CONFIGURATION OF α,β-DIHYDROXY-β-METHYLVALERIC ACID, AN ISOLEUCINE PRECURSOR[1]

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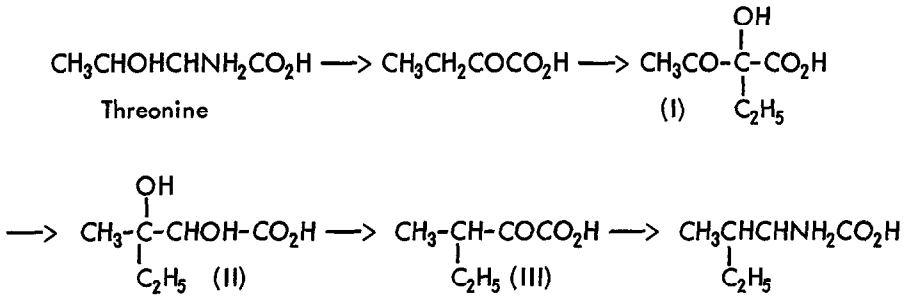
Biosynthesis of isoleucine in bacteria has been shown, by a combination of tracer studies (Strassman et al, 1956) and enzymatic methods (Radhakrishnan and Snell, 1960; Tatum and Adelberg, 1951; Adelberg et al, 1951) to proceed by way of the intermediates shown in Scheme 1; the detailed evidence has been summarized recently (Meister, 1965). Compound II, α,β-dihydroxy-β-methylvaleric acid, is formed by the action of reductoisomerase on α-aceto-α-hydroxybutyric acid (I) (Radhakrishnan et al, 1960) and converted by a dihydroxyacid dehydrase to α-keto-β-methylvaleric acid (III) (Umbarger and Adelberg, 1951), the immediate precursor of isoleucine.

Scheme 1. Biosynthesis of Isoleucine



Acid II, with two asymmetric centers, is capable of existence in four optically active forms. Though the biosynthetic intermediate is apparently a single isomer and has been isolated[3] and synthesized as a pure quinine salt (Sjolander et al, 1954), no evidence

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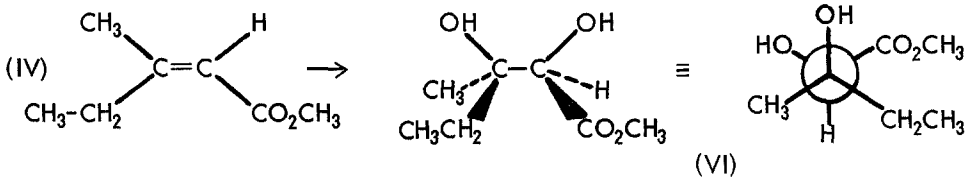
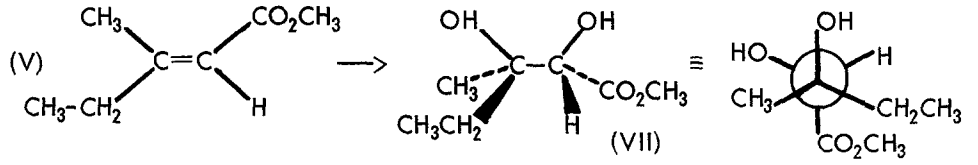
(3) An identical acid has been isolated (Mattocks, 1964) as an ester of a pyrrolizidine alcohol in the alkaloid strigosine.

has been presented concerning its stereochemistry. This question is of considerable significance because of the intimate dependence of biological activity on geometric configuration. We report here the elucidation, by stereospecific synthesis, of the relative configuration of the two asymmetric centers of II.

Method and Results.

The approach chosen to determine the relative configuration of II was to synthesize stereospecifically both racemates for comparison with the natural acid. Wittig reaction of 2-butanone with carbomethoxymethylenetriphenylphosphorane (Fodor and Tomoskozi, 1961) gave a mixture of cis- and trans- isomers (IV and V) of methyl β-ethylcrotonate in a 5:12 ratio. Vapor phase chromatography on an SDC-710 column at 155° cleanly separated the esters. Configurations could be assigned conclusively on the basis of their nmr spectra: the olefinic methyl in IV appears as a doublet (J = 1.5 Hz) at δ 1.86, while the corresponding methyl protons of V, deshielded by the cis ester function, (Jackman and Wiley, 1960) are shifted downfield to δ 2.12. Conversely, the methylene group of IV is deshielded and appears as a quartet (J = 8 Hz) at δ 2.63, while the methylene protons of V fall at δ 2.17. Both esters gave similar mass spectra with parent peaks at m/e 128.





Stereospecific cis hydroxylation of IV and V was achieved with neutral $KMnO_4$ in aqueous ethanol at -40° (Riiber, 1915), and the diols purified by vapor phase chromatography. On the basis of the known stereochemistry of permanganate hydroxylation (Gunstone, 1960), the diol from IV may be assigned configuration VI, while that from V is assigned configuration VII.

To enable comparison with the natural acid, a sample of II was synthesized by the published method (Sjolander et al, 1954); the quinine salt had m.p. 203-204°, $[\alpha]^{24}D$ -139°; reported m.p. 203-204°, $[\alpha]^{23}D$ -144°. The acid was freed from the

quinine salt and converted to the methyl ester, $[\alpha]^{25}D$ -14.4° (c= 1.0 in dil. HCl, pH 1), with diazomethane. Though the infrared spectrum of this ester of the natural acid was indistinguishable from those of both synthetic racemates VI and VII, a clear differentiation could be made by comparing nmr and mass spectra. The mass spectrum and nmr spectrum of the methyl ester of II were identical with those of VI and showed slight but distinct differences from those of VII. These results establish that the natural α,β-dihydroxy-β-methylvaleric acid has the relative configuration indicated in VI. Studies to elucidate the absolute configuration are in progress.

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