trituration with alcohol yielded small amounts of 1,6-bis-(phenylthio)-hexane.

The thiophenol was characterized as its 9-phenylxanthyl sulfide, m.p. 164° dec.[15]

Authentic phenyl propyl sulfide is reported to boil at 218.5–219.5°.[16] The sulfone prepared by oxidation of our crude sample (b.p. 215–220°) with hydrogen peroxide in acetic acid melted at 41.5–43.5°; Ipatieff, Pines and Friedman[16] report m.p. 44°.

γ-(Phenylthio)-propyl Bromide (V) and Lithium.—V was prepared by the slow addition of 0.5 mole of potassium phenyl mercaptide in alcohol solution to 215 g. (1.06 moles) of 1,3-dibromopropane in refluxing alcohol. The addition required 1.25 hours, after which the mixture was refluxed one-half hour more. After cooling, the potassium bromide was filtered and the filtrate diluted with several volumes of water. The product was separated and the aqueous phase extracted with ether. The combined extracts were dried over potassium carbonate and distilled to yield 99.3 g. (0.49 mole) of unreacted 1,3-dibromopropane, b.p. 58–60° (16 mm.), 46.6 g. (0.20 mole, 40%) of V, b.p. 155–160° (16 mm.),[17] and 34.1 g. of pot residue which may be 1,3-bis-(phenylthio)-propane.

To 1.9 g. (0.27 g. atom) of lithium in 100 ml. of ether was added 31.1 g. (0.135 mole) of V in 60 ml. of ether while stirring. The reaction started spontaneously and the addition was complete in one hour. The mixture was stirred and refluxed 3 hours more and was then allowed to stand overnight. To detect the possible presence of any unreacted lithium reagent, it was then carbonated and extracted with 5% sodium bicarbonate. Subsequent work-up of these washings yielded none of the corresponding acid. The ether phase was dried over potassium carbonate and the ether removed from it to yield 4.4 g. (22%) of 1,6-bis-(phenylthio)-hexane (VI) as white plates, m.p. 81–82° from methanol, and 7.9 g. of an oil which gave no discrete fraction on molecular distillation, and no crystalline sulfone on oxidation with hydrogen peroxide in acetic acid.

Oxidation of VI with hydrogen peroxide in acetic acid yielded the disulfone, 1,6-bis-(phenylsulfonyl)-hexane, m.p. 112–114°.

1,6-Bis-(phenylthio)-hexane (VI).—VI was prepared by mixing 25 g. (0.1 mole) of 1,6-dibromohexane with 0.3 mole of potassium phenyl mercaptide in alcohol solution. An exothermic reaction occurred, after which the mixture was refluxed one-half hour more. The product (VI) as well as potassium bromide were separated by filtration, and the latter removed by trituration with water. After recrystallization from alcohol the product weighed 29 g. (96%) and melted at 81–82°. Mixed m.p.'s of this compound with material from the reaction of phenylithium and thiacyclobutane or with the material from the reaction of γ-(phenylthio)-propyl bromide with lithium showed no depression. The disulfone of VI, 1,6-bis-(phenylsulfonyl)-hexane (prepared in 98% yield by oxidation with hydrogen peroxide in acetic acid), also showed no depression in m.p. when mixed with the corresponding derivatives of the other samples of VI.

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α,β-Dihydroxyisovaleric Acid and α,β-Dihydroxy-β-methylvaleric Acid, Precursors of Valine and Isoleucine[1,2]

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The synthesis and resolution of DL-α,β-dihydroxyisovaleric acid and of a DL-α,β-dihydroxy-β-methylvaleric acid are described. An optical isomer of each acid has been shown to be identical with the precursor of valine and of isoleucine previously obtained from a *Neurospora* mutant.

By the use of a mutant strain of *Neurospora crassa*, which requires valine and isoleucine for growth, an isomer of α,β-dihydroxyisovaleric acid[3] has been found to be a precursor of valine, and an isomer of α,β-dihydroxy-β-methylvaleric acid[4] has been shown to be a precursor of isoleucine. Confirmatory evidence for the structure of the precursors has now been obtained by synthesis of these two dihydroxy acids.

DL-α,β-Dihydroxyisovaleric acid,[5] the valine precursor, was prepared from ethyl DL-α,β-oxidoisovalerate[5] by hydrolysis of the ester and the oxide groups. Resolution of the acid was accomplished by formation of the diastereoisomeric quinine salts, one of which was crystalline. This crystalline salt was identical with the quinine salt of the dihydroxy acid obtained from the *Neurospora* mu-

TABLE I

α,β-DIHYDROXYISOVALERIC ACID

	Quinine salt of synthetic α,β-dihydroxyisovaleric acid	Quinine salt of natural α,β-dihydroxyisovaleric acid
M.p., °C.	208–209 dec.	209–210 dec.
Mixed m.p., °C.	209–210 dec.	
Anal. Calcd. for $C_{23}H_{34}N_2O_6$:	Found:	Found:
C, 65.47	65.36	65.13
H, 7.47	7.21	7.23
N, 6.11	5.91	6.00
$[\alpha]^{23}D$ in MeOH	−142° (*c* 1)	−141° (*c* 1)

Infrared spectra: The spectra of the two salts as a solid mull in petrolatum were identical from 2–15 μ.

	Synthetic α,β-dihydroxyisovaleric acid	Natural α,β-dihydroxyisovaleric acid
$[\alpha]^{23}D$	−12.5° (*c* 2 in 0.1 *N* HCl)	−12.4° (*c* 2 in dilute HCl, *p*H 1)
	+ 9.5° (*c* 2 in water, *p*H 5.5–6.5)	+10° (*c* 2 in water, *p*H 5.5–6.5)

Biological activity: The two substances exhibited identical threshold concentrations for inhibition of *Escherichia coli* K-12, and supported in each case the same growth rate of valine-less *E. coli* 6B9.

(1) Part of this work was supported by a contract between the Office of Naval Research and the Regents of the University of California.

(2) These acids in previous reports (ref. 3, 4) were named α,β-dihydroxy-β-methylbutyric acid and α,β-dihydroxy-β-ethylbutyric acid, respectively, to emphasize their origin from the β-acetylation of a 4-carbon precursor.

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tant. Regeneration of the acid from its salt yielded an optically active α,β-dihydroxyisovaleric acid which had the same biological activity as the natural compound (Table I).

The isoleucine precursor, α,β-dihydroxy-β-methylvaleric acid [6] was synthesized from ethyl DL-β-methyl-α,β-oxidovalerate,[6] an oil which was isolated by distillation and was a DL-modification of either one or both of the two possible stereoisomeric oxides. Hydrolysis of the ester and the oxide groups yielded a racemic α,β-dihydroxy-β-methylvaleric acid[6]; this acid was obtained as an oil and was assumed to be one or both of the two stereoisomeric DL-dihydroxy acids. Resolution of the acid with quinine yielded a crystalline salt of an optical isomer which was identical with the quinine salt of the natural acid isolated from the *Neurospora* mutant. The acid, when regenerated from its salt, was obtained as an optically active α,β-dihydroxy-β-methylvaleric acid which also was identical with the natural compound in biological activity (Table II).

TABLE II

α,β-DIHYDROXY-β-METHYLVALERIC ACID

	Quinine salt of synthetic α,β-dihydroxy-β-methylvaleric acid	Quinine salt of natural α,β-dihydroxy-β-methylvaleric acid
M.p., °C.	203 dec.	204 dec.
Mixed m.p., °C.	203 dec.	
Anal. Calcd. for $C_{26}H_{36}N_2O_6$:	Found:	Found:
C, 66.09	66.47	66.16
H, 7.68	7.39	7.48
N, 5.93	5.98	5.81
$[\alpha]^{23}D$ in MeOH	−144° (*c* 1)	−144° (*c* 1)

Infrared spectra: The two substances gave identical spectra from 2–15 μ as solid mulls in petrolatum.

	Synthetic α,β-dihydroxy-β-methylvaleric acid	Natural α,β-dihydroxy-β-methylvaleric acid
$[\alpha]^{23}D$	+ 3° (*c* 2.3 in H_2O containing 1 eq. of $Ca(OH)_2$)	+3° (*c* 2.3 in H_2O containing 1 eq. of $Ca(OH)_2$)
	−15° (*c* 2.3 in dilute HCl, *p*H 1)	−16.7° (*c* 2.3 in dilute HCl, *p*H 1)

Biological activity: The two acids supported the same growth rate of the isoleucine-deficient mutant of *Escherichia coli* 58-336.

Experimental

Ethyl DL-α,β-Oxidoisovalerate.—The method of Rutowski and Dajew,[7] involving the Darzens condensation of acetone with ethyl chloroacetate using powdered sodium as the base, gave poor yields. Sodium ethoxide gave a smoother reaction and better yields (45–50%).

α,β-Dihydroxyisovaleric Acid.—Saponification of the ester and hydrolysis of the epoxide ring according to Kögl, Duisberg and Erxleben[5] gave a colorless sirup rather than the crystalline substance described by them. It is to be noted that the amount of alkali recorded by them for saponification of five grams of their ester is stoichiometrically inadequate. The hydrolysis reaction was accompanied by some decomposition to isobutyraldehyde, and we found it advantageous to do this step using boiling water rather than 0.4 *N* sulfuric acid. Over-all yields from the ester to dihydroxy acid averaged 60%.

Quinine Salt of Synthetic α,β-Dihydroxyisovaleric Acid.—DL-α,β-Dihydroxyisovaleric acid (8.64 g., 0.065 mole) and anhydrous quinine (24 g., 0.073 mole) were dissolved in 175 ml. of absolute ethanol. After standing at 5° overnight, the crystalline salt was filtered and dried. The crude material (10.3 g.) melted at 198–200° dec. After two recrystallizations from ethanol, the lustrous needles (5.8 g., 39%) melted at 208–209° dec. Additional pure salt could be obtained by concentrating the mother liquors from the recrystallizations and recrystallizing the resulting solids. Analytical data are given in Table I.

Quinine Salt of Natural α,β-Dihydroxyisovaleric Acid.—The dihydroxy acids produced by *Neurospora crassa* strain 16117 were isolated from culture filtrates as described by Adelberg, Bonner and Tatum.[4] They were then separated by ascending chromatography on a chromatopile,[8] the solvent consisting of 70% ether, 30% benzene, made 3 *M* with formic acid and saturated with water. A column using about 1200 pieces of Whatman No. 1 filter paper separated a mixture containing one gram of each acid. The sheets holding the dihydroxy acids were extracted with water in a Waring blendor; the resulting solutions were concentrated, made alkaline and extracted continuously with ether for 24 hours. The aqueous phase in each case was then brought to *p*H 1.5 with sulfuric acid and extracted with fresh ether for another 24 hours. The second ether extract was dried over sodium sulfate, evaporated to dryness, and the sirupy free acid was dissolved in water. This solution was decolorized with Norite, treated with an excess of calcium carbonate, filtered and evaporated to dryness. The calcium salt of the acid (0.7 g.) was dissolved in 5 ml. of water. The *p*H was adjusted to 1–2 with concentrated hydrochloric acid, and the solution was continuously extracted with ether for 30 hours. After drying over magnesium sulfate, the ether was removed *in vacuo*, and the residue was dissolved in absolute ethanol and treated with anhydrous quinine (1.48 g.) in ethanol. The total volume of the solution was 12 ml. From the solution, 1.32 g. (63%) of crystalline material melting at 206.5–208° dec. was obtained. One recrystallization from ethanol gave a product melting at 209–210° dec. Analytical data are given in Table I.

Optically Active α,β-Dihydroxyisovaleric Acid.—A solution of the quinine salt of α,β-dihydroxyisovaleric acid (1.55 g.) in 30 ml. of water was basified with 2.5 *N* sodium hydroxide. The quinine was removed on a filter, and the filtrate was concentrated *in vacuo* to a volume of 5–10 ml. The solution was acidified to *p*H 1–2 with concentrated hydrochloric acid and was then continuously extracted with ether for 24 hours. After the solution was dried over magnesium sulfate, the ether was removed *in vacuo* leaving 0.29 g. (64%) of the sirupy acid (see Table I).

Biological Activity of α,β-Dihydroxyisovaleric Acid.—Both the synthetic and natural free acids were regenerated from the quinine salts by passage through columns of Dowex No. 1 cation-exchange resin. The resulting solutions were neutralized with sodium hydroxide, sterilized by autoclaving five minutes at 15 p.s.i., and used directly in the growth experiments.

Preliminary experiments with crude calcium salt showed that the compound's activity was extremely weak compared to that of valine, and only demonstrable at *p*H values below 6.0. For this reason the more precise method of turbidimetry was put aside in favor of the safer method of inspection of colony size on solid medium.[9] Two systems were employed: inhibition of the valine-sensitive strain K-12 of *E. coli* and growth stimulation of the valine-dependent mutant *E. coli* 6B9.[10]

Approximately 100 cells of K-12, 6B9 and the valine-resistant wild-type *E. coli*, strain 9637, were spread on minimal agar,[11] *p*H 5.9, supplemented with varying concentrations of synthetic α,β-dihydroxyisovaleric acid and natural α,β-dihydroxyisovaleric acid. The synthetic and natural acids, at 120 μg./ml., supported slow growth of *E. coli* 6B9, colonies becoming visible at 4 days and reaching maximum size at six days. No colonies appeared on control plates with no supplementation. Neither preparation showed the slightest inhibition of *E. coli* 9637 at concentrations up to 333 μg./ml. The effect on growth of K-12 is indicated in Table III. The two preparations thus showed identical valine-like activity, including inhibition of *E. coli* K-12 but not of 9637 and stimulation of *E. coli* 6B9.

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TABLE III

EFFECT ON GROWTH OF *E. coli* K-12

(Colony size after six days incubation at 37°; 1+ indicates barely visible colonies; 5+ indicates maximum size for this strain under these conditions.)

Concn., γ/ml.	α,β-Dihydroxyisovaleric acid	
	Natural	Synthetic
0	5+	5+
83	3+	3+
167	1-2+[a]	1-2+[a]
250	1+	1+
333	1+	1+

[a] Colonies not uniform in size.

Ethyl DL-β-Methyl-α,β-oxidovalerate.—The following modification of the procedure of Linstead and Mann[12] was used. A mixture of redistilled methyl ethyl ketone (130 g., 1.8 moles) and ethyl chloroacetate (220 g., 1.8 moles) was added dropwise, over a period of three hours, to a stirred suspension of sodium ethoxide (68 g., 2.0 moles) in 350 ml. of dry ether. The reaction mixture, cooled in an ice–salt-bath during the addition, was allowed to warm gradually to room temperature and was kept overnight, the openings to the system protected with Drierite tubes. The orange mixture was heated on the steam-cone for three hours, the ether being allowed to distil away gradually to raise the temperature. The liquid was poured on ice containing 30 ml. of 50% sulfuric acid; ether was added, and the layers were separated. The aqueous phase was extracted once with ether and the combined ether extracts were washed with water, four times with dilute bicarbonate and twice with water. After drying over sodium sulfate and magnesium sulfate, the ether was removed and the residue was distilled *in vacuo* through a glass-spiral column 12 inches long. A forerun of material (52.9 g.) boiling at 30–65° (2–3 mm.) was collected, followed by a pale yellow liquid (118.7 g.) boiling at 65–68° (2–3 mm.). The red-brown residue was discarded and the forerun and main fraction were redistilled. The colorless product (119.5 g., 42%) was collected at 60–64° (2–3 mm.), $n^{21}D$ 1.4255.

DL-α,β-Dihydroxy-β-methylvaleric Acid.—A solution of ethyl DL-β-methyl-α,β-oxidovalerate (15.8 g., 0.1 mole) in 42 ml. of 2.5 *N* sodium hydroxide was heated at reflux temperature for two hours. The solution was extracted once with ether, acidified with hydrochloric acid, and the oily layer which separated was dissolved in ether. The aqueous phase was extracted twice more with ether, the combined ether extracts were dried over magnesium sulfate, and the ether was removed. The residual liquid in 300 ml. of water was heated at reflux temperature for four hours. The water was removed *in vacuo* leaving a colorless sirup (7.5 g., 51%).

Quinine Salt of Synthetic α,β-Dihydroxy-β-methylvaleric Acid.—DL-α,β-Dihydroxy-β-methylvaleric acid (4.0 g., 0.027 mole) was dissolved in ethanol and treated with anhydrous quinine (8.75 g., 0.027 mole) in ethanol. The solution (about 50 ml.) was seeded with the quinine salt of the natural acid from *Neurospora* and kept at 3° for three days. The sticky precipitate was recrystallized from ethanol to give a product (2.15 g.) melting at 188–191° dec. After seven more recrystallizations from ethanol, the compound (0.5 g.) melted at 203° dec. For analytical data, see Table II.

Quinine Salt of Natural α,β-Dihydroxy-β-methylvaleric Acid.—The calcium salt was first prepared, followed by regeneration of the acid and treatment with quinine according to the procedure described for the quinine salt of natural α,β-dihydroxyisovaleric acid. From 0.2 g. of calcium salt, 0.27 g. (50%) of quinine salt melting at 200–202° dec. was obtained. One recrystallization from ethanol gave material melting at 203–204°. For analytical data, see Table II.

Optically Active α,β-Dihydroxy-β-methylvaleric Acid.—The optically active acid was regenerated in 70% yield from the quinine salt according to the procedure described for α,β-dihydroxyisovaleric acid.

Biological Activity of α,β-Dihydroxy-β-methylvaleric Acid.—Both synthetic and natural acids were prepared for testing by the procedure described for α,β-dihydroxyisovaleric acid.

The preparations were compared for ability to support growth of the isoleucine-deficient mutant of *Escherichia coli*, strain 58-336. The procedure used was that of Adelberg,[13] except that the 10-ml. cultures were incubated on a rotary shaker in 125-ml. erlenmeyer flasks equipped with Klett-tube sidearms. Readings of turbidity were made at frequent intervals, and growth rates were found to be identical at comparable concentrations of the natural and synthetic preparations. Maximum turbidities are shown in Table IV, indicating equal activity of the two preparations within experimental error.

TABLE IV

EFFECT ON GROWTH OF *E. coli* 58-336

(Values are maximum Klett readings. The maximum on 0.1 mg. of DL-isoleucine was 126; on 1.0 mg. of DL-isoleucine 430.)

Concn. (mg./10 ml.)	Natural α,β-dihydroxy-β-methylvaleric acid	Synthetic α,β-dihydroxy-β-methylvaleric acid
0.00	10	10
1.00	175	184
0.05 (plus 0.10 mg. DL-isoleucine)	170	167
0.10 (plus 0.10 mg. DL-isoleucine)	188	179

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