and conversely the conversion of the *d*-isomer to a form which no longer reacts directly with DPN+ in the presence of *d*-BOH-S-CoA dehydrogenase but which does react on addition of the ox liver fraction. Since (a) the liver fraction contains only 0.5–1 γ of DPN+ per mg. protein[9] (final DPN+ concentration $\leq 1.3 \times 10^{-6}$ *M*), and (b) the rate of interconversion of the isomers is not affected by addition of excess DPN+ although the *KM* for DPN+ of *d*-BOH-S-CoA dehydrogenase is 4.3×10^{-5} *M*,[6] the presence of a racemase rather than a specific *l*-BOH-S-CoA dehydrogenase is indicated. Preparations of ox and rat liver possessing racemase activity were found to be devoid of DPN+-linked dehydrogenases acting on *d*- or *l*-BOH and of enzymes racemizing these isomers. BOH-S-CoA racemase activity, measured as DPN+ reduction by *l*-BOH-S-CoA, has been found in extracts of mitochrondria from rat liver, kidney, heart and brain and in extracts of *R. rubum* and *Cl. acetobutylicum*, all of which contain the *d*-BOH-S-CoA dehydrogenase.

(8) J. R. Stern, M. J. Coon and A. del Campillo, THIS JOURNAL, **75**, 1517 (1953).

(9) Assayed catalytically according to an unpublished procedure of Dr. S. Korkes.

TABLE I

100 μM. diol buffer, *p*H 9.5, BOH-S-CoA (as indicated) and 1 mg. of ox liver fraction (final volume, 1.0 ml.) were incubated 30 minutes at 25°. Assays performed after acid heat deproteinization.

Addition	μM. recovered: *d*-BOH-S-CoA[b]	μM. recovered: *l*-BOH-S-CoA[c]
l-BOH-S-CoA[a] (0.21 μM.)	0.06	0.13
d-BOH-S-CoA[a] (0.20 μM.)	0.10	0.12

[a] Assayed by conversion to citrate in a multi-enzyme system.[2] [b] Assayed with heart *d*-BOH-S-CoA dehydrogenase fraction. [c] Measured by reduction of DPN on addition of ox liver fraction after oxidation of *d*-BOH-S-CoA by heart dehydrogenase fraction.

Crystalline crotonase[10] was found to dehydrate *d*-BOH-S-CoA but not *l*-BOH-S-CoA as determined by direct optical test.[11] Dehydration of *l*-BOH-S-CoA occurred only in the presence of the liver racemase system and crotonase. We find that crystalline crotonase hydrates *cis*-crotonyl-S-CoA as well as *trans*-crotonyl-S-CoA. With both isomers the product of hydration has been identified as *d*-BOH-S-CoA by enzymatic assay.

The enzymatic racemization of BOH-S-CoA may play a special role in linking ketone body metabolism to the fatty acid cycle. The properties of the racemase system and its significance are currently being investigated.

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RECEIVED NOVEMBER 1, 1954

(10) J. R. Stern, I. Raw and A. del Campillo, *Fed. Proc.*, **13**, 304 (1954).

(11) Compare F. Lynen and S. Ochoa, *Biochim. Biophys. Acta*, **12**, 299 (1953).

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A MECHANISM FOR THE PHOTO-OXIDATION OF WATER BY CERIC ION

Sir:

Chemical evidence has been obtained for the intermediate formation of OH radical in the photoreduction of ceric ion as proposed by Weiss and Porret.[1] The evidence is the effect of added solutes which have been demonstrated to react with OH radical in aqueous solution. Photoreduction of ceric ions by ultraviolet radiation, previously re-

(1) J. Weiss and D. Porret, *Nature*, **139**, 1019 (1937).