850. *Peptides. Part XII.*[1] *The Stereoisomers of 4-Methylproline.*

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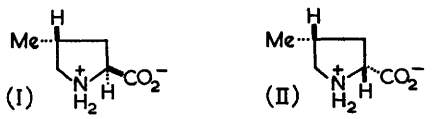
Both the *cis*- and the *trans*-isomer of 4-methyl-L-proline have been isolated from natural sources. *trans*-4-Methyl-L-proline (I) and the *cis*-D-isomer (II) have been synthesised, and their absolute configurations established by optical rotatory dispersion and enzymic methods. A convenient synthesis of the totally racemic amino-acid is also described.

The first description of 4-methylproline as a natural product is due to Hulme and Arthington.[2] Structure (I) (without the stereochemical connotations) was assigned to their product from young apple fruits on the basis of elemental analysis, colour tests characteristic of the pyrrolidine ring, and a chromatographic similarity to synthetic 4-methylproline. Subsequently, two of us isolated an amino-acid with similar properties from an antibiotic (I.C.I. 13,959),[3] and this was likewise tentatively regarded as 4-methylproline. However, in neither case was definite evidence obtained for the location of the methyl group, and later we showed that 3-, 4-, and 5-methylprolines were indistinguishable in a number of paper-chromatographic solvent systems. Further, it was found that methylproline isolated from perry, which was almost certainly identical with Hulme and Arthington's compound, differed in infrared spectrum and optical rotation from the amino-acid isolated from antibiotic 13,959. These results underlined the need for more rigorous identification of the natural amino-acids, a task complicated by their diastereoisomerism. We now show that the amino-acids from perry and antibiotic 13,959 are, respectively, the *trans*- (I) and the *cis*-isomer (mirror-image of II) of 4-methyl-L-proline, by synthesis of the diastereoisomers (I) and (II) of defined absolute configuration.



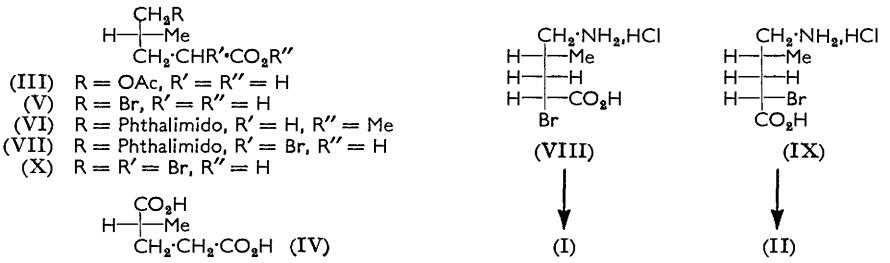
Several methods are available for the determination of the absolute configuration at the α-carbon of asymmetric α-amino-acids. These include enzymic methods,[4] measurement of the change in optical rotation on passing from neutral to acidic solution,[5] and correlations based on the anomalous optical rotatory dispersion of appropriate amino-acid derivatives (*e.g.*, dithiocarbamates [6] and ethoxythiocarbonyl derivatives [7]). However, determination of configuration at other asymmetric centres is less easy. For this reason we chose as starting material the methylpentanoic acid (III) in which the absolute configuration of the single asymmetric centre (destined to become C-4 of 4-methylproline) is known. Determination of the configuration at C-2 (C-α) in the product would then define its complete stereochemistry.

(+)-5-Acetoxy-4-methylpentanoic acid (III) is formed as a by-product in the industrial degradation of sapogenins.[8] Our sample, which was generously provided by Glaxo Laboratories Ltd., had been obtained from hecogenin (partial structure XIII) *via* ψ-hecogenin acetate. The absolute configuration of the acid (III) has been established by its conversion into (−)-α-methylglutaric acid (IV),[9] of defined configuration.[10] These

[1] Part XI, *Tetrahedron*, 1960, **11**, 39.
[2] Hulme and Arthington, *Nature*, 1952, **170**, 659; 1954, **173**, 588.
[3] Kenner and Sheppard, *Nature*, 1958, **181**, 48.
[4] Greenstein, *Adv. Protein Chem.*, 1954, **9**, 121.
[5] Neuberger, *Adv. Protein Chem.*, 1948, **4**, 297.
[6] Sjöberg, Fredga, and Djerassi, *J. Amer. Chem. Soc.*, 1959, **81**, 5002.
[7] Djerassi, Undheim, Sheppard, Terry, and Sjöberg, *Acta Chem. Scand.*, 1961, **15**, 903.
[8] Cameron, Evans, Hamlet, Hunt, Jones, and Long, *J.*, 1955, 2807.
[9] Bailey, Price, Horne, and Polgar, *J.*, 1959, 661.
[10] James, *J.*, 1955, 637.

assignments have now been confirmed by conversion of the acid (III) into 4-methylproline, and oxidation of the latter to (+)-methylsuccinic acid.

Cleavage of (+)-5-acetoxy-4-methylpentanoic acid (III) with a mixture of hydrobromic and sulphuric acid afforded (−)-5-bromo-4-methylpentanoic acid (V), which after esterification condensed smoothly with potassium phthalimide to yield (+)-methyl 4-methyl-5-phthalimidopentanoate (VI). The free acid obtained by acidic hydrolysis was treated with bromine and phosphorus, yielding the monobromo-acid (VII), $[\alpha]_D$ +26·1°. Little stereospecificity was expected in the bromination step which introduces a second asymmetric centre, and the product was shown to be a mixture of the diastereoisomers epimeric at C-2. Crystallisation of the derived brucine salt and decomposition with sodium hydroxide afforded the acid (VII) with enhanced optical rotation, $[\alpha]_D$ +46·6°, while a third form, $[\alpha]_D$ +10°, was obtained from the original mother-liquors. However, separation of diastereoisomers at this stage was of no synthetic advantage, since removal of the phthaloyl protecting group by acidic hydrolysis resulted in extensive racemisation at C-2. The crude 5-amino-2-bromo-4-methylpentanoic acid hydrochloride (VIII + IX) thus obtained had $[\alpha]_D$ +14—15° regardless of the optical rotation of the starting phthaloyl derivative.



Initially this amino-acid hydrochloride was obtained as a non-crystallisable oil, and its cyclisation to 4-methylproline was examined without extensive purification or separation of the stereoisomers (VIII) and (IX). Cyclisation in aqueous sodium hydroxide and isolation through the hydrochloride yielded a crystalline mixture of the 4-methylproline-diastereoisomers (I) and (II). The composition of this mixture varied slightly from experiment to experiment. On one occasion a specific rotation of −4° was found, which in the light of the constants subsequently obtained for the pure amino-acids, corresponded to a 20% excess of the *trans*-isomer (I). Normally, however, the optical rotation (*ca.* +12°) corresponded more closely to an equimolecular mixture of (I) and (II), and the rotation was unchanged on repeated crystallisation.

A shorter but less efficient route to the mixture of methylproline isomers passed through the *N*-toluene-*p*-sulphonyl derivatives. Treatment of the acetoxy-acid (III) with bromine and phosphorus resulted in simultaneous α-bromination and replacement of the acetoxyl group. The product, 2,5-dibromo-4-methylpentanoic acid (X) was esterified and condensed with potassium toluene-*p*-sulphonamide, affording crystalline 4-methyl-*N*-toluene-*p*-sulphonylproline methyl ester, again a mixture of diastereoisomers epimeric at C-2. Removal of the ester and tosyl groups afforded the methylproline mixture (I + II), $[\alpha]_D$ +16°.

Two of many attempts to separate this mixture of diastereoisomers were partly successful, and provided the first synthetic samples of pure *trans*-4-methyl-L-proline (I). In reaction of the mixed methylproline isomers with copper carbonate, it was observed that the copper ion appeared to bind preferentially one diastereoisomer of each type, thus removing an equal number of the *cis*- and *trans*-molecules from solution. A predominance of either isomer then combines with copper ion, forming either pure *cis*- or pure *trans*-copper salts. These copper salts, containing two molecules of one

isomer, were found to be very much more soluble in ethanol than the salt containing one molecule of each of the diastereoisomers of 4-methylproline, and hence they could be separated readily. Thus, provided that the original mixture of isomers contained an excess of one form or the other, this form could be separated by means of its soluble copper salt. When the mixture of isomers, $[\alpha]_D$ −4°, was treated in this manner, and the ethanol-soluble copper derivative was decomposed by passage through Dowex-50 (NH_4^+) cation-exchange resin, there was obtained a 17% yield of *trans*-4-methyl-L-proline (I), $[\alpha]_D$ −50°, which was readily recrystallised to a constant optical rotation of −59·9° (in H_2O). The L-configuration of this product, and hence the complete stereochemistry depicted in (I), was inferred initially from the positive shift in optical rotation on passing from neutral to acidic solution [5] (Δ = +33°; cf. L-proline, Δ = +32°; hydroxy-L-proline, Δ = +28°). Further evidence on this point is presented below.

A second method for the isolation of *trans*-4-methyl-L-proline (I) (which did not require initial enrichment of the mixture of isomers) was found in the use of the enzyme D-amino-acid oxidase. This enzyme catalyses the oxidation of D-amino-acids (including D-proline) to the corresponding α-keto-acids, and has previously been used both for the resolution and stereochemical assignment of amino-acids.[4] Oxidation of the mixed methylproline isomers with oxygen in the presence of this enzyme yielded the *trans*-isomer (I), $[\alpha]_D$ −50°, identical with the crude product obtained previously. This result confirms the assignment of the L-configuration to the amino-acid obtained *via* the soluble copper salt.

At this stage we returned to the possible separation of isomers before cyclisation. The immediate precursor of 4-methylproline, 5-amino-2-bromo-4-methylpentanoic acid hydrochloride (VIII + IX), had been obtained previously only as a viscous oil, $[\alpha]_D$ +15°, which could not be satisfactorily purified. However, a preparation which was carefully freed from all traces of excess hydrochloric acid crystallised, and recrystallisation afforded a single stereoisomer (VIII) in 45% yield, whose $[\alpha]_D$ +31·6° was unchanged on further recrystallisation. Subsequent preparations of this compound crystallised without difficulty. Cyclisation of the crystalline bromo-acid (VIII) with either aqueous sodium hydroxide or barium hydroxide proceeded stereospecifically, and yielded directly 67% of *trans*-4-methyl-L-proline (I), $[\alpha]_D$ −57°, corresponding to 98% optical purity.

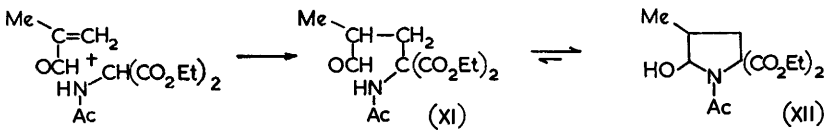
The bromo-acid of $[\alpha]_D$ +31·6° may be assigned the configuration depicted in (VIII) since inversion at C-2 is expected in the cyclisation. This follows from the observations by Hamilton,[11] that L-ornithine may be converted into D-proline through an intermediate 5-amino-2-bromopentanoic acid, and that the initial stage, treatment of the amino-acid with nitrosyl bromide, proceeds with retention of configuration. Accordingly, the crystallisation liquors of (VIII) must have been heavily enriched in the epimeric α-bromo-acid (IX). Cyclisation of the crude product obtained by evaporation of these liquors yielded methylproline of $[\alpha]_D$ +31° which after purification through the copper salt furnished pure *cis*-4-methyl-D-proline (II), $[\alpha]_D$ +85°.

Final confirmation of the absolute stereochemistry assigned to the synthetic amino-acids comes from the optical rotatory dispersion curves of their dithiocarbamate derivatives. Djerassi and his collaborators [6,7] have recently developed a useful method for the determination of configuration of α-amino-acids, based on the anomalous optical rotatory dispersion properties ("Cotton effects") of appropriate ultraviolet-absorbing derivatives. The *N*-alkylthio(thiocarbonyl) derivatives (dithiocarbamates) appear to be the most generally useful. The range of amino-acids so far examined (including L-proline,[6] hydroxy- and allohydroxy-L-proline [7]) suggests that only the asymmetry at the α-carbon atom is important in determining the sign of the Cotton effect curve, positive curves being associated with L-amino-acid derivatives. In agreement with these generalisations, the dithiocarbamates of the synthetic L- (I) and D-amino-acid (II) showed, respectively, positive and negative Cotton effect dispersion curves of very similar amplitude.

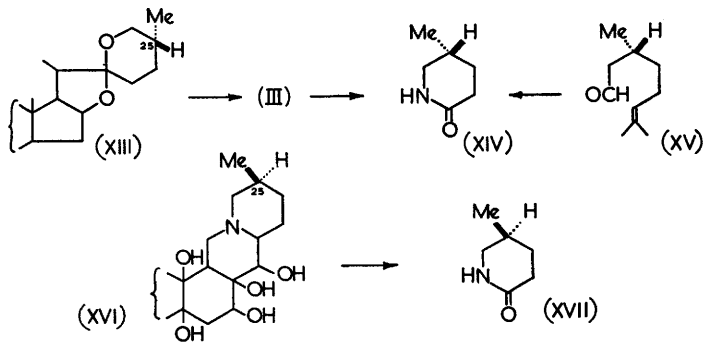
[11] Hamilton, *J. Biol. Chem.*, 1952, **198**, 587.

With both stereoisomers in hand, we were able to establish finally that the methylprolines isolated from both perry and antibiotic 13,959 were indeed the 4-methyl derivatives, and to define their complete stereochemistry. 4-Methylproline from perry was identical with the synthetic *trans*-L-isomer (I).[12] The synthetic *cis*-4-methyl-D-proline (II) could be obtained in dimorphic forms with different (solid state) infrared spectra but identical nuclear magnetic resonance spectra * (in D_2O solution). One form of synthetic *cis*-4-methyl-D-proline was identical in infrared spectrum with that of the natural amino-acid from antibiotic 13,959, but the optical rotations were of opposite sign. This second natural amino-acid is therefore *cis*-4-methyl-L-proline.

For some limited purposes the procurement of optically pure amino-acids is unnecessary. We have therefore also developed a convenient two-stage synthesis of 4-methylproline, which provided the totally racemic amino-acid hydrochloride in 46% yield.† 2-Methylacraldehyde and diethyl acetamidomalonate afforded the cyclic adduct (XII), characterised as the phenylhydrazone of the tautomeric aldehyde (XI). Reduction of the adduct with tin and hydrochloric acid simultaneously removed the acetyl and the ester groups and decarboxylated the resulting malonic acid derivative. The 4-methylproline was conveniently isolated as its hydrochloride.



One of the early routes envisaged for the synthesis of 4-methylproline involved the initial conversion of the acetoxy-acid (III) into the methylpiperidone (XIV). This route was in fact superseded by those described above, and conversion of the piperidone (XIV) into 4-methylproline was not attempted. However, the preparation of this compound (XIV) is recorded here since it confirms a number of stereochemical correlations described in the



literature. The stereoidal alkaloid cevine (partial structure XVI) has been oxidised to (—)-5-methyl-2-piperidone (XVII),[15] the absolute configuration of which (and hence of cevine itself) was deduced by synthesis of its enantiomer (XIV) from citronellal (XV).[16]

* The nuclear magnetic resonance spectra of *cis*- and *trans*-4-methylproline have been described elsewhere in another connection.[13]

† Totally racemic 4-methylproline was first obtained by Dakin[14] during attempts to prepare δ-hydroxyleucine. The racemic amino-acid has also been synthesised by Drs. G. W. Alderson and V. M. Clark (University of Cambridge) whose results will be published separately.

[12] Burroughs, Dalby, Kenner, and Sheppard, *Nature*, 1961, **189**, 394.
[13] Abraham, McLauchlan, Dalby, Kenner, Sheppard, and Burroughs, *Nature*, 1961, **192**, 1150.
[14] Dakin, *J. Biol. Chem.*, 1946, **164**, 615.
[15] Craig and Jacobs, *J. Biol. Chem.*, 1941, **141**, 258, 267.
[16] Jeger, Prelog, Sundt, and Woodward, *Helv. Chim. Acta*, 1954, **37**, 2302.

This same (+)-piperidone has now been obtained from the acetoxy-acid (III) by amination of the methyl ester of the derived bromo-acid (V). These transformations, therefore, demonstrate the identical configurations of C-25 in hecogenin (XIII) (a steroidal sapogenin of the iso-series) and the terpene citronellal (XV), and the enantiomeric relationship with C-25 of the alkaloid cevine (XVI), and they confirm the absolute configurations previously proposed.[10,16]

Experimental

The stereochemistry of compounds *other than α-amino-acids* is specified by the (*R*)-, (*S*)-system of Cahn, Ingold, and Prelog.[17]

Isolation of 4-Methylproline from Perry (with L. F. Burroughs).—In a chromatographic survey of 19 perries, only one (a seedling variety of the Huffcap type) contained a detectable amount of 4-methylproline. Ethanol was added to the perry (5 l.) to a concentration of 35% v/v, and all the amino-acids were adsorbed on a column of ZeoKarb 225 cation-exchange resin (H^+ form; 700 milliequiv.). The column was washed with N-aqueous ammonia (1 l.), the eluate was concentrated *in vacuo* to 100 ml., and the amino-acids were re-adsorbed on a column (42 × 1 cm.) of Dowex 2 × 10 anion-exchange resin (OH^- form). The amino-acids were displaced with 0·1N-hydrochloric acid (0·4 ml./min.; 3-ml. fractions). Fractions 36—49 contained 4-methylproline, proline, 4-hydroxymethylproline,* α- and β-alanine, γ-amino-butyric acid, and three other trace constituents. The residue (400 mg.) from evaporation of fractions 36—49 was dissolved in N-hydrochloric acid (10 ml.) and applied to a column (62 × 3 cm.) of Dowex 50 × 4 cation-exchange resin (H^+ form). The column was washed with N-hydrochloric acid (0·5 ml./min.; 10-ml. fractions). Fractions 225—336 contained 4-methylproline and reduced amounts of proline and 4-hydroxymethylproline, and the residue from evaporation (64·7 mg.) was re-chromatographed on the same column. Fractions 263—289 contained only 4-methylproline (12·4 mg.). The amino-acid was dissolved in dilute acetic acid and liberated from its hydrochloride by passage through a column of Dowex 1 × 4 anion-exchange resin (acetate form). Recrystallisation from ethanolic ether afforded *trans*-4-methyl-L-proline (5 mg.) as colourless needles, $[\alpha]_D^{16\cdot5}$ −52° (*c* 0·35 in H_2O), $[\alpha]_D^{19}$ −21° (*c* 0·19 in 3N-hydrochloric acid). The infrared spectrum was identical with that of synthetic *trans*-4-methyl-L-proline described below.

Isolation of 4-Methylproline from Antibiotic I.C.I. 13,959.—The antibiotic (0·95 g.) was hydrolysed with 6N-hydrochloric acid (25 ml.) in a sealed tube at 100—105° for 48 hr. The hydrolysate was diluted, filtered, and washed with ether, and the aqueous layer was evaporated to dryness. The residue was applied in N-hydrochloric acid (20 ml.) to a column of ZeoKarb 225 × 8 cation-exchange resin (H^+ form; 10·5 × 2·8 cm.), which was washed with N-hydrochloric acid (10-ml. fractions; 0·5 ml./min.). Fractions 54—102 contained 4-methylproline and leucine, and the residue from evaporation (303 mg.) was rechromatographed on a second column of Dowex 50 × 4 cation-exchange resin (H^+ form; 62 × 3 cm.). Fractions 292—334 (120 mg.) contained only 4-methylproline, which was recovered from its hydrochloride as previously described. *cis*-4-Methyl-L-proline, crystallised from ethanolic ether, had $[\alpha]_D^{18}$ −83° (*c* 0·53 in H_2O), $[\alpha]_D^{18}$ −43° (*c* 0·51 in 3N-hydrochloric acid). The infrared spectrum was identical with that of synthetic *cis*-4-methyl-D-proline described below.

(*R*)-5-*Bromo*-4-*methylpentanoic Acid* (V).—A mixture of 5-acetoxy-4-methylpentanoic acid (42 g.), 48% aqueous hydrobromic acid (163 ml.), and concentrated sulphuric acid (20 ml.) was heated at 90° for 7 hr. After cooling, the solution was poured into water, and the product isolated with ether. Distillation yielded the bromo-acid (28·3 g., 60%), b. p. 96—100°/0·2 mm., $[\alpha]_D^{18}$ −1·79° (*c* 14·9 in ether). The *dicyclohexylammonium salt*, crystallised from benzene–light petroleum, had m. p. *ca.* 80° (decomp.) (Found: C, 57·5; H, 9·1; Br, 21·6; N, 3·6. $C_{18}H_{34}BrNO_2$ requires C, 57·45; H, 9·1; Br, 21·2; N, 3·7%).

(*R*)-*Methyl* 5-*Bromo*-4-*methylpentanoate*.—The foregoing bromo-acid (20 g.) was heated under reflux with methanol (200 ml.) and concentrated sulphuric acid (2 ml.) for 20 hr. The solution was concentrated to 100 ml. and poured into saturated aqueous sodium hydrogen

* The structure of this further derivative of proline has been established recently by both nuclear magnetic resonance spectroscopy [13] and mass spectrometry.[18]

[17] Cahn, Ingold, and Prelog, *Experientia*, 1956, **12**, 81.
[18] Biemann, Deffner, and Steward, *Nature*, 1961, **191**, 380.

carbonate solution (200 ml.). Distillation of the product, isolated with ether, yielded the *methyl ester* (16 g., 80%), b. p. 110—112°/20 mm., $[\alpha]_D^{18}$ 0° (*c* 17·7 in EtOH) (Found: C, 40·35; H, 6·3; Br, 37·8. $C_7H_{13}BrO_2$ requires C, 40·2; H, 6·3; Br, 38·2%). When the methyl ester was prepared from (III) without purification of the intermediate bromo-acid, the overall yield was 60%.

(*R*)-*Methyl* 4-*Methyl*-5-*phthalimidopentanoate* (VI).—A stirred mixture of the foregoing bromo-ester (14·2 g.), potassium phthalimide (13·0 g.), and dimethylformamide (90 ml.) was warmed at 90° for 3 hr. The cooled solution was poured into water and extracted with chloroform (2 × 75 ml.). The extracts were washed with 0·1N-sodium hydroxide and water, dried (Na_2SO_4), and evaporated. The residual oil crystallised on trituration with iced water and yielded the *phthalimido-derivative*, m. p. 41—42° (18 g., 98%). A sample recrystallised from cold aqueous ethanol had m. p. 43—44°, $[\alpha]_D^{20}$ +15·3° (*c* 3·97 in EtOH) (Found: C, 65·3; H, 6·2; N, 5·1. $C_{15}H_{17}NO_4$ requires C, 65·4; H, 6·2; N, 5·1%).

(*R*)-4-*Methyl*-5-*phthalimidopentanoic Acid*.—The methyl ester (16·8 g.) was hydrolysed by 2N-hydrochloric acid (120 ml.) under reflux for 2½ hr. On cooling to 0°, an oil, which slowly crystallised, separated. The solid product was collected and partitioned between aqueous sodium hydrogen carbonate solution and chloroform. Acidification of the aqueous layer yielded the *phthalimido-acid* (11·5 g., 72%), m. p. 80—81° unchanged on recrystallisation from aqueous methanol, $[\alpha]_D^{14}$ +15·0° (*c* 8·28 in EtOH) (Found: C, 64·5; H, 5·7; N, 5·4. $C_{14}H_{15}NO_4$ requires C, 64·4; H, 5·8; N, 5·4%).

(2*RS*,4*R*)-2-*Bromo*-4-*methyl*-5-*phthalimidopentanoic Acid* (VII).—A solution of bromine (24 g.) in carbon tetrachloride (50 ml.) was added dropwise in 15 min. to 4-methyl-5-phthalimidopentanoic acid (9·5 g.) and red phosphorus (0·7 g.) in carbon tetrachloride (50 ml.). The mixture was heated under reflux for 4 hr., the carbon tetrachloride and excess of bromine were removed by evaporation *in vacuo*, and ice (*ca.* 150 g.) was added to the oily residue The mixture crystallised when agitated at 0° for 2 hr. and the product (13·8 g.), m. p. 125—132°, $[\alpha]_D^{17}$ +24·4° (*c* 1·48 in EtOH), was collected. Recrystallisation from benzene yielded colourless prisms of the bromo-acid (6·5 g.), m. p. 142—144°, $[\alpha]_D^{19}$ +26·1° (*c* 2·16 in EtOH); a second crop (2·6 g.; total 9·1 g., 72%) had m. p. 136—138°, $[\alpha]_D^{19}$ +29·0° (*c* 1·64 in EtOH). A sample, crystallised once more for analysis, had m. p. 141—142° (Found: C, 49·5; H, 4·2; Br, 23·3; N, 3·8. Calc. for $C_{14}H_{14}BrNO_4$: C, 49·3; H, 4·1; Br, 23·4; N, 4·1%). From one preparation, a third crop of colourless needles, m. p. 149—150°, $[\alpha]_D^{20}$ +10° (*c* 1·41 in EtOH), was obtained (Found: C, 49·6; H, 4·1; Br, 23·35; N, 3·8%).

Brucine 2-*Bromo*-4-*methyl*-5-*phthalimidopentanoate*.—A solution of brucine dihydrate (12·5 g.) in 2-methoxyethanol (30 ml.) was warmed to 90°, and 2-bromo-4-methyl-5-phthalimidopentanoic acid (10 g.; $[\alpha]_D^{19}$ +30°) was added. The solution was set aside at room temperature for 2 hr., and a few drops of water and more 2-methoxyethanol (20 ml.) were added. Crystallisation commenced immediately, and the product (11·4 g.), m. p. 120—122°, $[\alpha]_D^{17}$ +10·0° (*c* 2·25 in 2-methoxyethanol), was collected after 30 min. Recrystallisation to constant optical rotation yielded the pure *salt* (4·5 g.), $[\alpha]_D^{16}$ +11·6° (*c* 1·79 in 2-methoxyethanol) (Found: C, 57·8; H, 5·75; N, 5·2. $C_{37}H_{40}BrNO_8,2H_2O$ requires C, 57·7; H, 5·7; N, 5·45%).

Decomposition of the Brucine Salt.—The foregoing salt (4·5 g.) was shaken with N-aqueous sodium hydroxide (7 ml.) and chloroform (15 ml.). The aqueous layer was separated, washed with chloroform, and acidified. The precipitated product, m. p. 139—141°, was collected and recrystallised to constant optical rotation, yielding (2*R* or *S*,4*R*)-2-*bromo*-4-*methyl*-5-*phthalimidopentanoic acid* (377 mg.), m. p. 142—144°, $[\alpha]_D^{18}$ +45·8° (*c* 0·85 in EtOH) (Found: C, 49·3; H, 4·4; Br, 23·8; N, 4·0%).

(2*R*,4*R*)-5-*Amino*-2-*bromo*-4-*methylpentanoic Acid Hydrochloride* (VIII).—(2*RS*,4*R*)-2-Bromo-4-methyl-5-phthalimidopentanoic acid, $[\alpha]_D$ +26° (three 10-g. batches), was heated under reflux with 6N-hydrochloric acid (3 × 140 ml.) during 17 hr. Phthalic acid was removed by filtration and ether-extraction of the combined solutions, which were then evaporated *in vacuo*, finally at 60°/0·2 mm. The crude mixture of isomers (VIII and IX), $[\alpha]_D^{18}$ +14·9° (*c* 2·2 in H_2O), crystallised after 40 hr. at room temperature, and recrystallisation from propan-2-ol–ether yielded (2*R*,4*R*)-5-amino-2-bromo-4-methylpentanoic acid hydrochloride (9·8 g., 45%), m. p. 145—146°, $[\alpha]_D^{19}$ +31·6° (*c* 2·1 in H_2O). The m. p. and optical rotation were unchanged on recrystallisation, but the elemental analyses revealed the presence of the 2-chloro-compound, which could very likely be formed during hydrolysis of the phthaloyl group (Found: C, 30·6; H, 5·4; Br, 28·7; Cl, 16·7; N, 5·6. Calc. for $C_6H_{13}BrClNO_2$: C, 29·3; H, 5·3; Br, 32·4; Cl,

14·4; N, 5·7%). The mother-liquors from the crystallisation of (VIII) were carefully treated with ether until no more crystals were obtained. Evaporation of the filtered solution yielded the crude (2*S*)-bromo-isomer (IX) (12 g.) as an oil, $[\alpha]_D^{20}$ +7·3° (*c* 8·9 in H_2O).

4-*Methylproline* (*Mixture of Diastereoisomers* I *and* II).—A solution of the crude (2*RS*,4*R*)-5-amino-2-bromo-4-methylpentanoic acid hydrochloride, $[\alpha]_D^{18}$ +14·9° (5 g.), in water (20 ml.) containing sodium hydroxide (5 g.) was set aside for 4 days at room temperature. The solution was then acidified with hydrochloric acid and evaporated *in vacuo*. The residue was extracted with boiling ethanol, and the extracts were evaporated. The residue was heated under reflux for 30 min. with concentrated hydrochloric acid (to hydrolyse traces of ethyl ester formed during the extraction procedure), and the solution was evaporated *in vacuo*. Recrystallisation of the residue from ether–propan-2-ol afforded 4-methylproline hydrochloride (2·76 g.) as colourless plates, m. p. 156—160°. A solution of the hydrochloride in dilute acetic acid (10 ml.) was poured through a column of Dowex 1 × 4 anion-exchange resin (acetate form), and the column was washed with dilute acetic acid until no more ninhydrin-reacting material was eluted. Evaporation of the total eluate and recrystallisation of the residue from ethanol–ether yielded 4-methylproline (I and II), m. p. 232—234° (decomp.; chars at 210°) (1·65 g., 43%) (Found: C, 56·0; H, 8·7; N, 10·7. Calc. for $C_6H_{11}NO_2$: C, 55·8; H, 8·6; N, 10·85%). The chloroacetyl derivative crystallised from cyclohexane–diethyl carbonate, then having m. p. 107—108°, $[\alpha]_D^{18}$ 20·3° (*c* 1·4 in EtOH) (Found: C, 47·0; H, 5·75; Cl, 17·3; N, 6·6. Calc. for $C_8H_{12}ClNO_3$: C, 46·8; H, 5·8; Cl, 17·3; N, 6·8%).

trans-4-*Methyl*-L-*proline* (I).—Crystalline (2*R*,4*R*)-5-amino-2-bromo-4-methylpentanoic acid hydrochloride (VIII), $[\alpha]_D$ +31·6° (5 g.), was added to a hot solution of barium hydroxide (9 g.) in water (500 ml.). The mixture was heated under reflux for 10 min., cooled, and filtered. The filtrate was shaken with freshly precipitated silver oxide and filtered, before addition of dilute sulphuric acid and further filtration. The residue from evaporation of the filtrate was dissolved in dilute aqueous ammonia (15 ml.) and passed through a column of Dowex 50 cation-exchange resin (NH_4^+ form). The column was washed with dilute ammonia solution until samples spotted on filter paper and dried gave a negative ninhydrin reaction, and the total eluate was then evaporated. The crystalline residue (2·4 g.) recrystallised from ethanol–ether, yielding colourless needles of trans-4-*methyl*-L-*proline* (1·6 g., 67%), m. p. 239—240° (decomp.), $[\alpha]_D^{18}$ −56·6° (*c* 1·03 in H_2O), $[\alpha]_D^{20}$ −23·9° (*c* 1·54 in 3N-hydrochloric acid), $\nu_{max.}$ 2941, 2732, 2392, 1613, 1570, 1456, 1443, 1389, 1351, 1321, 1309, 1285, 1244, 1226, 1172, 1139, 1103, 1037, 1003, 974, 936, 915, 864, 850, 831, 800, and 691 $cm.^{-1}$ (Found: C, 55·4; H, 8·4; N, 10·5%). Cyclisation in aqueous sodium hydroxide at room temperature for 4 days similarly yielded the *trans*-amino-acid (60%), $[\alpha]_D$ −54° (*c* 1·2 in H_2O).

Separation of trans-4-*Methyl*-L-*proline* via *its Copper Salt*.—The mixture of methylproline isomers (I + II) (1·36 g.), $[\alpha]_D$ −3·8°, was dissolved in water (70 ml.), and copper carbonate (1·6 g.) was added. The suspension was heated under reflux for 2 hr., then filtered hot, and the filter pad was washed with warm water. The filtrate and washings were combined and evaporated, finally at 100°/0·2 mm. The residue was treated with warm ethanol (50 ml.) and filtered and the filtrate was evaporated. The yield of crude *trans*-4-methyl-L-proline copper salt was 275 mg. The copper salt was decomposed by passage of its solution in dilute aqueous ammonia through a column of Dowex 50 cation-exchange resin (NH_4^+ form) and elution with aqueous ammonia. Evaporation yielded a crystalline residue (233 mg.), $[\alpha]_D^{22}$ −49·7° (*c* 0·43 in H_2O), which was recrystallised from ethanol–ether to constant optical rotation, $[\alpha]_D^{23}$ −59·9° (*c* 0·24 in H_2O) (Found: C, 55·65; H, 8·6; N, 11·0%). The insoluble copper salt, decomposed in a similar manner, yielded 4-methylproline (820 mg.), $[\alpha]_D^{23}$ +13° (*c* 1·62 in H_2O).

Action of D-*Amino-acid Oxidase on* 4-*Methylproline*.—A preparation of hog-kidney D-amino-acid oxidase (Nutritional Biochemicals Corporation, Cleveland, Ohio, 15 units/mg.) (175 mg.), suspended in 0·1M-pyrophosphate buffer of pH 6·8 (2 ml.), was added to a solution of 4-methylproline (I + II), $[\alpha]_D$ +12° (258 mg.), and potassium cyanide (6 mg.) in water (8 ml.). One drop of octanol was added to prevent frothing, and a gentle stream of oxygen was bubbled through the mixture for 24 hr. at 37°, the pH being maintained at 8·2 by gradual addition of 0·1N-lithium hydroxide. The mixture was then acidified to pH 5 and dialysed against 4 changes of water (100 ml. each). The combined dialysates were concentrated to 10 ml., and the solution was desalted by adsorption of the amino-acid successively on Dowex 50 × 4 (H^+ form) (eluted with dilute ammonia solution), and Dowex 1 × 4 (OH^- form) (eluted with dilute acetic acid) ion-exchange resins. The residue from evaporation of the final eluate was extracted with

ethanol, and the ethanol-soluble part was recrystallised three times from ethanol–ether. The resulting *trans*-4-methyl-L-proline had $[\alpha]_D^{11}$ −49·6° (*c* 0·73 in H_2O) and was identical in infra-red spectrum with the product described above.

cis-4-*Methyl*-D-*proline* (II).—The crude 5-amino-2-bromo-4-methylpentanoic acid hydrochloride (IX), $[\alpha]_D$ +7·3° (12 g.), in water (50 ml.) was added to a hot solution of barium hydroxide (21 g.) in water (800 ml.), and the mixture was heated under reflux for 10 min. The cooled solution was filtered, shaken with freshly precipitated silver oxide, filtered, and treated with dilute sulphuric acid until precipitation of the barium was complete. The filtrate was concentrated to *ca.* 10 ml., filtered again, and evaporated to dryness. The crystalline residue, $[\alpha]_D^{17}$ +30·6°, was combined with the residues from three additional experiments (total 15 g.) and heated under reflux with water (500 ml.) and copper carbonate (30 g.) for 45 min. The mixture was filtered hot and the filtrate evaporated to dryness. The residual copper salt (18 g.) was extracted with warm ethanol (200 ml.), which was filtered and evaporated. The ethanol-soluble copper salt was dissolved in dilute aqueous ammonia and passed through a column of Dowex 50 (NH_4^+) ion-exchange resin, which was washed with dilute aqueous ammonia. Evaporation of the total eluate yielded a crystalline residue (6 g.); recrystallisation from ethanol–ether yielded cis-4-*methyl*-D-*proline* (3·5 g.), m. p. 238—239° (decomp.), $[\alpha]_D^{20}$ +85·2° (*c* 1·68 in H_2O), $[\alpha]_D^{20}$ +47·9° (*c* 1·28 in 3N-hydrochloric acid), $\nu_{max.}$ (Nujol mull) 2941, 2421, 1623, 1582, 1451, 1379, 1351, 1323, 1289, 1250, 1224, 1168, 1140, 1110, 1079, 1045, 1030, 984, 960, 935, 919, 877, 833, 779, and 677 cm.$^{-1}$ (Found: C, 55·5; H, 8·5; N, 10·8%). A second crop (500 mg.) was obtained in a dimorphic form, m. p. 237—238°, $[\alpha]_D^{20}$ +88·4° (*c* 1·37 in H_2O), $[\alpha]_D^{20}$ +47° (*c* 1·15 in 3N-hydrochloric acid, $\nu_{max.}$ 2907, 2404, 1623, 1582, 1453, 1376, 1333, 1256, 1229, 1176, 1142, 1111, 1038, 1013, 992, 955, 941, 884, 832, 771, and 685 cm.$^{-1}$) (Found: C, 55·4; H, 8·3; N, 10·6%). This polymorph slowly reverted to the stable form above.

trans-4-*Methyl*-1-*methylthio*(*thiocarbonyl*)-L-*proline*.—Carbon disulphide (80 mg.) was added to an ice-cold solution of *trans*-4-methyl-L-proline (128 mg.) in water (3 ml.) and N-sodium hydroxide (2 ml.). After the mixture had been shaken at room temperature for 40 min., methyl iodide (180 mg.) was added and shaking was continued for 2 hr. further. After being washed with ether, the aqueous solution was acidified, and the product (175 mg.) was isolated with ether. Crystallisation from chloroform–light petroleum yielded the *methylthio*(*thiocarbonyl*) *derivative*, m. p. 84° (Found: C, 43·6; H, 6·1; N, 6·45. $C_8H_{13}NO_2S_2$ requires C, 43·8; H, 6·0; N, 6·4%). R.D. (MeOH), *c* 0·12, $[\alpha]_{589}$ −40°, $[\alpha]_{355}$ +710°, $[\alpha]_{315}$ −2980°.

cis-4-*Methyl*-1-*methylthio*(*thiocarbonyl*)-D-*proline*.—Prepared in a similar manner, this *derivative* had m. p. 115—117° (Found: C, 44·1; H, 6·0; N, 6·2%). R.D. (MeOH), *c* 0·086 (0·0086 below 310 mμ), $[\alpha]_{589}$ +46°, $[\alpha]_{351}$ −1185°, $[\alpha]_{300}$ +2800°.

(2*RS*,4*R*)-2,5-*Dibromo*-4-*methylpentanoic Acid* (X).—Bromine (40 ml.), diluted with carbon tetrachloride (40 ml.), was added dropwise to a stirred mixture of 5-acetoxy-4-methylpentanoic acid (34·8 g.), red phosphorus (2·8 g.), and carbon tetrachloride (50 ml.). The solution was then heated under reflux for 4 hr., and after evaporation the residual liquid was poured into water. The oil which separated was extracted into ether, which was washed with water, dried ($MgSO_4$), and evaporated. The residual dibromo-acid (55 g.) was sufficiently pure for use in the next stage. A sample was distilled for analysis at 160—170° (bath-temp.)/15 mm. (Found: C, 26·05; H, 3·9. Calc. for $C_6H_{10}Br_2O_2$: C, 26·3; H, 3·65%).

(2*RS*,4*R*)-*Methyl* 2,5-*Dibromo*-4-*methylpentanoate*.—(*a*) The above dibromo-acid (20 g.) was heated under reflux with methanol (200 ml.) containing concentrated sulphuric acid (2 ml.) for 18 hr. The solution was concentrated to 100 ml. and poured into aqueous sodium hydrogen carbonate solution (200 ml.). The product was isolated with ether, and distillation yielded the dibromo-ester (6·5 g., 26% from III), b. p. 84—86°/15 mm. (Found: C, 29·0; H, 4·5. Calc. for $C_7H_{12}Br_2O_2$: C, 29·2; H, 4·2%).

(*b*) 5-Bromo-4-methylpentanoic acid (V) (28·5 g.) was dissolved in thionyl chloride (25 ml.), and the solution was heated under reflux until evolution of hydrogen chloride ceased (40 min.). A mixture of bromine (24 g.) and thionyl chloride (10 ml.) was then added dropwise to the boiling solution, which was then boiled for a further 4 hr. Thionyl chloride and the excess of bromine were evaporated, and the residue was poured into methanol (200 ml.). Next morning the methanolic solution was poured into aqueous sodium hydrogen carbonate solution (300 ml.). The product was isolated with ether, and distillation yielded the dibromo-ester (29 g., 76%), b. p. 87—89°/20 mm.

4-*Methyl*-1-*toluene*-p-*sulphonylproline*.—Methyl (2*RS*,4*R*)-2,5-dibromo-4-methylpentanoate

(29 g.), potassium toluene-*p*-sulphonamide (21 g.), and dry dimethylformamide (120 ml.) were stirred at 90° for 4½ hr. A solution of sodium methoxide (6 g.) in methanol was then added, and the mixture was kept at 90° for a further 2½ hr. After evaporation of the solvent *in vacuo*, the residue was partitioned between ethyl acetate and water. The organic phase was washed successively with 2N-aqueous sodium hydroxide, dilute hydrochloric acid, and water. Evaporation of the dried ($MgSO_4$) ethyl acetate solution and recrystallisation of the residue from benzene–light petroleum yielded lustrous plates of the toluene-*p*-sulphonyl methyl ester (5·5 g., 17%), m. p. 71—73°. A sample recrystallised once further had m. p. 74—75°, $[\alpha]_D^{16}$ +14·2° (*c* 1·82 in EtOH) (Found: C, 56·6; H, 6·6; N, 4·5; S, 11·1. Calc. for $C_{14}H_{19}NO_4S$: C, 56·6; H, 6·4; N, 4·7; S, 10·8%). Saponification of this ester with sodium hydroxide (1·25 equiv.) in boiling aqueous dioxan during 2 hr. and isolation in the usual manner afforded 4-methyl-1-toluene-*p*-sulphonylproline (62%), m. p. 129—130°, $[\alpha]_D^{20}$ +16·9° (*c* 1·9 in EtOH) (Found: C, 55·2; H, 6·1; N, 4·75; S, 11·5. Calc. for $C_{13}H_{17}NO_4S$: C, 55·1; H, 6·0; N, 4·95; S, 11·3%). Removal of the toluene-*p*-sulphonyl group with hydrogen bromide in acetic acid [19] yielded 4-methylproline (I + II), $[\alpha]_D$ +16° (*c* 1·65 in H_2O).

Diethyl 1-Acetyl-5-hydroxy-4-methylpyrrolidine-2,2-dicarboxylate (XII).—2-Methylacraldehyde (0·4 g.) was added to a solution of diethyl acetamidomalonate (1 g.) in benzene (25 ml.) containing sodium methoxide (5 mg.), and the solution was kept at room temperature for 2 hr. After acidification with acetic acid (a few drops) and filtration, the solution was evaporated *in vacuo* to yield the oily pyrrolidine derivative (1·3 g.), $\nu_{max.}$ (liquid film) 3333, 2959, 1748, 1639, 1437, 1389, 1361, 1342, 1285, 1242, 1199, 1152, 1119, 1094, 1053, 1029, 919, 865, 746, and 682 $cm.^{-1}$. With phenylhydrazine hydrochloride and sodium acetate in the usual manner, the pyrrolidine yielded *diethyl α-acetamido-α-(2-methyl-3-phenylhydrazonopropyl)-malonate* as colourless prisms, m. p. 150—151° (Found: C, 60·5; H, 7·1; N, 11·3. $C_{19}H_{27}N_3O_5$ requires C, 60·5; H, 7·2; N, 11·1%).

4-Methylproline Hydrochloride (Totally Racemic).—The foregoing pyrrolidine derivative (1·07 g.) was heated under reflux for 45 min. with 6N-hydrochloric acid (20 ml.) and granulated tin (1·8 g.). The excess of tin was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. Hydrogen sulphide was bubbled through a solution of the residue in warm water until precipitation of stannic sulphide was complete. Evaporation *in vacuo* of the filtered solution left a colourless crystalline residue (500 mg.), which was recrystallised from propan-2-ol–ether, yielding totally racemic 4-methylproline hydrochloride (280 mg., overall yield 46%), m. p. 192—193° (lit.,[20] m. p. 195°) (Found: C, 43·3; H, 7·3; Cl, 21·2; N, 8·2. Calc. for $C_6H_{12}ClNO_2$: C, 43·5; H, 7·25; Cl, 21·45; N, 8·5%).

(R)-5-Methylpiperidone (XIV).—A solution of (*R*)-methyl 5-bromo-4-methylpentanoate (8 g.) in methanol (100 ml.) was saturated with anhydrous ammonia and set aside in a stoppered flask for 14 days. The solution was then evaporated, and the residue dissolved in water, which was brought to pH 7 and extracted once with ether (discarded). The aqueous solution was then extracted continuously with ether for 60 hr., and the ethereal extract was dried ($MgSO_4$) and evaporated. The residual oil was chromatographed on alumina (activity III; 100 g.), and the product (187 mg.) was eluted with ether–methanol (9 : 1). After distillation (short-path) at 110—120°, the piperidone had $[\alpha]_D^{18}$ +78° (*c* 1·5 in EtOH) (lit.,[16] $[\alpha]_D^{22}$ +84°). The infrared spectrum was identical with the published spectrum for (+)-5-methylpiperidone prepared from citronellal.[16]

Oxidation of 4-Methylproline to (R)-Methylsuccinic Acid.—Potassium permanganate (4·2 g.) in water (100 ml.) was added dropwise during 30 min. to 4-methylproline (I + II) (1·29 g.) in water (10 ml.), and the mixture was then kept at 80° for 10 min. Precipitated manganese dioxide was filtered off and washed with warm water. The combined filtrates were acidified with 2N-hydrochloric acid (20 ml.) and evaporated *in vacuo*. The residue was extracted with ethanol (20 ml.), which was evaporated before extraction with ether (20 ml.). Crystallisation of the product from light petroleum–benzene yielded methylsuccinic acid, m. p. 108—109°, $[\alpha]_D^{18}$ +9·0° (*c* 1·2 in H_2O). The infrared spectrum was identical with that of authentic (±)-methylsuccinic acid.

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