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Stereochemistry of Valine and Isoleucine Biosynthesis II. Absolute Configuration of (−)α,β-Dihydroxyisovaleric Acid and (−)α,β-Dihydroxy-β-Methylvaleric Acid.[1]

RICHARD K. HILL AND SHOU-JEN YAN

Department of Chemistry, University of Georgia, Athens, Georgia 30601

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The valine biosynthetic precursor (−)α,β-dihydroxyisovaleric acid has been converted to (S) (+)-2,3-dihydroxy-2-methylbutane, demonstrating that the dihydroxy-acid intermediate has the (R) configuration. The corresponding isoleucine precursor, (−)α,β-dihydroxy-β-methylvaleric acid, has been degraded to (R) (−)-2-hydroxy-2-methylbutyric acid, establishing the (R) configuration at C-3; the natural intermediate is consequently the (2R; 3R) isomer. The stereochemistry of the enzymatic reactions involving these intermediates is discussed.

The biosynthetic pathways to valine and isoleucine in bacteria and yeast have been elucidated in considerable detail through the application of a variety of isotopic, mutant, and enzymatic experiments (*1*). The routes to these two essential amino acids have proved to be remarkably parallel, as illustrated in Scheme 1.

The common sequence in the two pathways begins with an α-keto acid: pyruvic acid in the case of valine, α-ketobutyric acid (derived from threonine by the action of threonine deaminase) in the case of isoleucine. Condensation with the "active acetaldehyde" unit of α-hydroxyethyl thiamine pyrophosphate, mediated by an acetohydroxy acid synthetase, forms α-acetolactic acid (I) and α-aceto-α-hydroxybutyric acid (IV), respectively. These acids are then converted by an isomeroreductase (or alternatively, as has recently been demonstrated (*2*) to be possible in *Mycobacterium tuberculosis* $H_{37}Rv$, by the successive action of a separate acetohydroxy acid isomerase and a reductase) to the dihydroxy acids α,β-dihydroxyisovaleric acid (II) and α,β-dihydroxy-β-methylvaleric acid (V). Each of these is converted by a dihydroxy acid dehydratase to the α-keto acids, α-ketoisovaleric acid (III), and α-keto-β-methylvaleric acid (VI), which are finally transaminated to valine and isoleucine. Evidence has been adduced that the same enzymes mediate corresponding reactions in the two pathways.

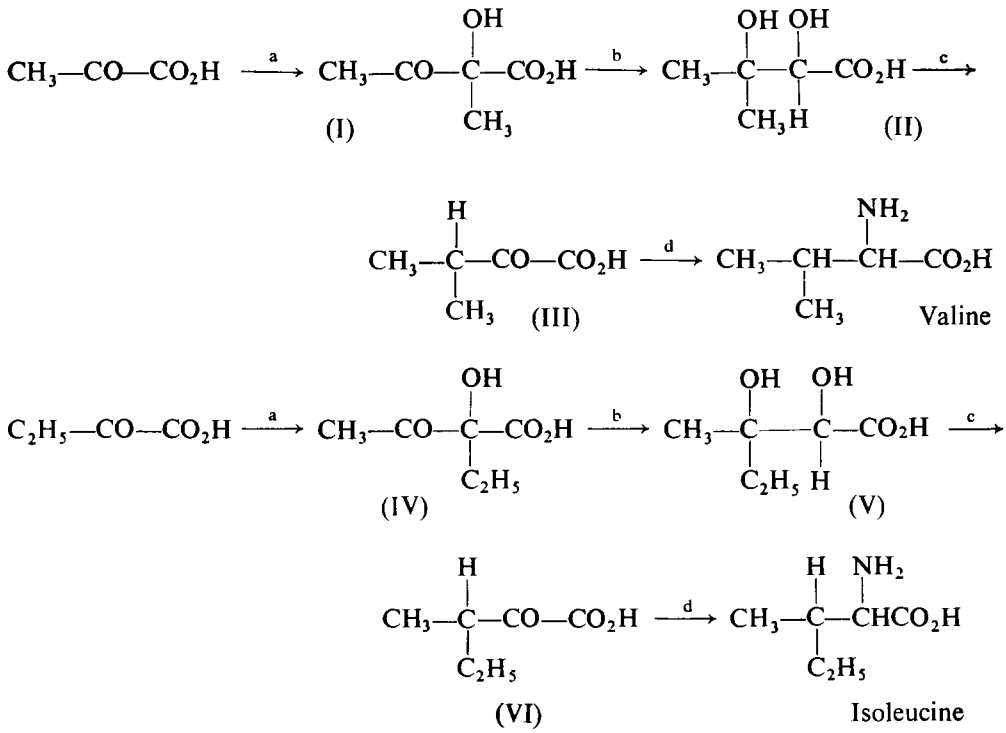
These two closely related biosynthetic pathways pose a number of stereochemical questions intimately related to the mechanisms of the enzymatic transformations involved:

1. In the thiamine-catalyzed condensations of pyruvic acid and α-ketobutyric acid with "active acetaldehyde" to form α-acetolactic acid (I) and α-aceto-α-hydroxybutyric acid (IV), what is the configuration at the newly formed asymmetric center? Do acids (I) and (IV) belong to the same configurational series, as anticipated by their formation from a common enzyme?

[1] For paper I in this series, see R. K. HILL and P. J. FOLEY, JR., *Biochem. Biophys. Res. Commun.* **33**, 480 (1968).

2. In the conversions of (I) to α,β-dihydroxyisovaleric acid (II) and of (IV) to α,β-dihydroxy-β-methylvaleric acid (V), the step which involves an unusual acyloin-like rearrangement, to which face of the carbonyl does the migrating alkyl group move; i.e. what is the absolute configuration at the β-carbons of (V) and (assuming the two methyls could be differentiated) of (II)?

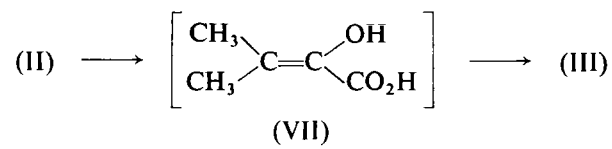
SCHEME 1. Biosynthesis of Valine and Isoleucine.



a: acetohydroxy acid synthetase;
b: acetohydroxy acid isomeroreductase;
c: dihydroxy acid dehydratase;
d: transaminase.

3. What is the stereochemical result of the NADPH reduction, catalyzed by either a reductase or an isomeroreductase, which creates the chiral secondary carbinol carbon in acids (II) and (V); i.e. what is the absolute configuration at the α-carbons of (II) and (V)?

4. Finally, Arfin has recently shown (*3*) that the dehydration of α,β-dihydroxyisovaleric acid (II) to α-ketoisovaleric acid (III) involves an enol intermediate (VII), and it is likely that the dehydration of (V) to (VI) in isoleucine biosynthesis proceeds by a similar enol intermediate. In the presumably stereospecific protonation of the enols to



(III) and (VI), is the β-carbon protonated from the same face of the double bond from which the β-hydroxyl departed, i.e., with overall retention, or from the opposite face, with overall inversion of configuration?

It is clear that the answers to these questions would provide valuable clues to the mechanisms and conformational requirements of these enzyme-catalyzed reactions, and we have accordingly undertaken an investigation of these stereochemical problems. This paper reports the determination of absolute configuration of α,β-dihydroxyisovaleric acid (II) and of α,β-dihydroxy-β-methylvaleric acid (V), and discusses the bearing which the results have on the answers to questions 2, 3, and 4.

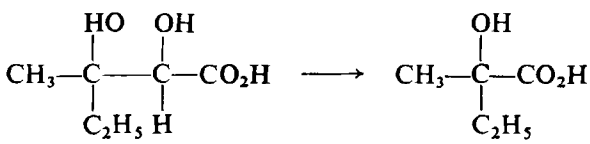
The dihydroxy acids used in this study were synthesized by a modification of the method of Sjölander et al. (*4*) and resolved to optical purity with quinine; the synthetic optically active acids have been shown to be identical with the naturally occurring biosynthetic intermediates (*4*). Our approach to the determination of absolute configuration was to convert each acid, by straightforward reactions not involving the asymmetric center, to a compound of established configuration.

α,β-Dihydroxyisovaleric acid. The dihydroxyacid II involved in valine biosynthesis possesses a single asymmetric center, so the immediate stereochemical problem is the absolute configuration at C-2. A suitable standard of established configuration to which to relate (II) is 2,3-dihydroxy-2-methylbutane (*5*) (XIII), and accordingly acid (II) was converted to (S)-(+)-(XIII) as outlined in Scheme 2.

The levorotatory acid (II) was methylated with diazomethane and the resulting ester (VIII) converted to the dextrorotatory acetonide (IX). Lithium aluminum hydride reduction afforded the carbinol (X), which gave a crystalline *p*-toluenesulfonate (XI). Attempts to reduce the tosylate by lithium aluminum hydride yielded only the starting alcohol, by S—O rather than C—O cleavage, so a somewhat longer route was necessary. Displacement of the tosylate by sodium benzylmercaptide gave the benzyl thioether (XII), which was desulfurized with W-7 Raney nickel. The desulfurization product was hydrolyzed directly to diol (XIII), $[\alpha]_D^{25} + 5.4°$. Confirming the results of Kjaer (*5*) the same diol, $[\alpha]_D^{24} +5.6°$, was prepared by the action of methyl Grignard reagent on (S)-(+)-methyl lactate. The latter reaction allows assignment of the (S)-configuration to the dextrorotatory diol (XIII), and accordingly the levoratory α,β-dihydroxyisovaleric acid (II) has the (R) configuration.

α,β-Dihydroxy-β-methylvaleric acid. The dihydroxy acid (V) which acts as an intermediate in isoleucine biosynthesis has two asymmetric centers, and so might be one of four possible stereoisomers. Although the naturally occurring form has been synthesized and shown to be one pure stereoisomer (*4*), the mode of synthesis gave no information on its configuration. It is of interest that the same acid occurs naturally as an ester of a pyrrolizidine alcohol in the alkaloid strigosine (*6*).

The relative configuration at carbons 2 and 3 has previously been established in our laboratory[2]; the natural acid was shown by stereospecific synthesis to be either the

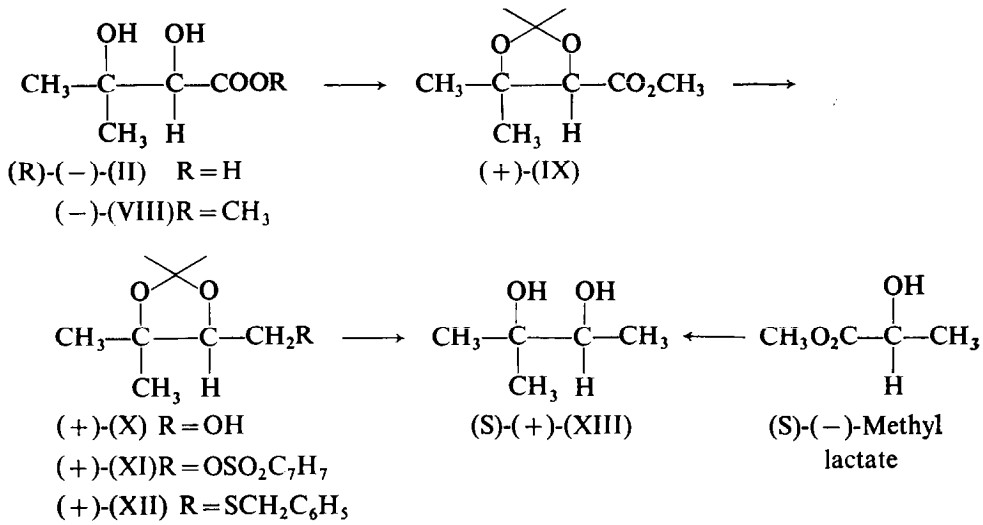


(V) (XVIII)

[2] See footnote 1.

(2R;3R) or the (2S;3S) isomer. Consequently the determination of absolute configuration at either of the asymmetric centers would complete the stereochemical assignment. The approach chosen in this investigation was to eliminate the asymmetry at C-2 by converting (V) to α-hydroxy-α-methylbutyric acid (XVIII) of known (7) configuration.

SCHEME 2. Correlation of (−)α,β-Dihydroxyisovaleric Acid with (S)-(−)-Methyl Lactate.



Four lines of attack were investigated in attempts to accomplish this conversion by removing the carboxyl group of (V):

(a) Acid V was converted to the hydrazide and the amide, and attempted Curtius and Hofmann degradations carried out in efforts to prepare 2-hydroxy-2-methylbutanal, but no aldehyde product could be isolated from either sequence.

(b) Attempts were made to dehydrate the amide of (V) or its diacetate to a cyanohydrin which could then be induced to lose HCN, but neither thionyl chloride, phosphorus pentoxide, phosphorus oxychloride, or refluxing acetic anhydride effected dehydration in acceptable yield.

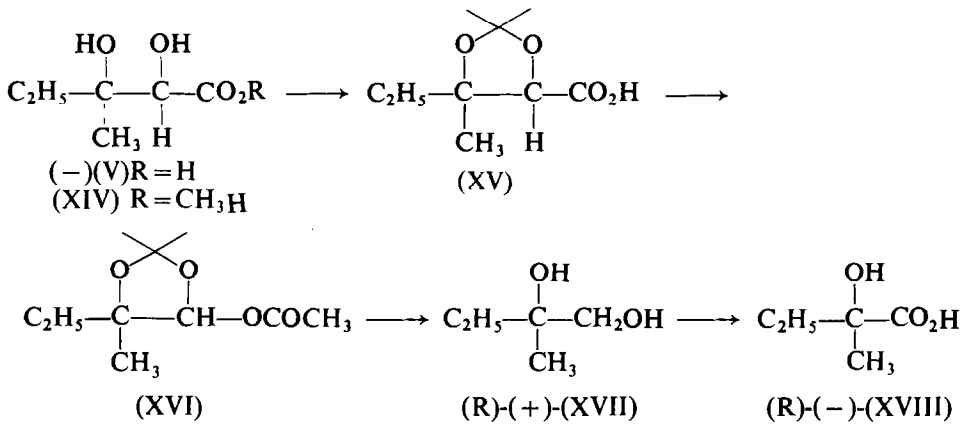
(c) Oxidation of the secondary alcohol group of (V) was essayed in the hope of obtaining the α-keto acid which might be further degraded with hydrogen peroxide, but both silver carbonate–Celite oxidation and platinum-catalyzed oxygenation were unsuccessful.

(d) Success was achieved only after first protecting the hydroxyl groups as the acetonide, after which the carboxyl could be removed by lead tetraacetate oxidation. The route used is shown in Scheme 3. Acid (V) could be converted to the crystalline acetonide (XV) directly, though better yields were obtained if the acid was first esterified, converted to the acetonide, and then saponified. Oxidation with lead tetraacetate in benzene proceeded in good yield to furnish the acetate (XVI), obtained, as anticipated, as a mixture of epimers at C-1. Lithium aluminium hydride reduction led directly to (+)-2-methyl-1,2-butanediol (XVII), identical with the hydride reduction product of α-hydroxy-α-methylbutyric acid (XVIII). Finally, diol (XVII) could be oxidized to (R)-(−)-(XVIII) by the action of oxygen over platinum. The product was shown identical with authentic samples of the levorotatory acid, using the crystalline *p*-phenylphenacyl esters for comparison.

Since the (R) configuration for (−)-(XVIII) has been established unambiguously (7),

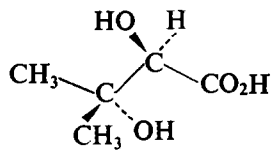
this correlation proves the (R)-configuration at C-3 of (V), and consequently the levorotatory biosynthetic intermediate (V) is the (2R;3R) isomer.

SCHEME 3. Degradation of α,β-Dihydroxy-β-methylvaleric Acid to (R)-(−)-α-Hydroxy-α-methylbutyric Acid.

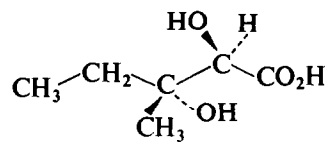


DISCUSSION

With the elucidation of the configuration of the dihydroxy acid biosynthetic intermediates as (R)-(−)-α,β-dihydroxyisovaleric acid (II) and (2R;3R)-(−)-α,β-dihydroxy-β-methylvaleric acid (V) it is possible to examine several consequences of their configurations.



(R)-(−)-(II)

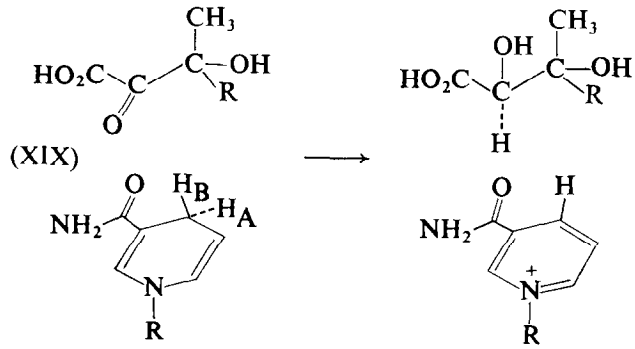


(2R; 3R)-(−)-(V)

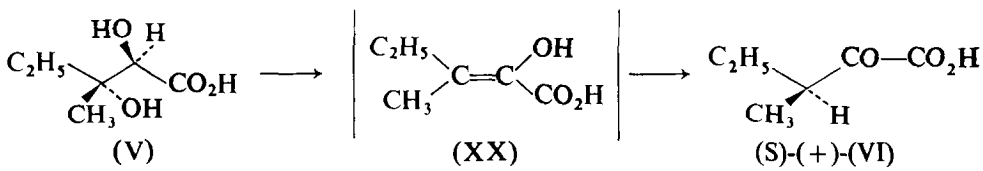
1. Both dihydroxy acids have an asymmetric center at C-2 which is generated by NADPH reduction of a keto group during formation of the dihydroxyacids. Arfin and Umbarger have shown (*8*) that the hydrogen transferred is the 4B hydrogen of NADPH. It would be anticipated that reduction of two such similar substrates catalyzed by the same enzyme would lead to the same configuration at the chiral carbinol carbon. This study has shown that such is indeed the case; in both dihydroxy acids C-2 has the (R)-configuration.

It is interesting to note that these results are consistent with Prelog's model (9) for substrate-pyridine nucleotide interactions in oxidoreductases. Making Prelog's assumptions that the carbonyl oxygen of the substrate points toward the pyridine ring nitrogen of NADPH, and that nonbonded interactions are minimized by placing the bulkier group of the substrate opposite the hydrogen at C-5 on the pyridine ring, it can be seen that the transition state geometry thus derived (XIX) correctly predicts the (R)-configuration at the carbinol carbon. The results also conform to the general model of Karabatsos *et al.* (10), independent of the nature of the enzyme.

Not too much significance should be attached to the geometry shown in (XIX), since not all reductions proceed in accord with this stereochemical model (*10*) and other transition state arrangements have been suggested (*11*, *12*).



2. Arfin has demonstrated (*3*) that the enzymatic dehydration of dihydroxy acid II to α-ketoisovaleric acid in the valine series involves an enol intermediate. Assuming the same mechanism holds true in the isoleucine series, dehydration of (−)-α,β-dihydroxy-β-methylvaleric acid (V) to an enol (XX) would momentarily destroy both asymmetric centers:



Since the enol likely remains bound to the enzyme, however, its two faces do not become identical, and the intermediate retains its chirality in the bound form. Stereospecific protonation to (S)-(+)-α-keto-β-methylvaleric acid regenerates the asymmetric center at C-3.

Elucidation of the (R)-configuration at C-3 of (−)-V permits the conclusion that the overall replacement of hydroxyl by hydrogen at C-3 during dehydration occurs with net retention of configuration. This result is consistent with the enol intermediate mechanism, but is not in agreement with a reasonable alternative possibility, an intramolecular hydride shift from C-2 to C-3. An intramolecular hydride transfer (ruled out by Arfin's tracer experiments (*3*)) would have been expected, by analogy with related nonenzymatic 1,2-hydride shifts (*13*), to proceed with inversion of configuration at C-3.

The stereochemical course of dihydroxy acid dehydration would be expected to be the same in the valine series, but experimental proof must await the preparation of samples in which the methyls have been differentiated by isotopic labeling.

The remaining stereochemical questions in valine and isoleucine biosynthesis can be answered only by the elucidation of configuration of the acetohydroxy acids I and IV, and experiments toward this end are in progress.

Acknowledgments. This research was supported by Research Grant GM 16944-03 from the U.S. Public Health Service, to whom we express our warm appreciation. We thank Professor Anders Kjaer, Technical University of Denmark; Dr. Albert Hofmann, Sandoz AG; and Dr. Norman G. Brink, Merck Sharp and Dohme, for samples of (+)-α-hydroxy-α-methylbutyric acid.

EXPERIMENTAL SECTION

Infrared spectra were recorded on Perkin–Elmer Models 257 or 621 spectrophotometers. The nmr spectra were recorded on Varian A-60 and HA-100 instruments. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter, while ORD spectra were recorded on a Cary-60 recording spectropolarimeter. Mass spectra were obtained on a Hitachi–Perkin–Elmer RMU-6 spectrometer.

(±)-*Ethyl α,β-oxidoisovalerate.* The following modification of the procedure of Sjölander *et al.* (*4*) gave better yields than the original. In a 1-liter flask fitted with a condenser protected by a drying tube, thermometer, and mechanical stirrer was placed a mixture of 23.2 g (0.55 mole) of a 57.2% dispersion of sodium hydride in mineral oil together with 550 ml of *n*-hexane. The slurry was cooled to 10° and a solution of 61.6 g (0.50 mole) of ethyl chloroacetate in 42 ml (0.57 mole) of acetone added with stirring. The addition of 2.0 ml of absolute ethanol caused the reaction to begin immediately. The mixture was allowed to warm to 15°, kept between 15 and 23° for 1.5 hr, then stirred at room temperature until hydrogen evolution had ceased (3.5 hr) and finally heated to 55° for 20 min.

Ethanol (20 ml) was added to destroy any remaining sodium hydride and the mixture poured onto 1 kg of ice containing 5 ml of 50% sulfuric acid. The organic layer was separated and the aqueous layer extracted with three 200-ml portions of *n*-hexane. The combined hexane solutions were washed with 250 ml each of 50% sodium bicarbonate solution and water, dried over sodium sulfate, and distilled through a 10-cm Vigreux column. The glycidic ester (43 g, 60% yield) was collected at 43–44° (2 mm); nmr (CCl_4): δ 1.23, *t*($J = 7$ Hz), 3H; 1.28, s, 3H; 1.33, s, 3H; 3.13, s, 1H; 4.3, q($J = 7$ Hz), 2H; ir (neat): 1730 cm^{-1}.

(±)α,β-*Dihydroxyisovaleric acid* (*II*). A suspension of 36.2 g (0.25 mole) of ethyl α,β-oxidoisovalerate in 500 ml of water was acidified with 3 ml of 1 *N* hydrochloric acid and refluxed with stirring for 2 hr. Sodium hydroxide (52.5 ml of 5 *N*) was added and reflux continued for 2.5 hr. The cooled mixture was washed with three 200-ml portions of ether, acidified to pH 1 with 10% hydrochloric acid, and evaporated at reduced pressure. The residue was treated with 25 ml of absolute ethanol, filtered to remove sodium chloride, and the filtrate and washings concentrated to leave a colorless syrup, 36.1 g.

Resolution of α,β-Dihydroxyisovaleric acid. The crude acid was resolved with quinine as described by Sjölander *et al* (*4*). The quinine salt, after four recrystallizations from ethanol, melted at 208–209° dec (lit(4) mp 208–209° dec), $[\alpha]_D^{25}$ −141.8° ($c = 1$, methanol), lit (*4*) $[\alpha]_D^{23}$ −142° ($c = 1$, methanol).

A 2.2-cm column was filled with 80 g of Bio-Rad cation-exchange resin (AG 50 W-XI, 50–100 mesh, hydrogen form) in water and washed until the washings were neutral. A solution of 9 g of the resolved quinine salt in 600 ml of warm water was passed through the column, using an additional 300 ml of water for washing the column. Concentration of the eluates at reduced pressure left 2.55 g (98%) of (R) (−)-α,β-dihydroxyisovaleric acid; nmr (D_2O): δ 1.37, s, 6H; 4.16, s, 1H; 5.05, s, 3H; ir (neat): 3650–2300 (broad), 1720 cm^{-1}; $[\alpha]_D^{25}$ −16° ($c = 2$, 0.1 *N* HCl), −14.3° ($c = 2.5$, water), +8.0° ($c = 2.5$

in water with 1 equiv of NaOH added). This agrees well with the reported rotation of $[\alpha]_D^{23}$ −12.5° ($c = 2$, 0.1 *N* HCl), but the reported (*4*) value of +9.5° ($c = 2$, water) appears to be in error. The acid showed a plain negative ord curve between 589 and 300 nm in water, 0.1 *N* HCl, or methanol.

(*R*)-(−) *Methyl α,β-dihydroxyisovalerate* (*VIII*). A solution of 2.5 g of (*R*)-(−) α,β-dihydroxyisovaleric acid in 10 ml of methanol was cooled in an ice bath and esterified with an ethereal solution of diazomethane. Removal of the solvents gave 2.2 g (95%) of methyl ester. Distillation through a 10 cm Vigreux column gave an 85% recovery of pure ester, bp 42.5° (0.03 mm); $[\alpha]_D^{25}$ −28.1° ($c = 4$, chloroform); nmr ($CDCl_3$): δ 1.25, s, 3H; 1.31, s, 3H; 3.41, s, 1H; 3.86, s, 3H; 4.08, s, 2H; addition of D_2O to the nmr sample caused the peak at 3.41 to disappear and reduced the intensity of the peak at 4.08 to 1 H. ir (neat) 3650–3050 (broad), 1725 cm^{-1}.

Anal. Calcd for $C_6H_{12}O_4$: C, 48.64; H, 8.11. Found: C, 48.61; H, 7.99.

(*R*)-(+)-*2,2,4,4-Tetramethyl-5-carbomethoxy-1,3-dioxolane* (*IX*). A solution of (R)-(−) methyl α,β-dihydroxyisovalerate (7.1 g, 0.048 mole) and 0.5 g of *p*-toluenesulfonic acid in 200 ml of 2,2-dimethoxypropane was kept at room temperature for 60 hr, then diluted with 500 ml of chloroform. The mixture was washed with 100 ml of 5% sodium bicarbonate and 150 ml of water, dried over sodium sulfate, and concentrated at reduced pressure. Distillation of the residue gave 7.03 g (73%) of the acetonide, bp 51.5° (1.75 mm), $[\alpha]_D^{25}$ +24.9° ($c = 4$, chloroform); nmr (CCl_4): 1.08, s, 3H; 1.30, s, 3H; 1.39, s, 3H; 1.43, s, 3H; 3.70, s, 3H; 4.23, s, 1H; ir (neat): 1756, 1727 cm^{-1}.

Anal. Calcd for $C_9H_{16}O_4$: C, 57.44; H, 8.51. Found: C, 57.52; H, 8.69.

(*S*)-(+)-*2,2,4,4-Tetramethyl-5-hydroxymethyl-1,3-dioxolane* (*X*). To a suspension of 0.83 g of lithium aluminum hydride in 85 ml of anhydrous ether, stirred in a waterbath at 35°, was added dropwise a solution of 3.76 g of (R)-(+)-2,2,4,4-tetramethyl-5-carbomethoxy-1,3-dioxolane in 20 ml of ether. The mixture was refluxed with stirring for an additional 3 hr and stirred at room temperature overnight. After cooling in ice the excess reagent was destroyed by the addition of 5 ml of 3% sodium hydroxide, followed by stirring for 1 hr. The precipitate was filtered and washed well with ether, and the combined ether solutions dried and concentrated. Distillation of the residue yielded 2.3 g (72%) of the alcohol, bp 49.5–50° (0.65 mm), $[\alpha]_D^{25}$ +14° ($c = 4$, chloroform); nmr ($CDCl_3 + D_2O$): δ 1.10, s, 3H; 1.30, s, 3H; 1.34, s, 3H; 1.40, s, 3H; 3.48–3.96, m, 3H; 4.78, s, (OH); ir (neat): 3650–3050 cm^{-1}. The mass spectrum did not show a parent peak, but displayed prominent peaks at *m/e* 145, 129, 103, 101, and 85.

A crystalline 1-naphthylurethan was prepared from racemic 2,2,4,4,-tetramethyl-5-hydroxymethyl-1,3-dioxolane (prepared as described above). Four recrystallizations from hexane gave the analytical sample, mp 110–110.5°.

Anal. Calcd for $C_{19}H_{23}NO_4$: C, 69.30; H, 6.99; N, 4.25. Found: C, 69.08; H, 7.08: N, 3.85.

Toxylate XI. To a solution of 2.00 g (0.0125) mole of (S)-(+)-2,2,4,4-tetramethyl-5-hydroxymethyl-1,3-dioxolane in 20 ml of dry pyridine, cooled to −15° in a salt–ice bath, was added dropwise with stirring a solution of 2.64 g (0.0138 mole) of *p*-toluenesulfonyl chloride in 10 ml of pyridine. The solution was stirred at −15° for 1 hr, kept at −3° for 18 hr, then stored at 5° for 24 hr. The mixture was poured into 200 ml of water and ice, and the precipitated solid filtered by suction and washed well with cold water. After drying in a vacuum desiccator overnight, the solid weighed 3.32 g (85%) and melted at 70–71°. Recrystallization from 100 ml of pentane gave 3.01 g of colorless needles, mp 74.5–75.5° $[\alpha]_D^{25}$ +2.4° ($c = 3$, chloroform); nmr ($CDCl_3$) 1.05, s, 3H; 1.26, s, 6H; 1.33, s, 3H; 2.42, s, 3H; 3.84–4.15, m, 3H; 7.28–7.83, q, 4H; ir (KBr) 1598, 1365, 1175 cm^{-1}.

Anal. Calcd for $C_{15}H_{22}O_5S$: C, 57.32; H, 7.00. Found: C, 57.01; H, 7.09.

(S)-(+)-5-(2,2,4,4-Tetramethyl-1,3-dioxa)-cyclopentylmethyl benzyl sulfide (XII). A solution of sodium *n*-butoxide was prepared from 0.21 g of sodium and 14 ml of *n*-butanol in a 50-ml round-bottomed flask. Benzyl mercaptan (1.24 ml) was added, followed by 2.2 g of (S)-(+)-5-(2,2,4,4-tetramethyl-1,3-dioxa) cyclopentylmethyl *p*-toluenesulfonate, and the mixture was stirred at reflux for 6.5 hr. After keeping at room temperature overnight, the precipitated sodium *p*-toluenesulfonate (1.39 g) was filtered and washed with ether. The combined filtrate and washings were diluted with 100 ml of ether, washed with three 30-ml portions of 5% sodium hydroxide and two 30-ml portions of water, dried, and concentrated. The residue was dissolved in 2 ml of hexane and chromatographed on 30 g of Stahl silica gel H, eluting with 9:1 hexane-ethyl acetate. The colorless sulfide (1.5 g), R_f 0.4, contained traces of benzyl mercaptan, R_f 0.6. The sulfide showed $[\alpha]_D^{21}$ +57° ($c = 3$, chloroform); nmr ($CDCl_3$) 1.01, s, 3H; 1.21, s, 3H; 1.29, s, 3H; 1.38, s, 3H; 2.25–2.73, octet, 2H; 3.75, s, 2H; 3.71–3.94, t, 1H; 7.21–7.33, m, 5H; ir (neat) 3040–3010, 1600, 1375 cm^{-1}.

(S)-(+)-2,3-Dihydroxy-2-methylbutane (XIII)

A. *From 5-(2,2,4,4-tetramethyl-1,3-dioxa) cyclopentylmethyl benzyl sulfide.* To a solution of 1.06 g of the (S)-(+)-sulfide in 120 ml of 95% ethanol was added 12 g (wet) of W-7 Raney nickel, and the mixture was stirred at reflux for 6 hr. The Raney nickel was removed from the hot solution by filtration through asbestos and washed with two 20-ml portions of hot ethanol. The combined filtrate and washings were cooled to room temperature, acidified with 14 ml of 5% hydrochloric acid and kept for 36 hr. After neutralization with 5% sodium bicarbonate the ethanol was removed at reduced pressure and the aqueous residue extracted continuously with ether for 24 hr. The ether extracts were dried and concentrated and the residue (0.27 g) purified by bulb-to-bulb distillation at 85–92° (8 mm) to yield the pure diol, $[\alpha]_D^{25}$ +5.4° ($c = 6$, chloroform); nmr ($CDCl_3$ + D_2O): δ 1.08, 1.14, d, 3H, $J = 6$ Hz; 1.11, s, 3H, 1.16, s, 3H; 3.58, q, 1H, $J = 6$ Hz; 3.88, broad, 2H; ir (neat): 3650–3050 cm^{-1}.

B. *From (S)-(−)-Methyl lactate.* (S)-(−)-Methyl lactate was prepared by esterification of (S)-(+)-lactic acid with diazomethane and distilled at 59.5° (23 mm); $[\alpha]_D^{22}$ −9.5° (neat).

A Grignard solution was prepared from 1.15 g of magnesium turnings and 7.66 g of methyl iodide in 40 ml of ether. The solution was cooled to 15° and 0.84 g of (S)-(−)-methyl lactate in 10 ml ether was added slowly over 30 min. The solution was refluxed for 2 hr and poured into 35 ml of saturated ammonium chloride solution and ice. The ether layer was separated and the aqueous layer extracted with ether. The combined ether extracts were dried and concentrated, and the residue (0.11 g) purified by distillation at 84–89° (10 mm) to yield 79 mg of pure diol, $[\alpha]_D^{24}$ +5.6° ($c = 7$, chloroform), $[\alpha]_{578}^{24}$ +5.9°, $[\alpha]_{546}^{24}$ +6.4°, $[\alpha]_{436}^{24}$ +10.2°, $[\alpha]_{365}^{24}$ +14.4°. The nmr and infrared spectra were identical with those from the product of part A.

(±)-Ethyl β-methyl-α,β-oxidovalerate. The Darzens condensation was carried out as described above for the preparation of ethyl α,β-oxidoisovalerate, substituting 2-butanone for acetone. The glycidic ester (*4*) was collected at 71.5–73° (2 mm) in 60% yield; ir (neat) 1750 cm^{-1}; nmr (CCl_4): δ 0.93, t, 3H, $J = 7$ Hz; 2.24, s, 3H; 2.24, t, 3H, $J = 7$ Hz; 2.56, q, 2H, $J = 7$ Hz; 3.18, s, 1H; 4.16, q, 2H, $J = 7$ Hz.

α,β-Dihydroxy-β-methylvaleric acid (V). The racemic acid was obtained as a pale yellow syrup in quantitative yield by the hydrolysis procedure described above for α,β-dihydroxyisovaleric acid, and resolved with quinine as described by Sjölander *et al.* (*4*). Seven recrystallizations of the quinine salt from absolute ethanol gave colorless needles (9.2%), mp 203–204° dec, $[\alpha]_D^{28}$ −144.8° ($c = 1$, methanol); lit. (*4*) mp 203–204° dec, $[\alpha]_D^{23}$ −144° ($c = 1$, methanol).

The acid was regenerated from the quinine salt in quantitative yield by passing through a column of cation-exchange resin at 55° as described above. Pure (2R:3R)-α,β-dihydroxy-β-methylvaleric acid was obtained as a colorless liquid, $[\alpha]_D^{28}$ −32.8° (c = 2.3, 0.1 N HCl); Sjölander *et al.* (*4*) reported $[\alpha]_D^{23}$ −15° (c = 2.3, 0.1N HCl), though a rotation of $[\alpha]_D^{23}$ −28° (c = 1.43, 1N HCl) has been reported for the acid obtained by hydrolysis of strigosine (*6*); ir (neat): 3700–2300 (broad), 1730 cm^{-1}; nmr (D_2O): δ 1.24, t, 3H, J = 7 Hz; 1.56, s, 3H; 1.90, m, 2H; 4.44, s, 1H; 5.06, s (OH).

(2R:3R)-(+)-4-Ethyl-2,2,4-trimethyl-1-3-dioxolane-5-carboxylic acid (XV). A solution of 2.41 g (0.016 mole) of (−)-α,β-dihydroxy-β-methylvaleric acid in 80 ml of 2,2-dimethoxypropane containing 0.20 g of *p*-toluenesulfonic acid was kept for 60 hr at room temperature. After diluting with 200 ml of chloroform the solution was washed with 5% sodium bicarbonate and water, dried over sodium sulfate, and concentrated at reduced pressure. The infrared spectrum of the residue showed both ester (1745) and anhydride (1800, 1775 cm^{-1}) bands. The residue was stirred under reflux with 15 ml of 2.5 N sodium hydroxide for 2 hr, the mixture washed twice with chloroform, then acidified to pH 1 in the cold with 2 N hydrochloric acid in the presence of 50 ml of cold chloroform. The mixture was shaken, the chloroform layer quickly drawn off, and the aqueous layer extracted with three 50-ml portions of chloroform. The extracts were dried and concentrated, leaving 0.93 g (30%) of colorless acid, mp 82–85°. Three recrystallizations from ethyl acetate and two from pentane gave the pure acid, mp 93–94.5°, $[\alpha]_D^{25}$ +58.6° (c = 3, chloroform); ir (KBr): 3300–2520, 1733 cm^{-1}; nmr ($CDCl_3$): δ 0.94, t, J = 6 Hz, 3H; 1.41, s, 3H; 1.44, s, 3H; 1.52, s, 3H; 1.68, m, 2H; 4.41, s, 1H; 9.91, s, 1H.

Anal. Calcd for $C_9H_{16}O_4$: C, 57.44; H, 8.51. Found: C, 57.29, H, 8.58.

The racemic acid was prepared by essentially the same procedure, and after four recrystallizations from ethyl acetate melted at 103–105°.

5-Acetoxy-4-ethyl-2,2,4-trimethyl-1,3-dioxolane (XVI). A solution of 0.376 g (2.0 mmoles) of (+)-(2R;3R)-4-ethyl-2,2,4-trimethyl-1,3-dioxolane-5-carboxylic acid in 25 ml benzene was placed in a three-necked flask equipped with stirrer, nitrogen inlet tube, and condenser topped with a gas outlet tube leading to a vial of 5% barium hydroxide solution. With nitrogen flowing through the system, 3 g of lead tetraacetate (containing 10–15% of acetic acid) was added and the mixture stirred while being heated to reflux. Evolution of carbon dioxide began when reflux temperature was reached and continued for 4.5 hr of refluxing. After cooling, the white precipitate was filtered and washed with benzene and the combined benzene solutions washed with 20 ml of 5% sodium carbonate. The brown precipitate was removed by filtering through a layer of Celite, and the benzene solution washed again with bicarbonate and with water. Concentration of the dried benzene solution left 0.32 g of yellow residue, which was distilled at 70–75° (0.2 mm) in a Kügelrohr apparatus, affording 0.23 g (57%) of acetate XVI, $[\alpha]_D^{25}$ +27.8° (c = 7.5, chloroform); ir (neat): 1745, 1225 cm^{-1}; nmr (CCl_4): δ 0.92, overlap of two triplets, J = 7 Hz, 3H; 1.17 and 1.23, two singlets, 3H; 1.38, aggregate of singlets, 6H; 1.53, two overlapping quartets, J = 7 Hz, 2H; 1.98, s, 3H; 6.01, s, 1H.

Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.40; H, 8.91. Found: C, 59.21; H, 9.00.

(R)-(+)-2-Methyl-1,2-butanediol (XVII). To a suspension of 0.076 g of lithium aluminum hydride in 10 ml ether under a nitrogen atmosphere was slowly added, with stirring, a solution of 0.182 g of (+)-XVI in 10 ml of ether. The mixture was stirred at reflux for 7 hr and at room temperature overnight, then stirred for 30 min with 0.6 ml of 3% sodium hydroxide solution to destroy the excess reagent. After filtering the precipitate and washing it with ether the combined ether solution was concentrated and the residue distilled in a Kügelrohr apparatus. The diol was collected at 95–100° (0.2 mm),

giving 0.072 g (77%), $[\alpha]_D^{25}$ +7.7° ($c = 4$, chloroform); ir (neat) 3700–3020 cm^{-1} (broad); nmr ($CDCl_3$): δ 0.89, t, $J = 7$ Hz, 3H; 1.11, s, 3H; 1.47, q, $J = 7$ Hz, 2H; 3.39, s, 2H; 3.71, broad, 2H. The ir and nmr spectra were identical with those of a sample prepared by lithium aluminum hydride reduction of α-hydroxy-α-methylbutyric acid.

(R)-(−)-α-Hydroxy-α-methylbutyric acid (XVIII). In an atmospheric pressure hydrogenation apparatus a solution of 0.052 g of (R)-(+)-2-methyl-1,2-butanediol in 10 ml of 0.2 *N* sodium hydroxide solution was stirred in an oxygen atmosphere over prereduced platinum (from 0.056 g of platinum oxide). After 18 hr the oxygen uptake was 8.3 ml. After filtration of the catalyst, the filtrate was successively washed with three 20-ml portions of ether, acidified with 1 *N* hydrochloric acid to pH 1 and continuously extracted with ether for 42 hr. Concentration of the dried ether extracts left 0.050 g of oily XVIII, $[\alpha]_D^{25}$ −2.8° ($c = 5$, chloroform, lit. (7) $[\alpha]_D^{25}$ −8.5°, whose infrared spectrum was identical with that of an authentic sample.

Since the hydroxy acid from this experiment did not crystallize[3], it was converted to the *p*-phenylphenacyl ester. The crude ester was separated from unreacted *p*-phenylphenacyl bromide by chromatography over silica gel, eluting with ethyl acetate, and recrystallized once from ethyl acetate–hexane to give 21 mg of colorless needles, mp 118.5–119.5°, $[\alpha]_D^{25}$ −2.0° ($c = 2.1$, chloroform), $[\alpha]_D^{25}$ +1.4° ($c = 13.8$, methanol); the chloroform solution showed a plain negative ord curve. The ir and nmr spectra were identical with those of samples of the racemic *p*-phenylphenacyl ester, mp 119–120°, and of the (S)-(+)-ester, mp 117.5–118°, $[\alpha]_D^{25}$ +2.5° ($c = 1.5$, chloroform), $[\alpha]_D^{25}$ −1.5° ($c = 1.87$, methanol), plain positive ord curve in chloroform, prepared from (S)-(+)-2-hydroxy-2-methylbutyric acid, mp 72–73°, $[\alpha]_D^{25}$ +7.1° ($c = 3$, chloroform).

α,β-Dihydroxyisovaleramide. A solution of 0.486 g of (−) VIII in 5 ml methanol was mixed with 8 ml of liquid ammonia in a pressure bottle and kept 13 days at room temperature. The residue remaining after evaporation of the solvents was taken up in ethyl acetate and chromatographed over silica gel, eluting with 20% methanol in benzene. The amide (0.36 g, 81%) melted at 93–94° after six recrystallizations from ether.

Anal. Calcd for $C_6H_{13}NO_3$: C, 49.00; H, 8.84; N, 9.52. Found: C, 49.13, 49.23; H, 8.90, 8.93; N, 9.43, 9.66.

[3] Repetition of the oxidation with racemic diol XVII gave α-hydroxy-α-methylbutyric acid in crystalline form, mp 63–66° after sublimation.

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