THE ABSOLUTE CONFIGURATION OF METHYLMALONYL CoA AND STEREOCHEMISTRY OF THE METHYLMALONYL CoA MUTASE REACTION*

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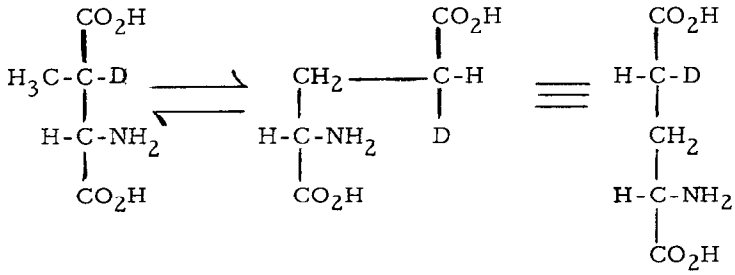
In a previous investigation the enzymic rearrangement of threo-L-β-methylaspartate to L-glutamate (Iodice and Barker, 1963) was studied with the aid of methylaspartate-3-D (I). The absolute



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configuration of the resulting glutamate-4-D (II) showed that the reaction had occurred with inversion of configuration of the β-carbon of methylaspartate (Sprecher, Switzer, and Sprinson, unpublished observations;

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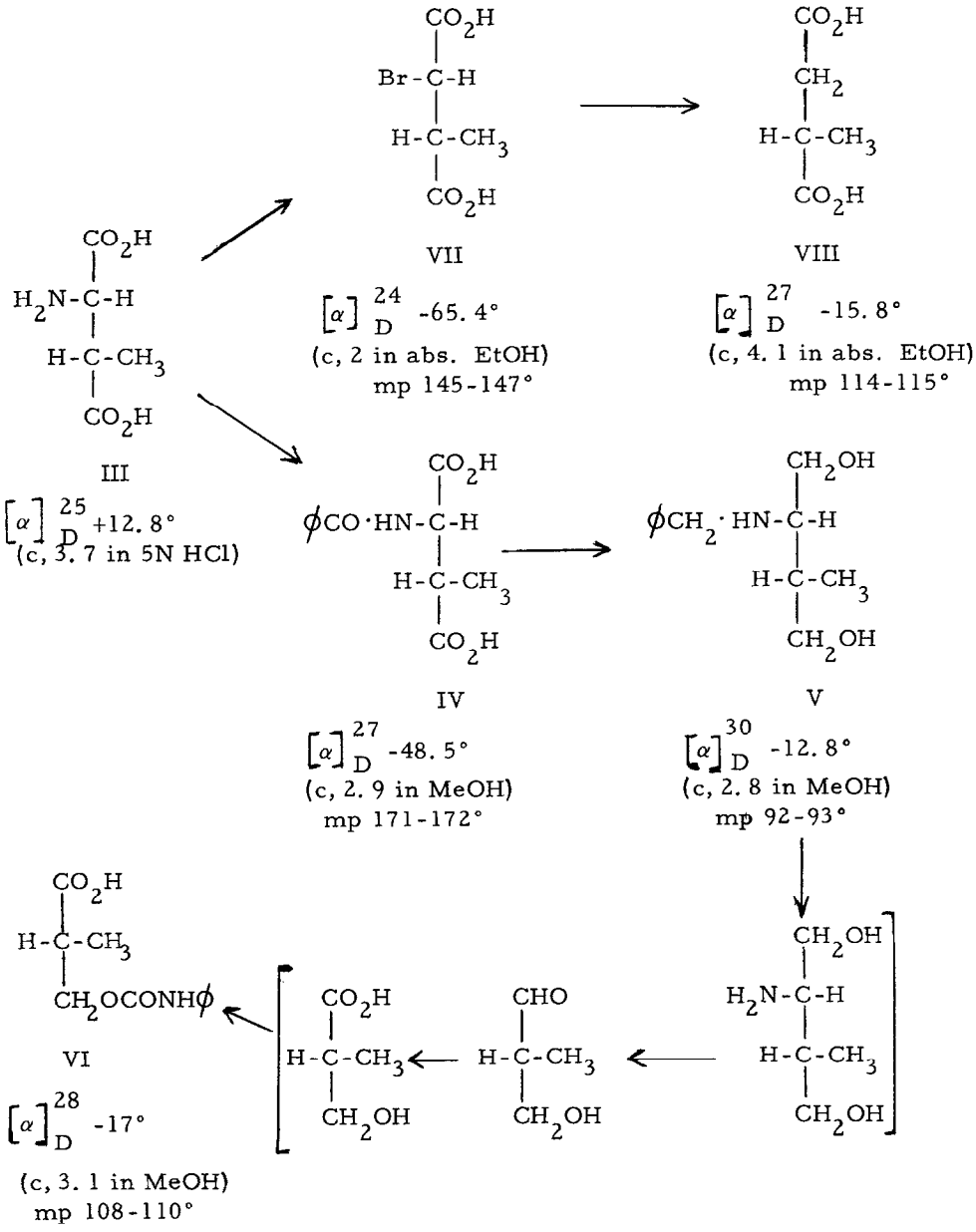
Sprecher and Sprinson, 1963). It was considered of interest to determine also the steric course of the similar rearrangement of methylmalonyl CoA (b)[1] to succinyl CoA (Kaziro and Ochoa, 1964). Since a cobamide coenzyme is required for activity in both reactions it was thought that a knowledge of their stereochemistry would throw some light on the mechanism of action of the coenzyme.

A solution to the problem was planned along the following lines: (1) reduction of methylmalonyl CoA (a) (formed in the propionyl CoA carboxylase reaction) with Raney nickel to an optically active 3-hydroxy-2-methylpropionic acid (β-hydroxyisobutyric acid); (2) preparation of a 3-hydroxy-2-methylpropionic acid of known configuration; (3) epimerization in D_2O of methylmalonyl CoA (a) by its racemase to methylmalonyl CoA (b)-2-D, isomerization of the latter to succinyl CoA-2-D, and determination of the configuration of the deuteriosuccinate from its optical rotatory dispersion curve (Cornforth et al, 1962).

Preparation of $\underline{D}_s$-3-hydroxy-2-methylpropionic acid N-phenylcarbamate. — Threo-$\underline{L}$-β-methylaspartate (III) was benzoylated, and the N-benzoyl derivative (IV) was reduced with $LiAlH_4$ in boiling tetrahydrofuran (Scheme 1). After hydrogenolysis of the 2-benzylamino-1, 4-dihydroxy-3-methylpropane (V) over Pd, cleavage with sodium periodate, removal of iodate with $Ba(NO_3)_2$, and oxidation with Ag_2O (or with Br_2), the resulting $\underline{D}_s$-3-hydroxy-2-methylpropionic

1. According to accepted convention (cf. Mazumder et al., 1962) methylmalonyl CoA (a) refers to the product of the propionyl CoA carboxylase reaction, and methylmalonyl CoA (b) refers to the epimer (produced by methylmalonyl CoA racemase) which is the substrate of methylmalonyl CoA mutase.



Scheme 1[2]

acid was removed by extraction with ether, treated with phenylisocyanate, and isolated as the crystalline N-phenylcarbamate (VI).

Although there is considerable evidence (Barker et al, 1958; Bright and Ingraham, 1960) in favor of the configuration of

[2]Compounds IV to VIII gave satisfactory analytical data, and their IR spectra were in accord with the assigned structures.

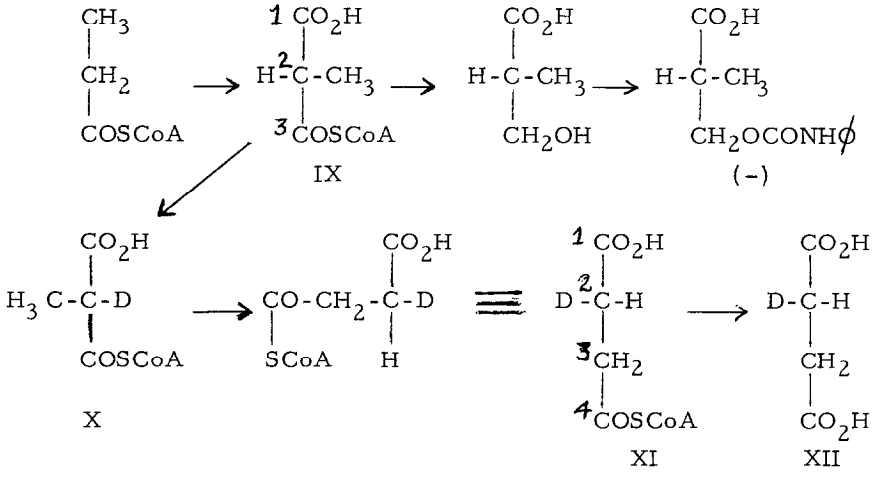
threo-L-β-methylaspartate (III), it was thought desirable to confirm it by correlation with a compound of known configuration. This was done by the converting III with $NaNO_2$-HBr-NaBr (Holmberg, 1927) to threo-L_s-2-bromo-3-methylsuccinic acid (VII), and reducing VII with hydrogen over Pt to the known (Fredga, 1942) L_s-2-methylsuccinic acid (VIII). The absolute configuration of VI is, therefore, established by the method of its preparation.

Reduction of methylmalonyl CoA (a) to D_s-3-hydroxy-2-methylpropionic acid. – Beef heart propionyl CoA carboxylase (22 units in 1.3 ml) of specific activity 9 and free of racemase (Kaziro et al., 1961) was added to 96 ml of a solution containing 0.30 mmoles of propionyl CoA, 1.60 mmoles of ATP, 0.06 mmoles of versene, 0.24 mmoles of glutathione, 1.2 mmoles of $MgCl_2$, 6.0 mmoles of $KHCO_3$, and 12 mmoles of potassium phosphate buffer, pH 7.8, and incubated for 15 minutes at 25°. A simultaneous control with 0.01 ml of enzyme solution and $Na_2C^{14}O_3$ showed a yield of 55% methylmalonyl CoA. The solution was treated immediately with Raney nickel-T1 (Dominguez et al., 1961) under hydrogen for 2.5 hours, the catalyst was removed by filtration, and the filtrate was acidified and extracted with ether. After careful removal of the solvent, the residue was treated with 1.2 ml of phenylisocyanate, and the 3-hydroxy-2-methylpropionic acid N-phenylcarbamate was isolated and purified by partition chromatography on celite. Yield 13 mg. On recrystallization from benzene 12 mg were obtained, mp 122-123°, sintering at 105° (the mp of racemic VI is 123°; that of (-)VI is 108-110°). It had a negative plain optical rotatory dispersion curve in the 525 to 290 mμ region parallel to the curve obtained with VI. The specific rotation was 20% of that shown by VI (80% racemization).

Conversion of propionyl CoA in D_2O to deuterio succinate. – An acetone powder (11.7 g) of beef liver mitochondria was extracted at 4° for 20 minutes by stirring with 117 ml of 0.005 M Tris hydrochloride buffer (pH 7.8) in D_2O, containing 0.005 M glutathione (Hegre et al., 1962). Insoluble material was removed by centrifugation at 20,000 x g for 20 minutes and discarded. The supernatant solution (690 mg of protein) was incubated for 15 minutes at 37° with 0.40 mmoles of propionyl CoA, 1.4 mmoles of ATP, 5 mmoles of $Na_2C^{14}O_3$ (20 μ curies), 1.4 mmoles of $MgCl_2$, 1.75 mmoles of glutathione, and 56 mmoles of Tris hydrochloride buffer, pH 7.8, in D_2O (final volume 400 ml). The propionyl CoA was added last to a solution of the other constituents at 37°. A radioactivity assay on an aliquot showed a 90% yield of succinate. After acidification and removal of precipitated proteins, succinic acid was isolated by ether extraction, and purified by chromatography on Dowex 1-Cl (Busch et al., 1952), sublimation in a vacuum, and crystallization from tetrahydrofuran-benzene. Yield 20 mg, mp 188-190°. This compound had 17.0 atom % excess D (1.02 atoms D) and a plain positive optical rotatory dispersion curve equivalent to 0.63 of the values obtained with stereospecifically labeled succinate-2-D. Approximately 0.4 atoms D were therefore introduced probably through exchange of two trans hydrogens by succinate dehydrogenase (Englard and Colowick, 1956; Tchen and Van Milligan, 1960) resulting in the formation of meso-succinate-2,3-D. In a control experiment succinyl CoA was incubated with the extract of the acetone powder in D_2O. The resulting succinic acid contained 0.22 atoms D, but was optically inactive.

Discussion. — The (–)3-hydroxy-2-methylpropionic acid N-phenylcarbamate (Scheme 2) obtained from methylmalonyl CoA (IX)

had 20% of the optical activity of VI, and must therefore have been about 80% racemized. [3] It is nevertheless clear from the sign of the



Scheme 2

residual optical activity that C-2 of IX , the product of the propionyl CoA carboxylase reaction, had the same configuration as the β-carbon of threo-L-methylaspartate (III).

When the series of reactions from propionyl CoA to succinate was carried out in D_2O, C-2 of D_s-methylmalonyl CoA (IX) was epimerized and its hydrogen was exchanged for D. (Mazumder et al., 1962; Overath et al., 1962). The L_s-methylmalonyl CoA-2-D (X) then underwent intramolecular rearrangement to succinyl CoA (XI) by migration of the -COSCoA group (Scheme 2). The deuteriosuccinate (XII) resulting from the deacylation of XI had a positive plain O. R. D. curve and is therefore L_s-succinate-2-D (Cornforth et al., 1962; Sprecher and Sprinson, 1963). As shown in the transformation of X to XI (Scheme 2) the methylmalonyl CoA mutase reaction must have occurred with retention of configuration of C-2.

3. Control experiments with DL-methylmalonyl CoA in D_2O showed extensive exchange of hydrogen on C-2 with D during reduction with Raney nickel.

It is of interest to compare the straight chain substrates of the glutamate and methylmalonyl CoA mutase reactions. The relevant carbon atoms of II and XI have opposite stereochemistry. In so far as the function of the cobamide coenzyme is concerned there is, therefore, no exclusive stereochemical requirement for activity. Whereas methylaspartate is rearranged with inversion, $\underline{\underline{L}}_S$-methylmalonyl CoA is rearranged with retention of configuration.

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