Experiments in the Synthesis of DL-isoLeucine.

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The crude products of numerous syntheses of DL-*iso*leucine have been found to contain substantial amounts of the *allo*-modification. Possible stereospecific routes to the amino-acid starting from 4-*sec.*-butylidene-2-phenyloxazol-5-one and 4-*sec.*-butylidene-2-thiothiazolid-5-one have been investigated and also found to give mixtures of the two forms.

In most of the recorded syntheses of *iso*leucine the simultaneous formation of *alloiso*leucine has been either cursorily dismissed or ignored altogether. We have therefore applied most of the classical and some newer methods of amino-acid synthesis to the preparation of this amino-acid and have assayed the crude products, microbiologically, for their *iso*leucine content. As shown in the following Table however, none led to *iso*leucine of high purity. In some routes minor modifications were made to the original published details and in most cases the isolation of the crude amino-acid mixture was effected by treating alcoholic solutions of the hydrochlorides with triethylamine.

Route	DL-*iso*Leucine (%) in crude material	Route	DL-*iso*Leucine (%) in crude material
Organic Syntheses [a]	89 *	Darapsky [e]	42
Oximino-acid [b]	66	Strecker [f]	68
Feofilaktov [c]	64	Bucherer–Bergs [g]	61
Schmidt † [d]	72		

a, Marvel, *Org. Synth.*, 1941, **21**, 60. *b*, Hamlin and Hartung, *J. Biol. Chem.*, 1942, **145**, 349. *c*, Feofilaktov, *Compt. rend. Acad. Sci., U.R.S.S.*, 1939, **42**, 755. *d*, Schmidt, *Ber.*, 1924, **57**, 704 (cf. *Organic Reactions*, 1946, **3**, 316). *e*, Gagnon *et al.*, *Canad. J. Res.*, 1947, **25**, *B*, 28. *f*, Gresham and Schweitzer, U.S.P. 2,520,312. *g*, Livak *et al.*, U.S.P. 2,553,055.

* The high proportion of *iso*leucine in the crude material arises here because the method of working up includes a crystallisation which increases the percentage of *iso*leucine in the product at the expense of overall yield.

† This reaction has not previously been applied to the preparation of *iso*leucine and details are given on p. 1720.

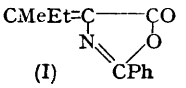
A convenient method for the separation of *iso*leucine from *alloiso*leucine by ion-exchange chromatography has recently been published (Piez, *J. Biol. Chem.*, 1954, **207**, 77), but before this only tedious methods were available (cf. Hood and Lyman, *J. Biol Chem.*, 1950, **186**, 195; Shabica, U.S.P. 2,456,742). We therefore examined two further syntheses involving αβ-unsaturated intermediates in the hope that the *cis-trans*-isomers could be separated, and then reduced and hydrolysed stereospecifically.

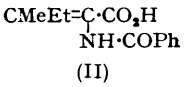
Lur'e and Vdovina (*Zhur. obshchei Khim.*, 1952, **22**, 1883) catalytically hydrogenated 4-*sec.*-butylidene-2-phenyloxazol-5-one (I), prepared from hippuric acid and ethyl methyl ketone, and hydrolysed the product to α-amino-β-methylvaleric acid. We obtained a mixture of both forms of the amino-acid by the action of red phosphorus and hydriodic acid on the oxazolone. Lur'e and Vdovina (*loc. cit.*) separated the isomers of the oxazolone (I) in an indirect manner and converted them into the two α-benzamido-β-ethyl-β-methylacrylic acids (II) and various derivatives thereof. We obtained the acid (II) as a mixture of isomers by hydrolysis of the crude oxazolone and separated one apparently pure isomer, m. p. 221—222°, by crystallisation.

This acid was readily hydrogenated over Raney nickel to α-benzamido-β-methylvaleric acid which on subsequent acid hydrolysis gave a specimen of amino-acid containing 78% of *iso*leucine. From the crystallisation mother-liquors of the acid (II), m. p. 221—222°, another acid, of m. p. 203°, was obtained which on hydrogenation gave a mixture of N-benzoyl*iso*leucine and *N*-benzoyl*alloiso*leucine.

Experiments in which pure specimens of *N*-benzoyl*iso*leucine and *N*-benzoyl*alloiso*leucine were hydrolysed under conditions identical with those employed for the reduction

products indicated that slight inversion occurred during hydrolysis, but insufficient to account for the low figure of 78% of *iso*leucine obtained from the pure acid of m. p. 221—222°, and this has led us to suspect that the reduction with Raney nickel may not be completely stereospecific. Taken in conjunction with the inversion during hydrolysis this would therefore limit the value of α-benzamido-β-ethyl-β-methylacrylic acid (m. p. 221—222°) as an intermediate for the preparation of pure *iso*leucine.


(I)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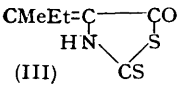
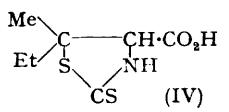

(III)

Possible syntheses via 4-*sec.*-butylidene-2-thiothiazolid-5-one (III), which is readily obtained by the condensation of 2-thiothiazolid-5-one with ethyl methyl ketone (cf. Billimoria and Cook, *J.*, 1949, 2323; Cook, Hunter, and Pollock, *J.*, 1950, 1892), were next examined. This compound, however, could not be separated into *cis-trans*-isomers, although reductive hydrolysis with red phosphorus and hydriodic acid according to the general procedure of Billimoria and Cook (*loc. cit.*) gave a mixture of approximately equal amounts of both forms of the amino-acid.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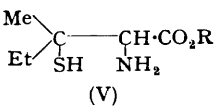
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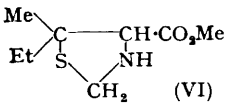
(VI)

Treatment of (III) with an excess of methanolic potassium hydroxide according to the general procedure of Chatterjee, Cook, Heilbron, and Levy (*J.*, 1948, 1337) readily yielded 5-ethyl-5-methyl-2-thiothiazolidine-4-carboxylic acid (IV), as a low-melting, deliquescent solid conveniently isolated as the highly crystalline phenethylamine salt. 4-Benzylidene-2-thiothiazolid-5-one yields one isomer of 5-phenyl-2-thiothiazolidine-4-carboxylic acid on treatment with methanolic potassium hydroxide, and the other isomer with aqueous sodium carbonate (Chatterjee *et al.*, *loc. cit.*), but the *sec.*-butylidene analogue (III) failed to react with cold aqueous sodium carbonate and after 2 hours' boiling gave only 23% of the acid obtained previously.

Vigorous treatment of the acid (IV) with hydrochloric acid (cf. Cook *et al.*, *J.*, 1948, 1060; 1949, 1437, 3007) afforded the hydrochloride of α-amino-β-mercapto-β-methylvaleric acid (V; R = H), previously obtained from 4-*sec.*-butylidene-2-methyloxazolone and hydrogen sulphide ("The Chemistry of Penicillin," Oxford, 1949, p. 469). The hydrochloride of the corresponding ester (V; R = Me) was obtained from the acid (IV) by successive *S*-methylation, esterification, reduction with aluminium amalgam, and treatment of the resulting methyl 5-ethyl-5-methylthiazolidine-4-carboxylate (VI) with mercuric chloride (cf. Cook, Hunter, and Pollock, *loc. cit.*). Unfortunately attempts to desulphurise the mercapto-amino-acid (V; R = H) by treatment with Raney nickel or with phosphorus and hydriodic acid failed to yield any *iso*leucine or *alloiso*leucine.

Experimental

The L-*iso*leucine assays were carried out by Hood and Lyman's method (*loc. cit.*) and the results doubled to give the percentages of DL-*iso*leucine shown in the Table: the organisms employed were *S. faecalis* and *Leuc. mesenteroides*.

Schmidt Reaction on Ethyl α-sec.-*Butylacetoacetate.*—Ethyl α-*sec.*-butylacetoacetate (9·3 g.) in dry chloroform (60 ml.) was treated with hydrazoic acid in chloroform (42·6 ml. of 7·58% w/v solution), and the mixture was added dropwise during 1 hr. to a vigorously stirred mixture of concentrated sulphuric acid (40 ml.) and chloroform (40 ml.) kept at −5° to 0°. The mixture was stirred for 30 min. at the same temperature and then poured on ice. The aqueous phase was extracted with chloroform, and the combined chloroform solutions were washed, dried (Na_2SO_4), and evaporated *in vacuo* to leave crude ethyl α-acetamido-β-methylvalerate (*ca.* 10 g.). This oil was refluxed overnight with 5N-hydrochloric acid (80 ml.) and, after treatment

with charcoal, the solution was evaporated *in vacuo*. The solid residue was dissolved in ethanol and treated with triethylamine to pH 6 to precipitate the amino-acid, which was collected and thoroughly washed with ethanol. The yield was 3·02 g. (46%).

4-sec.-*Butylidene-2-phenyloxazol-5-one*.—Acetic anhydride (76 ml.) was added dropwise during 30 min. to a stirred suspension of hippuric acid (54 g.) and anhydrous sodium acetate (24 g.) in ethyl methyl ketone (1300 ml.), and the mixture was refluxed for 2 hr. About 1050 ml. of ketone, b. p. 80—84°, were next distilled off during 2—3 hr. The oily residue was diluted with water (300 ml.), and sodium hydrogen carbonate (*ca.* 190 g.) was added until effervescence ceased. The product was extracted into ether and distilled, to give the mixed isomers as a pale yellow oil, b. p. 107—120°/0·15 mm. (43—49%), which partly solidified.

Reduction of the Oxazolone (I).—A mixture of the oxazolone (21·2 g.), acetic acid (140 ml.), hydriodic acid (100 ml.; 55% w/w), and red phosphorus (6 g.) was refluxed for 90 min. and filtered from unchanged phosphorus. The filtrate was evaporated to dryness *in vacuo* and the residue was dissolved in water and freed from benzoic acid by ether-extraction. After evaporation of the aqueous phase *in vacuo* the residue was dissolved in ethanol, and the solution adjusted to pH 6 with triethylamine (15 ml.). The white precipitate was collected and heated on the steam-bath for 1 hr. with an aqueous suspension of calcium hydroxide and, after removal of calcium phosphate, the filtrate was freed from calcium by addition of ammonium carbonate and filtration. Evaporation of the final filtrate *in vacuo* left 5·38 g. (42%) of amino-acid (washed with alcohol). Microbiological assay indicated the presence of 50% of *iso*leucine, raised to 58% by a single crystallisation from aqueous alcohol.

Hydrolysis of the Oxazolone (I).—The oxazolone (28 g.) was heated on the steam-bath for 45 min. with methanol (72 ml.) and 2N-sodium hydroxide (87 ml.), and the cooled solution was made strongly acid to precipitate α-benzamido-β-ethyl-β-methylacrylic acid, m. p. 193—195° (decomp.) (27 g., 89%). Three crystallisations from ethanol gave 7·9 g. of a component, m. p. 221—222° not increased by further crystallisation from ethanol or acetic acid (Found : C, 66·7; H, 6·6; N, 6·2. Calc. for $C_{13}H_{15}O_3N$: C, 66·9; H, 6·4; N, 6·0%), which with ethereal diazomethane yielded a methyl ester, m. p. 158° (Found : C, 68·0; H, 6·9; N, 5·8. Calc. for $C_{14}H_{17}O_3N$: C, 68·0; H, 6·9; N, 5·7%). Lur'e and Vdovina (*loc. cit.*) give the m. p. of these compounds as 223—224° and 155—156° respectively. From the ethanol mother-liquors were obtained 6·3 g. of a mixture of isomers, constant m. p. 203° (Found : C, 67·2; H, 6·8; N, 6·4%).

N-*Benzoyl*-iso*leucine and* -alloiso*leucine*.—Pure *iso*leucine (Hood and Lyman, *loc. cit.*) (13·1 g.) in 10% aqueous sodium hydroxide (80 ml.) was treated gradually at 0° with benzoyl chloride (20 ml.) and 20% aqueous sodium hydroxide (40 ml.) during 30 min., so that the mixture remained alkaline throughout. Acidification afforded N-*benzoyl*-DL-iso*leucine* (20·7 g., 88%) which after one crystallisation from 30% aqueous alcohol formed colourless plates, m. p. 136—138° unchanged on further crystallisation from this solvent or from benzene (Found : C, 66·5; H, 7·5; N, 6·1. $C_{13}H_{17}O_3N$ requires C, 66·3; H, 7·3; N, 6·0%). In the same way *alloiso*leucine (Greenstein, Levintow, Baker, and White, *J. Biol. Chem.*, 1951, **188**, 647) gave N-*benzoyl*-DL-alloiso*leucine*, colourless needles (from benzene), m. p. 126—127° (Found : C, 66·0; H, 7·0; N, 6·0%). A mixture of approximately equal amounts of the isomers had m. p. 112—114°. The only optically inactive α-benzamido-β-methylvaleric acid reported hitherto is the product, m. p. 118°, which Bouveault and Locquin (*Bull. Soc. chim. France*, 1906, **35**, 965) considered to be benzoyl*iso*leucine, but which probably contained the *allo*-modification.

iso*Leucine and* alloiso*Leucine*.—α-Benzamido-β-ethyl-β-methylacrylic acid, m. p. 221—222° (6·13 g.), in 1% aqueous sodium hydroxide (105 ml.) was hydrogenated at room temperature and pressure over Raney nickel (13 hr.). Acidification of the filtered reaction product gave α-benzamido-β-methylvaleric acid, m. p. 132—133° (6·06 g., 90%). Mixtures with *N*-benzoyl-*iso*leucine and *-alloiso*leucine melted at 135—137° and 112—114° respectively. The crude product (4·82 g.) and 20% hydrochloric acid (80 ml.) were refluxed together for 3 hr. and the resulting solution was evaporated to dryness *in vacuo* after removal of benzoic acid. The residue was dissolved in ethanol and treated with triethylamine to pH 6, and the precipitate (2·41 g., 89%) was collected and washed with ethanol until free from chloride. It was estimated to contain 78% of *iso*leucine, raised to 88% by one crystallisation.

Similar treatment of crude α-benzamido-β-ethyl-β-methylacrylic acid, m. p. 193—195° (decomp.), gave 86% of a mixture of *N*-benzoyl-*iso*leucine and *-alloiso*leucine, m. p. 112—114°, which on hydrolysis afforded 82% of amino-acid estimated to contain 62% of *iso*leucine. The acid, m. p. 203°, from the mother-liquors of the crystallisation of (II) gave 89% of a reduction product, m. p. 116—118°.

The specimens of amino-acid obtained when authentic *N*-benzoyl-*iso*leucine and -*alloiso*-leucine were hydrolysed as described were estimated to contain 90% and 17% respectively of *iso*leucine.

4-sec.-*Butylidene-2-thiothiazolid-5-one.*—(*a*) A suspension of 2-thiothiazolid-5-one (50 g.) in ethyl methyl ketone (800 ml.) was treated with benzylamine (1·5 ml.) and after agitation for a few minutes the clear purple solution was set aside with daily additions of 0·5-ml. quantities of benzylamine until, after 3—14 days, the presence of 2-thiothiazolid-5-one could no longer be detected (cf. Billimoria and Cook, *loc. cit.*). The mixture was diluted with water (1 l.), and the crude product (59—94%) was collected and crystallised from alcohol (charcoal), to give straw-yellow platelets, m. p. 180—181°. 4-sec.-*Butylidene-2-thiothiazolid-5-one* also crystallised well from ethyl methyl ketone (Found: C, 44·8; H, 5·2; N, 7·1. $C_7H_9ONS_2$ requires C, 44·9; H, 4·8; N, 7·5%).

(*b*) A suspension of 2-thiothiazolid-5-one (23 g.) and anhydrous zinc chloride (23 g.) in ethyl methyl ketone (207 ml.) was refluxed with stirring for 6 hr., and the product cooled and diluted with water (600 ml.). The crude green precipitate (22·6 g., 70%) was purified as in (*a*).

Reduction of the Thiazolidone (III).—A solution of 4-*sec.*-butylidene-2-thiothiazolid-5-one (6 g.) in boiling acetic acid (50 ml.) was treated with red phosphorus (3 g.) and hydriodic acid (20 ml.; 40% w/w). The mixture was refluxed for 6 hr. and worked up as described for the reduction of the oxazolone (I), to yield 2·60 g. (62%) of amino-acid, which was estimated to contain 48% of *iso*leucine.

Phenethylamine 5-Ethyl-5-methyl-2-thiothiazolidine-4-carboxylate.—4-*sec.*-Butylidene-2-thiothiazolid-5-one (28 g.) was heated on the steam-bath for 20 min. with potassium hydroxide (33·6 g.) in methanol (400 ml.), and the resulting yellow solution was set aside for 3 days, then evaporated *in vacuo*. The residue was treated with 5N-hydrochloric acid, and the gummy product was extracted into chloroform. The washed and dried chloroform solution was concentrated to small volume, cooled, and treated with phenethylamine (18 g.), to give a colourless precipitate of the almost pure *phenethylamine salt* (37·5 g., 77%) which crystallised from water in needles, m. p. 182—183° (decomp.) (Found: C, 55·4; H, 6·6; N, 8·4; S, 19·8. $C_{15}H_{22}O_2N_2S_2$ requires C, 55·2; H, 6·8; N, 8·6; S, 19·6%).

α-*Amino*-β-*mercapto*-β-*methylvaleric Acid Hydrochloride.*—The low-melting acid (IV), regenerated from the phenethylamine salt (3·63 g.), was heated with concentrated hydrochloric acid (40 ml.) in a sealed tube at 120° for 40 hr. After evaporation to dryness *in vacuo* the sticky residue was further dried in a vacuum-desiccator over potassium hydroxide, washed with ether, and crystallised from acetonitrile, to give small colourless crystals of the hydrated *hydrochloride* (54%), m. p. 112—115°. Recrystallisation raised the m. p. to 114—116° (Found: C, 33·3; H, 7·5; S, 14·7. Calc. for $C_6H_{14}O_2NSCl,H_2O$: C, 33·1; H, 7·4; S, 14·7%). The anhydrous material, considered to be a mixture of isomers, has been reported to be very hygroscopic and of indefinite m. p. (*op. cit.*, p. 469). The neutral amino-acid gave an intense purple colour with ferric chloride and a crimson colour with ninhydrin.

5-*Ethyl-5-methyl-2-methylthio-*Δ^2*-thiazoline-4-carboxylic Acid.*—The acid (IV), from 32·6 g. of the phenethylamine salt, was dissolved in dry acetone (100 ml.) and treated with anhydrous potassium carbonate (30 g.) and methyl iodide (7 ml.). The stirred mixture was refluxed for 2 hr. and, after removal of the solvent *in vacuo*, the residue was dissolved in water, and the solution was brought to pH 4 by cautious addition of dilute hydrochloric acid. The amphoteric product was extracted into ether (6 × 75 ml.), and the gum remaining after evaporation of the dried extracts was triturated with light petroleum (b. p. 40—60°) to give a cream-coloured powder (16·1 g.). Crystallisation from light petroleum (b. p. 60—80°) gave colourless needles of 5-*ethyl-5-methyl-2-methylthio-*Δ^2*-thiazoline-4-carboxylic acid*, m. p. 101—103° (Found: C, 43·3; H, 6·0; N, 6·7; S, 29·3. $C_8H_{13}O_2NS_2$ requires C, 43·8; H, 6·0; N, 6·4; S, 29·2%).

Methyl 5-Ethyl-5-methylthiazolidine-4-carboxylate.—The *S*-methylated acid (11 g.) was esterified with ethereal diazomethane, and the resulting oil was dissolved in methanol and added to aluminium amalgam (from 10 g. of aluminium foil and 200 ml. of 3% mercuric chloride solution). Methanethiol was evolved and the mixture boiled spontaneously. When the reaction had subsided the mixture was refluxed for 90 min. and filtered, and the solid was washed with hot methanol. The colourless filtrate and washings were evaporated *in vacuo* and the residual oily *ester* (6·05 g., 64%) was distilled at 75—95°/0·3—0·4 mm. A redistilled specimen had b. p. 74°/0·2 mm., n_D^{25} 1·4992 (Found: C, 50·9; H, 7·7. $C_8H_{15}O_2NS$ requires C, 50·8; H, 8·0%).

Methyl α-*Amino*-β-*mercapto*-β-*methylvalerate Hydrochloride.*—The ester (VI) (4·1 g.) was refluxed with mercuric chloride (20 g.) in methanol (200 ml.) for 2 hr. and the mixture was set

aside overnight, saturated with hydrogen sulphide, and filtered. Evaporation of the colourless filtrate *in vacuo* left the crude *hydrochloride* (3·28 g., 71%), which crystallised from ethyl acetate in colourless needles, m. p. *ca.* 153—155° (decomp.) dependent on the rate of heating (Found : C, 39·8; H, 7·8; S, 14·6; Cl, 16·7. $C_7H_{16}O_2NSCl$ requires C, 39·3; H, 7·6; S, 15·0; Cl, 16·6%). An aqueous solution neutralised with sodium hydrogen carbonate gave an intense purple colour with ferric chloride.

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