THE ABSOLUTE CONFIGURATION OF METHYLMALONYL-CoA

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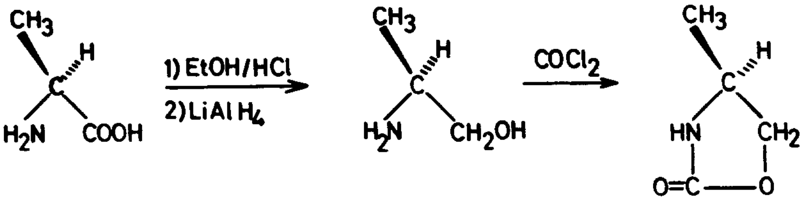
Received May 25, 1964

Methylmalonyl-CoA is an important intermediate in the metabolism of propionic acid. OCHOA and collaborators (1) found that the enzymic carboxylation of propionyl-CoA formed only one of the two possible diastereomeres of methylmalonyl-CoA, which was called "form a". On the other hand highly purified methylmalonyl-CoA-isomerase, which is a vitamin B_{12} dependent enzyme, was only able to produce succinyl-CoA, if the other diastereomere of methylmalonyl-CoA "form b" was present (1). Equilibrium between forms a and b can be accomplished by a specific enzyme (epimerase) or by heating at 100° C (2,3).

In the following communication the absolute configuration of "form a" of methylmalonyl-CoA is established. Aqueous solutions of S-methylmalonyl-N-capryl-cysteamine at pH 8.0 can be reduced by freshly prepared Raney nickel to ß-hydroxyisobutyric acid. Employing S-(2-deuterio-methylmalonyl)-N-capryl-cysteamine the ß-hydroxyisobutyric acid isolated after reduction contained 80 % of the original deuterium in position 2. This result showed that optically active 2-deuterio-methylmalonyl thiolesters are racemized to the extent of only 20 % during the reductive reaction.

($\pm$)-ß-hydroxyisobutyric acid was resolved into its optical isomeres by recrystallization of its quinine salt from absolute ethanol, to obtain the levorotatory form or by recrystallization of its (-)-α-phenylethylamine salt from absolute ethanol-ether solution to obtain the dextrorotatory form.

The absolute configuration of (-)-ß-hydroxyisobutyric acid (III) was related to (+)-(S)-alanine (I) by the following chemical reactions:



I

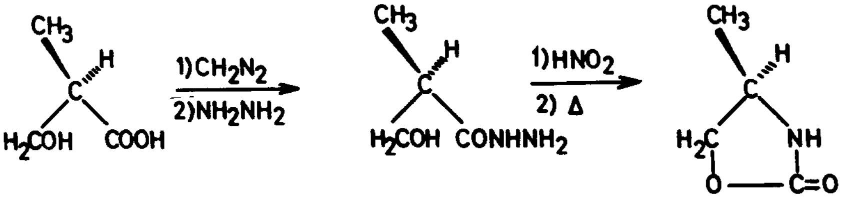
II

(+)-(S)-alanine

(+)-(S)-alaninol $[\alpha]^{20}_{578}$ - 7,8°

(c=4,88 in EtOH)

mp 53-54°



III

IV

$[\alpha]^{20}_{578}$ -7,6°

(c=11,95 in EtOH)

$[\alpha]^{20}_{578}$ -28,7°

(c=8,28 in water)

mp 132-133°

$[\alpha]^{20}_{578}$ + 7,9°

(c=7,74 in EtOH)

mp 53-54°

(+)- und (-)-methyloxazolidones (IV,II) had identical melting points (53-54°), IR spectra and microanalyses.

Their optical rotatory dispersion curves showed Cotton effects of opposite signs*.

Form a of methylmalonyl-CoA (V) was prepared by carboxylating 2,2-dideuterio-propionyl-CoA in the presence of C^{14}-bicarbonate, ATP, magnesium and potassium ions and crystalline propionyl-CoA-carboxylase from pig heart (specific activity 11,4 units/mg) (4). The C^{14}-2-deuterio-methylmalonyl-CoA showed a specific radioactivity of 12×10^6 counts/min · µmole (assay in dioxane-naphthalin solution in a Packard Tri-Carb liquid scintillation spectrometer). 6 µmoles of the C^{14}-methylmalonyl-CoA were treated immediately after formation with an excess of freshly prepared Raney nickel. After addition of 10 mg (-)-(R)-ß-hydroxyisobutyric acid (III) as a carrier, the solution was filtered. The Raney nickel was washed with n/10 sodium hydroxyde and combined with the filtrate. The solution was acidified and extracted with ether. The ether extract contained 24×10^6 counts/min. Aliquots of this product were mixed with 40 mg (385 µmoles) of (-)-(R)-, 20 mg (192 moles) of (+)-(S)- and 40 mg (385 moles) of (±)-ß-hydroxyisobutyric acid respectively. The hydrazids were formed and recrystallized from absolute ethanol. After each recrystallization the specific radioactivity was determined. The data are recorded in Table I.

Table I

Number of recrystallization	counts/min · µmole		
	(R)-hydrazid	(RS)-hydrazid	(S)-hydrazid
0	3120	3120	6240
1	2780	2750	1070
2	2740	2680	837
3	2750	2645	770

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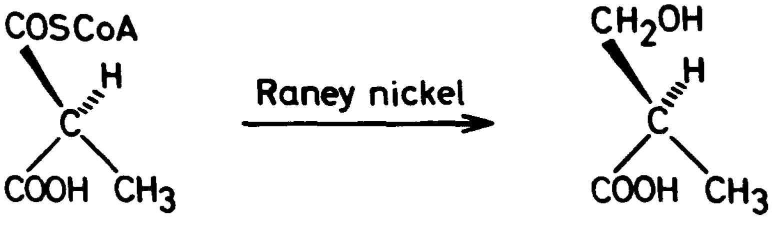
* We thank Prof. W. KLYNE for the optical rotatory dispersion spectra.

In an other experiment the quinine salt of (-)-(R)-ß-hydroxyisobutyric acid (III), when mixed with the radioactive ether extract did not change the specific radioactivity upon recrystallization, whereas the (-)-α-phenylethylamine salt of (+)-(S)-ß-hydroxyisobutyric acid subsequently lost radioactivity. After several recrystallizations of the latter and preparing the hydrazid of the regenerated (+)-(S)-ß-hydroxyisobutyric acid (3 x recrystallized) only 19 % of the original radioactivity was present.

These results prove that the reduction of "form a" of methylmalonyl-CoA (V) by Raney nickel formed (-)-(R)-ß-hydroxyisobutyric acid (III). It follows, therefore, that "form a" of methylmalonyl-CoA (V) has the S configuration.



V

III

(S)-methylmalonyl CoA

(-)-(R)-ß-hydroxyisobutyric acid

Note: Just before sending this manuscript to the editors, we received news that SPRECHER et al. (this Journal 15, No. 6) found methylmalonyl CoA (a) possessing the same configuration as described in this paper.

References

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