Absolute Configuration of 2,3-Dihydroxy-3-methylpentanoic Acid, an Intermediate in the Biosynthesis of Isoleucine

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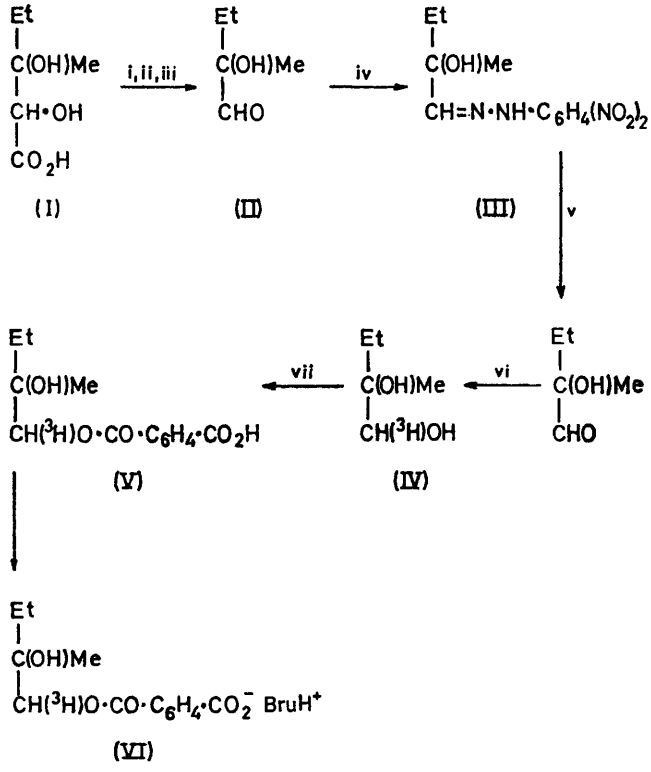
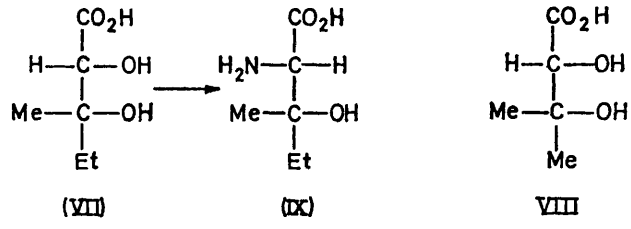
Summary The dihydroxy-acid precursor (VII) of isoleucine (IX) is shown to have the 2*R*,3*R*-configuration.

2,3-DIHYDROXY-3-METHYLPENTANOIC ACID (I) is a precursor of L-isoleucine both in micro-organisms[1] and in higher plants.[2] The isomer of the dihydroxy-acid (I) formed by Darzens condensation between butan-2-one and ethyl chloroacetate, followed by hydrolysis and purification of the acid as the quinine salt, was found to be identical in physical and biological properties with the natural (−)-acid (I) isolated from a *Neurospora crassa* mutant.[3] The *erythro*-configuration has recently been assigned to the synthetic acid.[4]

We have synthesised the (−)-*erythro*- and (−)-*threo*-acids (I) by stereospecific *trans*- and *cis*-hydroxylation, respectively, of *trans*-3-methylpent-2-enoic acid, followed by resolution of the quinine salts. It was intended to degrade the (−)-*erythro*-acid (I) to the aldehyde (II) by the method illustrated in Scheme 1, and to determine the absolute configuration of this aldehyde by comparison of the c.d. and o.r.d. curves of its 2,4-dinitrophenylhydrazone with those of the corresponding derivative prepared from (−)-2-hydroxy-2-methylbutanoic acid of known absolute configuration.[5] However, the derivative (III) prepared from the *erythro*-(−)-acid (I) was found, surprisingly, to give no detectable c.d. or o.r.d. Accordingly, the aldehyde (II) was recovered from the derivative by ozonolysis, reduced with [^{3}H]sodium borohydride to the diol (IV) which was converted into the phthaloyl ester (V) and thence into the corresponding brucine salt (VI) (Scheme 1). Aliquot portions of the labelled brucine salt were then co-crystallised and repeatedly recrystallised with the corresponding inactive brucine salts of the phthaloyl esters of the 2*R*- and 2*S*-diols (IV) prepared by standard methods from the enantiomers of 2-hydroxy-2-methylbutanoic acid. Activity was entrained with the salt of the 2*R*-derivative (V) but was lost from the salt of the 2*S*-derivative. It was concluded that the aldehyde (II) from the *erythro*-acid (I) had the 2*R*-configuration and therefore that the (−)-*erythro*-acid (I) had the 2*R*,3*R*-configuration (VII).



SCHEME 1. Reagents i, CH_2N_2; ii, $LiAlH_4$; iii, $NaIO_4$; iv, $NH_2{\cdot}NH{\cdot}C_6H_4(NO_2)_2$; v, O_3; vi, [^{3}H]$NaBH_4$; vii, $C_6H_4(CO)_2O$. Bru = brucine.



SCHEME 2.

This result indicates that the isoleucine precursor (VII) has the same absolute configuration at C-2 as the corresponding precursor (VIII) of valine,[3,6] a result which was expected since identical enzymes are involved in the formation and further metabolism of both precursors.[1]

The conclusion concerning the absolute configuration of the (−)-*erythro*-acid (VII) is supported by the c.d. curve which shows a negative maximum at 211 nm, $\Delta\epsilon$ − 1·45 (in acidic methanol). Negative Cotton effects are observed in the c.d. and o.r.d. curves of comparable 2*R*-2-hydroxy-acids.[7]

Since the absolute configuration of L-isoleucine (IX) is known, it can be concluded that the conversion of 2*R*,3*R*-2,3-dihydroxy-3-methylpentanoic acid (VII) into L-iso-

leucine (IX) (a process which requires the two enzymatic steps of dehydration and transamination) involves overall retention of configuration at C-3 (Scheme 2).

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