Absolute Configuration of 2,3-Dihydroxy-3-methylpentanoic Acid, an Intermediate in the Biosynthesis of Isoleucine, and its Identity with the Esterifying Acid of the Pyrrolizidine Alkaloid Strigosine [1]

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The (−)-*erythro*- and (−)-*threo*-isomers of 2,3-dihydroxy-3-methylpentanoic acid (I) have been synthesised. The (−)-*erythro*-isomer has been shown to have the absolute configuration 2*R*,3*R* and to be identical with the naturally occurring (−)-acid, a precursor of isoleucine (II), and with the esterifying acid of the pyrrolizidine alkaloid strigosine.

A LAEVOROTATORY isomer of 2,3-dihydroxy-3-methylpentanoic acid (I), an intermediate in the biosynthesis of isoleucine (II),[2] was accumulated by a blocked mutant of *Neurospora crassa*.[3] This isomer was synthesised by a non-stereospecific method and isolated from a mixture of isomers by fractional crystallisation of the quinine salts. The synthesised and natural acids (I) had similar properties; both isomers supported the same growth rate of an isoleucine-deficient mutant of *Escherichia coli*. The synthetic acid has been shown to have the *erythro*-configuration (2*R*,3*R* or 2*S*,3*S*).[4]

To investigate further the stereochemistry of the biologically active isomer of the acid (I), the racemic *erythro*- and *threo*-isomers were prepared. The (*Z*)- and (*E*)-ethyl 3-methylpent-2-enoates were synthesised and separated by fractional distillation. The *E*-isomer was hydrolysed with barium hydroxide to the corresponding acid (III). To show that no isomerisation had taken place on hydrolysis, a portion of the *E*-acid (III) was remethylated (diazomethane) and shown by g.l.c. to contain $<1\%$ of the *Z*-isomer. *trans*-Hydroxylation with tungstic oxide–hydrogen peroxide [5] gave the (±)-*erythro*-acid (IV), and *cis*-hydroxylation with osmium tetraoxide–barium chlorate [6] gave the (±)-*threo*-acid (V) (Scheme 1). The racemic acids were characterised as the crystalline dicyclohexylamine salts. Both acids were resolved as the quinine salts. From the *erythro*-acid a quinine salt of m.p. 208° was obtained and from the *threo*-acid a salt of m.p. 200°. The acids recovered from the salts were both laevorotatory. The *erythro*-isomer had $[\alpha]_D^{23}$ −23.1° and the *threo*-isomer $[\alpha]_D^{23}$ −15.6°. The c.d. curves of the two acids † were very similar [$\Delta\varepsilon_{211}$ −1.45 for the *erythro*- and $\Delta\varepsilon_{213}$ −1.84 for the *threo*-isomer (MeOH–HCl)]. The (±)-*threo*-acid was also prepared by *trans*-hydroxylation of (*Z*)-3-

† The c.d. curves of these acids and their salts will be described in greater detail in a forthcoming paper on the preparation and biological activity of all four isomers of the dihydroxy-acid (I).

[1] Preliminary report, D. H. G. Crout and D. Whitehouse, *J.C.S. Chem. Comm.*, 1972, 398.

[2] J. W. Myers and E. A. Adelberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1954, **40**, 493; R. L. Wixom, J. B. Shatton, and M. Strassman, *J. Biol. Chem.*, 1960, **235**, 128; R. L. Wixom, J. H. Wikman, and G. B. Howell, *ibid.*, 1961, **236**, 3257; R. L. Wixom, *Biochem. J.*, 1965, **94**, 427; H. S. Allaudeen and T. Ramakrishnan, *Arch. Biochem. Biophys.*, 1968, **125**, 199; R. L. Wixom and R. J. Hudson, *Plant Physiol.*, 1961, **36**, 598; M. Kanamori and R. L. Wixom, *J. Biol. Chem.*, 1963, **238**, 998; Z. S. Kagan and A. A. Maleina, *Doklady Akad. Nauk S.S.S.R.*, 1966, **166**, 235; Z. S. Kagan, G. Cheisner, and V. L. Kretovich, *Biokhimiya*, 1964, **29**, 624.

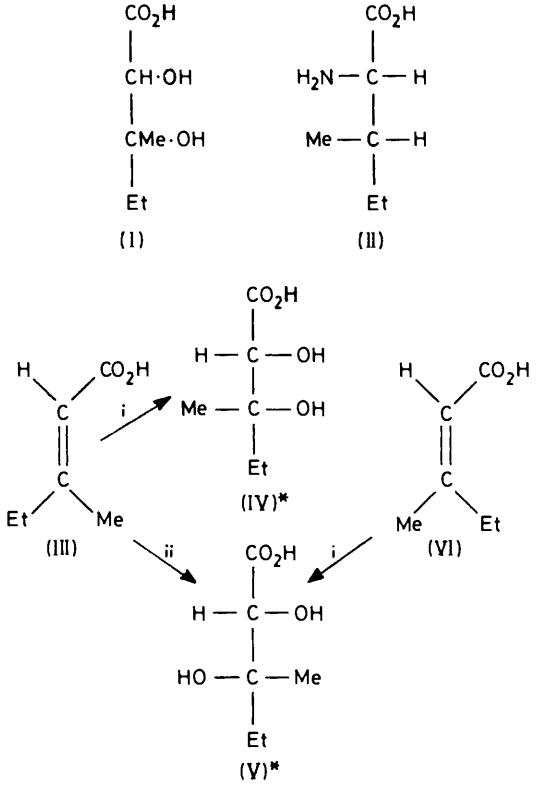
[3] J. R. Sjolander, K. Folkers, E. A. Adelberg, and E. L. Tatum, *J. Amer. Chem. Soc.*, 1954, **76**, 1085.

[4] R. K. Hill and P. J. Foley, *Biochem. Biophys. Res. Comm.*, 1968, **33**, 480.

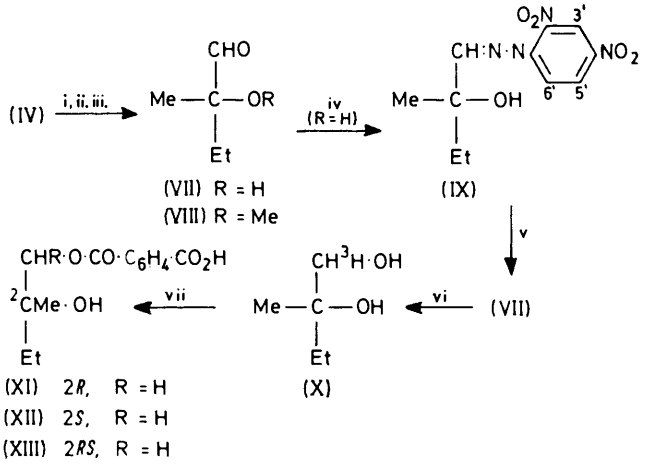
[5] R. Adams and B. L. Van Duuren, *J. Amer. Chem. Soc.*, 1952, **74**, 5349.

[6] G. Braun, *J. Amer. Chem. Soc.*, 1932, **54**, 1133.

methylpent-2-enoic acid (VI) (Scheme 1). The dicyclohexylamine salt of the product was identical (m.p. and mixed m.p.) with the corresponding salt of the acid



SCHEME 1 Reagents: i, WO_3, H_2O_2; ii, OsO_4, $Ba(ClO_3)_2$
* The 2*R*-isomer of the racemic product is illustrated.



SCHEME 2 Reagents: i, CH_2N_2; ii, $LiAlH_4$; iii, $NaIO_4$; iv, $H_2N{\cdot}NH{\cdot}C_6H_3(NO_2)_2$; v, O_3; vi, $NaB(^3H)_4$; vii, phthalic anhydride–C_5H_5N

obtained by *cis*-hydroxylation of the *E*-acid (III), whereas there was a considerable depression of the mixed m.p. with the salt of the dihydroxy-acid obtained by *trans*-hydroxylation of the *E*-acid (III). These results confirm the stereospecificity of the hydroxylation and agree with similar results [7] on analogous hydroxylations of isopropylcrotonic acid (2-isopropylbut-2-enoic acid).

To determine the absolute configuration of the biologically active, laevorotatory isomer of the *erythro*-acid (IV), the degradation shown in Scheme 2 was devised. Methylation (diazomethane) followed by reduction ($LiAlH_4$) and cleavage with periodate gave a mixture containing the aldehyde (VII) and its methyl ether (VIII), which were isolated as the corresponding 2,4-dinitrophenylhydrazones. It was intended to compare the optical properties of the derivative (IX) of the aldehyde (VII) with those of the corresponding derivative prepared from (−)-2-hydroxy-2-methylbutanoic acid of known (*R*) absolute configuration.[8] However, neither of the derivatives of the aldehydes (VII) and (VIII) gave detectable c.d. or o.r.d.

The aldehyde (VII) was therefore recovered from its 2,4-dinitrophenylhydrazone by ozonolysis [9] and reduced with sodium [^{3}H]borohydride to 2-methyl[1-^{3}H]butane-1,2-diol (X), which was converted into the phthaloyl ester (XI; R = ^{3}H). The (2*R*)-, (2*S*)- (of 74% optical purity),[10] and (2*RS*)-2-hydroxy-2-methylbutanoic acids were converted by methylation and reduction into the corresponding diols, which were similarly converted into the (2*R*)-, (2*S*)-, and (2*RS*)-phthaloyl esters (XI)—(XIII). The two enantiomeric phthaloyl esters were converted into their brucine salts, and the racemic ester was converted into its dicyclohexylamine salt. The radiochemical yield of the labelled phthaloyl ester obtained by degradation of the (−)-*erythro*-acid (IV) was obtained by dilution analysis of the crude reaction product with the dicyclohexylamine salt of the racemic ester (XIII). The reaction product was found to contain 14% of its activity as the phthaloyl ester.

A sample of the crude labelled phthaloyl ester was treated with brucine and co-crystallised with the brucine salt of the authentic (2*R*)-phthaloyl ester (XI). After seven recrystallisations the specific activity of the salt had reached a constant value equal to that calculated on the assumption that the labelled ester in the sample taken consisted solely of the 2*R*-isomer. It was concluded that the laevorotatory isomer of *erythro*-2,3-dihydroxy-3-methylpentanoic acid had the 2*R*,3*R*-configuration.

This assignment was confirmed by a corresponding dilution analysis with the brucine salt of the (2*S*)-phthaloyl ester (XII). This analysis was complicated by the inaccessibility of optically pure 2*S*-isomer. The (+)-2-hydroxy-2-methylbutanoic acid from which this isomer was prepared was a mixture of the 2*S*- and 2*R*-isomers in the ratio 74 : 26. Conversion into the phthaloyl ester (XII) was followed by purification by recrystallisation. This procedure resulted in a lowering of the optical purity which was attributable to the formation

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[9] R. E. Erickson, A. H. Riebel, A. M. Reader, and P. S. Bailey, *Annalen*, 1962, **653**, 129.
[10] A. R. Mattocks, *J. Chem. Soc.*, 1964, 1918.

of a racemic compound as indicated by the higher m.p. of the racemic ester (104°) as compared with the optically pure 2*R*-isomer (84°). The dicyclohexylamine salt of the (2*S*)-phthaloyl ester had a rotation corresponding to a mixture of the 2*S*- and 2*R*-isomers in the ratio 60 : 40. The enantiomeric compositions of these mixtures were confirmed by the n.m.r. spectra of the phthaloyl esters determined in the presence of the chiral shift reagent tris-[3-trifluoroacetyl-(+)-camphorato]europium(III).[11] At room temperature in deuteriochloroform with a molar ratio of ester to shift reagent of 3 : 2, a spectrum consisting of very broad signals was obtained. However, at 95 °C a sharp spectrum was obtained in which the signals due to the C-2 methyl protons of the enantiomeric esters (XIII) were almost completely resolved. By this technique the optical purity of the (2*R*)-phthaloyl ester was confirmed and it was demonstrated that the (2*S*)-ester consisted of a mixture of the 2*S*- and 2*R*-isomers in the ratio 62 : 38, in good agreement with the value determined polarimetrically. It was also found that after six recrystallisations of the brucine salt of the '2*S*'-ester the content of the 2*S*-isomer had increased to 82%. Since a dilution analysis cannot be performed with a mixed salt which has a constant composition on recrystallisation, the material used for dilution analysis was the mixture of brucine salts of the (2*S*)- and (2*R*)-phthaloyl esters containing 62% of the 2*S*-component. Since on recrystallisation the 2*R*-isomer content decreased from 38 to 18%, a maximum lowering of apparent specific activity of *ca.* 50% could be expected. Dilution of the recrystallised sample (now containing 82% 2*S*-isomer) with the 62 : 38 mixture followed by recrystallisation would again result in a decrease of 50% of the activity. In the event, the dilution–recrystallisation procedure was carried out three times. Recrystallisation of the brucine salt was continued until the specific activity, although still decreasing, was 13% of the activity expected if the original material diluted had contained labelled 2*S*- rather than 2*R*-isomer.*
The assignment of the 2*R*-configuration to the labelled isomer was thus confirmed.

Further supporting evidence is found in the c.d. curve of the (−)-*erythro* acid (IV), which shows a negative maximum at 211 nm, $\Delta\varepsilon$ −1.45. Similar negative Cotton effects are observed in the c.d. and o.r.d. curves of comparable (2*R*)-2-hydroxy-acids.[12]

In the original investigations on the identity of the dihydroxy acid (I),[3] the physical properties of the natural acid were compared with those of the synthetic acid, subsequently shown[4] to be the (−)-*erythro*-isomer. The points of comparison were the i.r. spectra, m.p.s and mixed m.p.s of the quinine salts, and the rotations of the quinine salts and of the free acids in ionised and un-ionised forms. However, it was not shown that these criteria were sufficient to distinguish between the isolated acid and the (−)-*threo*-isomer. Until this was done it would not be certain that the natural and synthetic acids of Sjolander *et al.* were identical, even though both acids were biologically active. In the Table, the m.p.s and rotations of the quinine salt and the rotation of the free acid to be found in the literature[2,13] are compared with those obtained in the present work. This comparison shows that these data alone do not permit a distinction to be made between the (−)-*erythro*- and (−)-*threo*-isomers of the acid (I). However, we have found that the i.r. spectra of the quinine salts both in Nujol mull and in KBr disc show distinct differences, particularly in the region 1 500—1 300 cm^{-1}. It is therefore unambiguously established that the (−)-*erythro*-acid (IV) is identical with the natural acid isolated by Sjolander *et al.*[3],†

Physical properties of isomers of 2,3-dihydroxy-3-methylpentanoic acid and their quinine salts

	Natural acid	Synthetic acid		
	Sjolander *et al.*	Sjolander *et al.*	Mattocks [a]	This work
M.p. of quinine salt (°C)	204	203	203	200 [b]
			203	208 [c]
$[\alpha]_D$ of quinine salt (°) (MeOH)	−144	−144	−133	−140 [b]
			−140	−140 [c]
$[\alpha]_D$ of free acid (°) (dil. HCl)	−16.7	−15	−22.9	−15.6 [b]
			−28	−23.1 [c]

[a] Results of two experiments. [b] (−)-*threo*-Acid (I). [c] (−)-*erythro*-Acid (I).

Mattocks isolated, from *Heliotropium strigosum*,[13] a pyrrolizidine alkaloid strigosine, the necic acid component of which is an isomer of the acid (I). However, he was unable to reach any conclusion as to the identity of this acid with the acid isolated by Sjolander *et al.*[3] because of discrepancies between the physical properties of the two acids (see Table). Through the courtesy of Dr. Mattocks we were able to examine the necic acid from

* If a sample of the labelled ester were diluted with a mixture containing x% *S*-isomer and $(100 - x)$% *R*-isomer and recrystallised until it had the composition y% *S*-isomer and $(100 - y)$% *R*-isomer, the apparent specific activity, assuming this to reside in the *S*-isomer, would be expected to change by a factor y/x. If a quantity m of the recrystallised material were diluted with a quantity M of material of composition x% *S*-isomer, $(100 - x)$% *R*-isomer, and the mixture were recrystallised to y% *S*-isomer, the apparent specific activity would be expected to change by a factor $ym/(ym + xM)$. The change in specific activity after three such dilutions and recrystallisations would therefore be $\frac{g}{x} \cdot \frac{ym}{(ym + xM)} \cdot \frac{ym}{(ym + xM)}$. By using this factor the final specific activity expected if all the activity resided in the *S*-isomer could be calculated. This value was compared with the observed final specific activity. The value of (observed specific activity × 100)/(calculated specific activity for initial labelled *S*-isomer) gave the maximum percentage of labelled *S*-isomer in the initial sample (*n.b.* this is a *maximum* value since the presence of labelled impurities is ignored in the above treatment).

† Although only the (−)-*erythro*-isomer supports growth of isoleucine-deficient mutants of *Salmonella typhimurium*, both (−)-*threo*- and (−)-*erythro*-isomers act as substrates for the enzyme αβ-dihydroxyacid dehydratase.[14]

[11] H. L. Goering, J. N. Eikenberry, and G. S. Koermer, *J. Amer. Chem. Soc.*, 1971, **93**, 5913.
[12] I. P. Dirkx and F. L. J. Sixma, *Rec. Trav. chim.*, 1964, **83**, 522; J. Cymerman Craig and S. K. Roy, *Tetrahedron*, 1965, **21**, 1847; F. W. Bachelor and G. A. Miana, *Canad. J. Chem.*, 1969, **47**, 4089; G. Barth, W. Voelter, E. Bannenberg, and C. Djerassi, *Chem. Comm.*, 1969, 355; P. M. Scopes, personal communication.
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[14] F. B. Armstrong, C. J. R. Hedgecock, J. B. Reary, D. Whitehouse, and D. H. G. Crout, *J.C.S. Chem. Comm.*, 1974, 351.

strigosine and to show from the i.r. spectra (Nujol and KBr) of its quinine salt that it was identical with the (−)-*erythro*-isomer of the acid (I).

EXPERIMENTAL

All m.p.s are corrected. I.r. spectra were determined with a Hilger H900 Infrascan spectrometer and n.m.r. spectra with a Perkin-Elmer R60 or JEOL MH-100 spectrometer, for solutions in deuteriochloroform with tetramethylsilane as internal standard. Mass spectra were determined with a Perkin-Elmer–Hitachi RMU spectrometer with an electron beam energy of 80 eV. Analytical g.l.c. was performed with a Pye Argon Chromatograph in connection with a Kent Chromalog 1 digital integrator. Paper chromatography of dihydroxy-acids was carried out on Whatman No. 1 paper in the solvent system toluene–butan-1-ol–acetic acid–water (2 : 2 : 1 : 5). Acids were made visible with a Methyl Red–borate buffer spray.[15] Radiochemicals were obtained from the Radiochemical Centre, Amersham. Radioactivity measurements were made with a Packard Tri-Carb Series 2 000 spectrometer in dioxan-based scintillation solutions [NE 250 or NE 220 (Nuclear Enterprises Ltd.)] or B.D.H. dioxan scintillator. Sufficient counts were taken to give a statistical error of <1% for each determination. All samples were counted in duplicate.

Synthesis of (Z)- *and* (E)-*Ethyl 3-Methylpent-2-enoates*.—The esters were synthesised by the method of Wadsworth and Emmons,[16] as follows. Triethyl phosphite (750 g, 4.52 mol) and ethyl bromoacetate (755 g, 4.52 mol) were induced to react together by mixing equimolar proportions (*ca.* 150 g of each) in a 500 cm³ flask fitted with a condenser. The mixture was heated to 60 °C, the source of heat was removed, and the temperature was allowed to rise spontaneously to 75 °C, at which temperature the mixture was maintained by intermittent cooling in ice–water. When the spontaneous reaction had subsided, the mixture was boiled for 15 min before being transferred to a 2 dm³ flask. This procedure was repeated until all the reactants had been added to the 2 dm³ flask, the contents of which were then boiled under reflux for 16 h. (The batchwise procedure was found advisable as the initial exothermicity of larger scale reactions was found difficult to control.) Sodium hydride (50% dispersion in mineral oil; 256 g, 5.3 mol) and 1,2-dimethoxyethane (700 cm³; freshly distilled over calcium hydride) were mixed and the cooled reaction mixture (above) was added dropwise with stirring, the temperature being maintained at 20 °C. Stirring was continued until evolution of hydrogen stopped (90 min). Butan-2-one (465 cm³, 4.8 mol) was added dropwise and the reaction mixture was stirred overnight at room temperature, during which time a solid precipitated. Water (500 cm³) was added and the resulting two layers were separated. The lower, aqueous layer was extracted with ether (3 × 250 cm³), and the extracts were combined with the organic phase, dried (Na_2SO_4) and distilled, first at atmospheric pressure and then at 20 mmHg. The fraction boiling between 56 and 72 °C was collected. The crude mixture was further purified on a spinning-band column (90 cm) at a distillation rate of 15 cm³ in 24 h. Fractions boiling between 74 and 79 °C (at 25 mmHg) were collected. G.l.c. [4 ft × $\frac{5}{32}$ in; 15% silicone grease on Chromosorb P (80—100 mesh); column temperature 125 °C; gas flow rate 30 cm³ min⁻¹] of the crude product showed the presence of (*E*)- and (*Z*)-ethyl 3-methylpent-2-enoate[17] in the ratio 3 : 1 (t_R 9.63 and 8.30 min, respectively).

(E)- (III) *and* (Z)-3-*Methylpent-2-enoic Acid* (VI).—*General procedure*. The esters were boiled under reflux with an excess of 5% barium hydroxide solution until the oily droplets of ester had disappeared. The cooled solution was extracted with ether, acidified (Congo Red) with dilute hydrochloric acid, and extracted with three portions of ether. The combined extracts were dried (Na_2SO_4) and evaporated. Hydrolysis of the *E*-ester gave the *E*-acid (III) as prisms, m.p. 45—47° (from light petroleum) (lit.,[18] 46°). Re-esterification with diazomethane gave the corresponding methyl ester, which was shown (g.l.c.) to consist solely (>99.5%) of the *E*-isomer. Hydrolysis of the *Z*-ester gave the *Z*-acid (VI) as an oil which crystallised; m.p. 33—35°. Treatment of a solution of the acid (1.4 g) in ether (15 cm³) with dicyclohexylamine (2.2 g) gave the corresponding *salt* as prisms, m.p. 114—116° (light petroleum) (Found: C, 73.85; H, 11.7; N, 4.8. $C_{18}H_{33}NO_2$ requires C, 73.15; H, 11.25; N, 4.75%). Recovery of the acid from an acidified aqueous solution of the salt as above gave the pure *Z*-acid (VI) as a crystalline solid, m.p. 34—36° (lit.,[18] 35.5°).

(2RS,3RS)-2,3-*Dihydroxy*-3-*methylpentanoic Acid* (IV).—(a) (*E*)-3-Methylpent-2-enoic acid (9 g) in acetone (60 cm³) and water (180 cm³) was treated with tungstic oxide (112 mg) and hydrogen peroxide solution (30%; 6.75 cm³). The mixture was stirred at 60 °C for 48 h, then extracted with benzene (3 × 50 cm³), and continuously with ether for 48 h. Evaporation of the dried (Na_2SO_4) benzene extracts gave starting material (3 g), shown by methylation (diazomethane) and g.l.c. to have undergone no isomerisation. The recovered acid in acetone (20 cm³) and water (60 cm³) was treated with tungstic trioxide (51 mg) in hydrogen peroxide (30%, 2.3 cm³) as before. Evaporation of the combined, dried (Na_2SO_4), ethereal extracts gave the dihydroxy-acid (IV) (4.0 g, 34%) as an oil. Paper chromatography revealed, in addition to a major product, R_F 0.65, a minor product, R_F 0.85. Treatment of the crude acid with an equimolar amount of dicyclohexylamine in acetone gave the *dicyclohexylamine salt* as needles (ethyl acetate), m.p. 180—182° (Found: C, 65.95; H, 10.7; N, 4.75. $C_{18}H_{35}NO_4$ requires C, 65.6; H, 10.7; N, 4.25%).

(b) *Resolution*. The dihydroxy-acid (24 g, 0.16 mol) in ethanol (150 cm³) was treated with a solution of quinine (61 g, 0.16 mol) in ethanol (200 cm³). The m.p. of the resulting salt (58 g) after six recrystallisations (ethanol) had reached a constant value of 208—210° (decomp.); $[\alpha]_D^{23}$ −140° (*c* 0.74 in MeOH) (Found: C, 65.55; H, 7.65; N, 5.95. Calc. for $C_{26}H_{36}N_2O_6$: C, 66.1; H, 7.7; N, 5.95%). A solution of the quinine salt (15 g) in water (100 cm³) was acidified (Congo Red) with dilute hydrochloric acid and extracted continuously with ether for 48 h. The dried (Na_2SO_4) ethereal extract was evaporated to give (2*R*,3*R*)-2,3-dihydroxy-3-methylpentanoic acid (IV) as a gum (3.9 g), $[\alpha]_D^{23}$ −23.1° [*c* 2.0 in HCl (0.1 mol dm⁻³)], τ (100 MHz) 4.39br (1 H, s, OH), 5.84 [1 H, s, C*H*(OH)], 8.33 (2 H, m, MeCH_2), 8.75 [3 H, s, *Me*C(OH)], and 9.02 (3 H, t, *J* 7.0 Hz, *Me*CH_2).

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[16] W. S. Wadsworth and W. D. Emmons, *J. Amer. Chem. Soc.*, 1961, **83**, 1733.
[17] L. Decaux and R. Vessière, *Compt. rend.*, 1968, **267C**, 738; S. Kobayashi, H. Takei, and T. Mukaiyama, *Chem. Letters*, 1973, 1097.
[18] T. A. Dobson and L. C. Vining, *Canad. J. Chem.*, 1968, **46**, 3007.

(2RS,3SR)-2,3-*Dihydroxy*-3-*methylpentanoic Acid* (IV).—(i) (*E*)-3-Methylpent-2-enoic acid (III) (50 g) in water (550 cm³) was treated with barium chlorate (32 g) and osmic acid (1%; 58 cm³); the resulting solution was stirred for 24 h, then extracted with benzene (3 × 200 cm³), and continuously with ether for 48 h. The ethereal extract was dried and evaporated to give the dihydroxy-acid as a gum (32 g). Paper chromatography revealed a major product, R_F 0.65, and a minor product, R_F 0.85. The *dicyclohexylamine salt* crystallised (acetone) and was recrystallised (ethyl acetate) to give needles, m.p. 175—178° (Found: C, 65.95; H, 10.9; N, 4.3. Calc. for $C_{18}H_{35}NO_4$: C, 65.6; H, 10.7; N, 4.25%).

(ii) (*Z*)-3-Methylpent-2-enoic acid (5 g) was hydroxylated with tungstic oxide and hydrogen peroxide as described in (a) above. The resulting dihydroxy-acid gave a dicyclohexylamine salt, m.p. 175—178°, not depressed on admixture with the corresponding salt prepared as in (a). The m.p. of a mixture with the salt of the 2*RS*,3*RS*-isomer, m.p. 180—182°, was 163—167°.

(iii) *Resolution*. The dihydroxy-acid (14 g) in ethanol (100 cm³) was treated with quinine (36 g) in ethanol (100 cm³). The mixture was set aside overnight at 3 °C to give the *quinine salt* (10 g). The mother liquor was kept at −10 °C for 5 days to give more (8.0 g) of the quinine salt. The fractions were combined and recrystallised (ethanol). After eight crystallisations the m.p. had reached a constant value of 200—202° (decomp.); $[\alpha]_D^{23}$ −140° (*c* 0.83 in MeOH) (Found: C, 65.95; H, 8.05; N, 5.95. $C_{26}H_{36}N_2O_6$ requires C, 66.1; H, 7.85; N, 5.95%). The free acid was recovered from the quinine salt as described above as a gum, $[\alpha]_D^{23}$ −15.6° [*c* 2.3 in HCl (0.1 mol dm⁻³)], τ (100 MHz) 4.34br (1 H, s, OH), 5.84 [1 H, s, C*H*(OH)], 8.30 (2 H, m, MeC*H*$_2$), 8.73 [3 H, s, *Me*C(OH)], and 9.04 (3 H, t, *J* 7.0 Hz, *Me*CH$_2$).

Degradation of (−)-erythro-2,3-*Dihydroxy*-3-*methylpentanoic Acid* (IV).—The (−)-*erythro*-acid (I) (581 mg), dissolved in ether–methanol (1 : 1; 20 cm³), was treated with an excess of diazomethane in ether. The solvents were evaporated off, the residue was dissolved in dry ether (30 cm³), and the solution was added dropwise to a stirred solution of lithium aluminium hydride (300 mg) in dry ether (30 cm³). The mixture was boiled under reflux for 16 h, the excess of lithium aluminium hydride was decomposed by dropwise addition of water, and the resulting mixture was treated with dilute sulphuric acid (6 mol dm⁻³; 20 cm³). The aqueous layer was extracted continuously with ether for 20 h. The combined ethereal solutions were dried and evaporated to give an oil (381 mg), which was dissolved in water (25 cm³) and treated dropwise with sodium periodate (430 mg) in water (25 dm³), with stirring, over 40 min. The solution was stirred for a further 30 min and extracted continuously with ether for 16 h. The extract was dried and evaporated to give a liquid residue (96 mg). This was dissolved in methanol (1 cm³) and treated with a solution of 2,4-dinitrophenylhydrazine (250 mg) in methanol (5 cm³) and sulphuric acid (1 cm³). The resulting solution was diluted with sulphuric acid (1 mol dm⁻³) to give an orange-red precipitate (199 mg). This was applied to a column of acid-washed alumina (grade II; 15 g) and the column was eluted with benzene–light petroleum. Material from the second band eluted crystallised (ethanol) to give the 2,4-*dinitrophenylhydrazone* of 2-*methoxy*-2-*methylbutanal* (20.1 mg) as yellow plates, m.p. 94° (Found: C, 48.85; H, 5.75; N, 19.15. $C_{12}H_{16}N_4O_5$ requires C, 48.65; H, 5.45; N, 18.9%), $\nu_{max.}$ (KBr) 3 330 and 1 595 (NH) and 1 620 cm⁻¹ (C:N), *m/e* 296 (M^+), τ (60 MHz) [for numbering see (IX)] 0.90 (1 H, d, *J* 3 Hz, 3′-H), 1.62 (1 H, dd, *J* 9 and 3 Hz, 5′-H), 2.07 (1 H, d, *J* 9 Hz, 6′-H), 2.52 (1 H, s, CH:N), 8.74 (3 H, s, MeO), 8.18 (2 H, m, MeC*H*$_2$), 8.56 [3 H, s, *Me*C(OH)], 9.20 (3 H, t, *J* Hz, *Me*CH$_2$). Material from the third band eluted crystallised (ethanol) to give the 2,4-*dinitrophenylhydrazone* of 2-*hydroxy*-2-*methylbutanal* (IX) (64 mg) as short orange needles, m.p. 130° (Found: C, 46.75; H, 5.15; N, 19.9. $C_{11}H_{14}N_4O_5$ requires C, 46.8; H, 5.0; N, 19.85%), $\nu_{max.}$ (KBr) 3 320 and 1 595 (NH), and 1 620 cm⁻¹ (C:N), *m/e* 282 (M^+), τ (60 MHz) 0.87 (1 H, d, *J* 3 Hz, 3′-H), 1.62 (1 H, dd, *J* 9 and 3 Hz, 5′-H), 2.08 (1 H, d, *J* 9 Hz, 6′-H), 2.40 (1 H, s, CH:N), 7.40 (1 H, s, OH), 8.22 (2 H, q, *J* 7 Hz, MeC*H*$_2$), 9.50 [3 H, s, *Me*C(OH)], and 9.40 (3 H, t, *J* 7 Hz, *Me*CH$_2$).

2-*Hydroxy*-2-*methyl*[1-³H]*butyl Hydrogen Phthalate* [*as* (XI)].— 2-Hydroxy-2-methylbutanal dinitrophenylhydrazone (20 mg), prepared from (−)-*erythro*-2,3-dihydroxy-3-methylpentanoic acid as described above, in dichloromethane (15 cm³), was ozonised at 0 °C until decomposition of the derivative was complete as determined by paper chromatography (Whatman No. 1 impregnated with formamide; mobile phase di-isopentyl ether saturated with formamide). The solvent was evaporated off, and the residue was dissolved in ethanol (1 cm³) and added to sodium [³H]borohydride (25 mCi; 524 mCi mmol⁻¹). After 90 min, an excess of inactive sodium borohydride was added and the mixture was set aside for a further 3 h. The excess of sodium borohydride was decomposed with dilute hydrochloric acid, and the solution was diluted with water (10 cm³) and extracted continuously with ether for 6 h. The extract was dried (Na_2SO_4) and evaporated. The residue was dissolved in pyridine (2.5 cm³) containing (2*RS*)-3-methylbutane-1,2-diol (25 mg) and phthalic anhydride (36 mg) was added. The solution was set aside for 4 days, the pyridine was removed under reduced pressure, the residue was dissolved in ether, and the solution was washed with water (5 cm³), sulphuric acid (5 cm³; 1 mol dm⁻³), and water (2 × 1 cm³). The ether was removed and the residue was dissolved in chloroform (15 cm³); the solution was dried (Na_2SO_4) and evaporated. The residue was dissolved in chloroform (10 cm³). This solution had an activity of 140 μCi. The content of 2-hydroxy-2-methyl[1-³H]butyl hydrogen phthalate was determined as described below.

Resolution of 2-*Hydroxy*-2-*methylbutanoic Acid*.—(±)-2-Hydroxy-2-methylbutanoic acid was resolved [10] with brucine to give a less soluble salt, m.p. 196—199°, $[\alpha]_D^{24}$ −18.4° (*c* 2.0 in EtOH), and a more soluble salt, m.p. 178—180°, $[\alpha]_D^{24}$ −17.4° (*c* 2.0 in EtOH). The free acids, recovered from acidified aqueous solutions of the salts by continuous extraction with ether, had, respectively, m.p. 75—77° $[\alpha]_{400}^{20}$ −15.4° (*c* 4.0 in EtOH) (lit.,[10] $[\alpha]_D$ −14.45°), and m.p. 72—75°, $[\alpha]_{400}^{20}$ + 7.5° (*c* 4.0 in EtOH), (lit.,[10] $[\alpha]_D$ + 3.75°).

(±)-2-*Hydroxy*-2-*methylbutyl Hydrogen Phthalate* (XIII).—(±)-2-Hydroxy-2-methylbutanoic acid [19] (8 g) in ether was treated with an excess of ethereal diazomethane. The resulting ester, dissolved in dry tetrahydrofuran (100 cm³), was slowly added to a stirred solution of lithium aluminium hydride (2.5 g) in tetrahydrofuran (100 cm³). The solution was then boiled under reflux for 12 h. The

[19] W. G. Young, R. T. Dillon, and H. J. Lucas, *J. Amer. Chem. Soc.*, 1929, **51**, 2528.

excess of reagent was decomposed by cautious addition of water (10 cm^3) and the tetrahydrofuran was evaporated off under reduced pressure. The residue was dissolved in sulphuric acid (1 mol dm^{-3}) and the solution was extracted continuously with ether for 16 h. The extract was dried (Na_2SO_4) and evaporated to give (±)-3-methylbutane-1,2-diol as a yellow liquid (5.8 g), $\nu_{max.}$ 3 390 (OH) and 2 980 cm^{-1} (CH). The diol (630 mg) dissolved in pyridine (25 cm^3) was treated with phthalic anhydride (896 mg) and stirred for 48 h. The solvent was removed under reduced pressure, the residue was dissolved in ether (25 cm^3), and the solution was washed with sulphuric acid (10 cm^3; 1 mol dm^{-3}) and water (2 × 10 cm^3). The ether was evaporated off and the residue was dissolved in chloroform and dried (Na_2SO_4). The solvent was removed to give an oil (480 mg) which slowly crystallised. Recrystallisation (ethyl acetate–light petroleum) gave the *derivative* (XIII) as small prisms, m.p. 105° (Found: C, 61.5; H, 6.4. $C_{13}H_{16}O_5$ requires C, 61.9; H, 6.4%), $\nu_{max.}$ 3 460 (OH), 1 705 cm^{-1} (CO_2H and CO_2R), τ 1.88 (2 H, s, 2 × OH), 2.0—2.6 (4 H, m, ArH), 5.63 (2 H, s, $CO_2{\cdot}CH_2$), 8.29 (2 H, q, *J* 7 Hz, $MeCH_2$), 8.68 [3 H, s, $MeC(OH)$], and 9.00 (3 H, t, *J* 7 Hz, $MeCH_2$). The *dicyclohexylamine salt* crystallised from acetone as small lozenges, m.p. 127—129° (Found: C, 69.8; H, 9.1; N, 3.2. $C_{25}H_{39}NO_5$ requires C, 69.25; H, 9.05; N, 3.25%).

(2R)-2-*Hydroxy*-2-*methylbutyl Hydrogen Phthalate* (XI).—(2*R*)-3-Methylbutane-1,2-diol (836 mg, 8 mmol) obtained from the corresponding acid by methylation (CH_2N_2) and reduction ($LiAlH_4$) was treated as above with phthalic anhydride (1.19 g, 8 mmol) in pyridine (25 cm^3) to give the phthaloyl ester (XI) (385 mg), m.p. 84°, $[\alpha]_{400}^{25}$ (as the dicyclohexylamine salt, m.p. 133—134°) +27° (*c* 1.0 in $CHCl_3$). The n.m.r. spectrum ($CDCl_3$) was identical with that of the racemic compound. The ester (300 mg, 1.9 mmol) was treated with brucine (469 mg, 1.19 mmol) in ethanol (15 cm^3). The *brucine salt* (520 mg) was precipitated by addition of ether and recrystallised (ethyl acetate); m.p. 130—132°, $[\alpha]_D^{22}$ −7.4° (*c* 2.0 in $CHCl_3$) (Found: C, 65.3; H, 6.6; N, 4.15. $C_{36}H_{42}N_2O_9,H_2O$ requires C, 65.05; H, 6.65; N, 4.2%).

(2S)-2-*Hydroxy*-2-*methylbutyl Hydrogen Phthalate* (XII).—2-Hydroxy-2-methylbutanoic acid, enriched in the 2*S*-isomer (972 mg) was converted into the hydrogen phthalate as above. The derivative (1.04 g) crystallised from ethyl acetate–light petroleum; m.p. 102—103°, $[\alpha]_{400}^{24}$ (as the dicyclohexylamine salt, m.p. 129.5—130.5°) −5.3° (*c* 1.0 in $CHCl_3$). The n.m.r. spectrum ($CDCl_3$) was identical with that of the racemate. The *brucine salt*, precipitated from an ethanolic solution on addition of ether, crystallised from ethyl acetate; m.p. 144—146°, $[\alpha]_D^{22}$ −11.0° (*c* 2.0 in $CHCl_3$) (Found: C, 65.2; H, 6.5; N, 4.2. $C_{36}H_{42}N_2O_9,H_2O$ requires C, 65.05; H, 6.65; N, 4.2%).

Dilution Analyses.—(*a*) A sample (0.1 cm^3) of the chloroform solution (10 cm^3) containing 2-hydroxy-2-methyl-[1-^{3}H]butyl hydrogen phthalate was treated with dicyclohexylamine (40 mg) followed by the dicyclohexylamine salt of the racemic derivative (XIII) (303 mg). The salt was recrystallised to constant activity from ethyl acetate–light petroleum. The final activity of the salt (6.33×10^5 disint. min^{-1} $mmol^{-1}$) indicated a total activity of 19.9 μCi for the labelled ester present in the original solution.

(*b*) The chloroform solution containing the labelled phthaloyl ester was concentrated to 5 cm^3, a sample (1 cm^3) was treated with brucine (5 mg), and the resulting brucine salt was treated with the inactive salt of the (2*R*)-ester (XI) (235 mg) and recrystallised from ethyl acetate–light petroleum. After seven recrystallisations the specific activity had reached a value (3.76×10^4 disint. min^{-1} mg^{-1}) which was unchanged on two further recrystallisations. The expected activity, calculated on the assumption that the labelled derivative in the original sample consisted entirely of the 2*R*-isomer, was 3.68×10^4 disint. min^{-1} mg^{-1}.

(*c*) A further sample (1 cm^3) of the solution of the labelled ester was subjected to dilution analysis as described above with the brucine salt of the 2*S*-enriched phthaloyl ester (2*S* : 2*R* 62 : 38) (290 mg). The diluted salt was recrystallised from ethyl acetate. The sample was further diluted after the sixth and eleventh crystallisations. After the sixteenth crystallisation the activity (corrected as described in the text) had fallen to 0.387×10^5 disint. $min.^{-1}$ mg^{-1}, whereas the expected activity, calculated on the assumption that the initial labelled material consisted solely of the 2*S*-isomer, was 2.93×10^4 disint. min^{-1} mg^{-1}.

Quinine Salt of the Necic Acid from Strigosine (XIV).—The acid was recovered from the brucine salt, m.p. 215° (165 mg), supplied by Dr. A. R. Mattocks, by acidification (dil. HCl) of an aqueous solution and continuous extraction with ether. The necic acid (41 mg) in ethanol (1 cm^3) was treated with quinine (100 mg) to give the quinine salt, which after three crystallisations (EtOH) had m.p. 208—211°. The i.r. spectra (KBr; nujol) were identical with the corresponding spectra of the quinine salt of (2*R*,3*R*)-2,3-dihydroxy-3-methylpentanoic acid and significantly different from those of the corresponding salt of the 2*R*,3*S*-acid.

We thank Dr. P. M. Scopes for c.d. determinations, Dr. A. R. Mattocks for a gift of the brucine salt of the necic acid from strigosine, and the S.R.C. for a research studentship (to D. W.).

[6/1395 *Received*, 16*th July*, 1976]