was dissolved in hot acetone, and cyclohexane was added to the point at which the first cloudiness developed. On standing, crystals formed, mp 209–211° dec.

Anal. Calcd for $C_{18}H_8F_3N_3O_3S_2$: C, 41.60; H, 2.15; F, 15.18; N, 11.20; S, 17.08. Found: C, 42.05; H, 2.10; F, 16.7; N, 11.96; S, 16.20.

The Stereochemistry of 3-Methylproline

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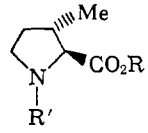
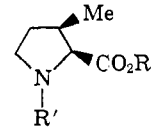
Abstract: The known product (VIII) of Michael condensation of crotonaldehyde with diethyl acetamidomalonate can be readily dehydrated to the enamide (VII), or converted to the related N-acetyl-4,5-dehydro-3-methylproline ethyl ester, which was separated into *cis* and *trans* forms. These diastereoisomers were key intermediates in the correlation of *cis*- and *trans*-3-methylprolines with alloisoleucine and isoleucine. For example, the *cis* enamide XI was converted, by hydrogenation and hydrolysis, to *cis*-3-methylproline (II), while reaction of XI with ethyl mercaptan gave a mercaptal (XV), which was desulfurized to N-acetylalloisoleucine ethyl ester (XVII). These correlations confirmed the stereochemical assignments based upon preferential saponification, in which isomeric mixtures of N-protected 3-methylproline esters gave a *cis* ester and a *trans* acid; the latter procedure was also useful for separation of the isomers. The nmr spectra of *cis*- and *trans*-3-methylprolines and their derivatives are discussed. By comparison with its published spectrum it is confirmed that the 3-methylproline in bottromycin A is *cis*.

The compound 3-methylproline has been shown to have a potent inhibitory effect upon the biosynthesis of actinomycin in *Streptomyces antibioticus*.[1] In order to study separately the effects of the stereoisomers of 3-methylproline, its separation into *cis* and *trans* forms was undertaken. A convenient synthesis of 3-methylproline was recently described;[2] the diastereoisomeric racemates were separated by crystallization, but not identified. The nmr coupling constants J_{23} of the N-*p*-toluenesulfonyl derivatives[3] were 4.6 and 7.2 cps and the melting points of the free amino acids were 218–219° and 210–211°, respectively. We now present evidence that the former isomer is *trans* (I) and the latter *cis* (II).[4]



I, R = R′ = H
IV, R = H; R′ = Tos
VI, R = Me; R′ = Tos
XIV, R = Me; R′ = Ac



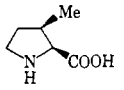
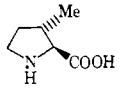
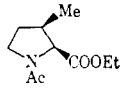
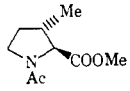
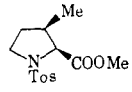
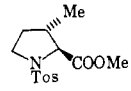
II, R = R′ = H
III, R = Me; R′ = Tos
V, R = H; R′ = Tos
XIII, R = Et; R′ = Ac

Separation of 3-methylproline into racemic *cis* and *trans* forms by ion-exchange chromatography was effective, but for large-scale separations, preferential saponification was more convenient. Saponification of N-*p*-toluenesulfonyl-3-methylproline methyl ester in methanolic sodium hydroxide was continued until one isomer was 96% hydrolyzed, while the other remained 95% intact. Glpc was used to follow the reaction and the acid and ester were separated by extraction. The more resistant ester was the sterically hindered *cis* form (III), while the acid fraction consisted principally of the *trans* form (IV). Hydrolysis of III to V was effected with a hot mixture of hydrochloric and acetic acids, and IV and V converted to the free amino acids by conventional means.[2]

The coupling constant J_{23} was greater for the *cis* derivatives III and V than for the *trans* derivatives IV and VI, and the same relationship held for all the derivatives of 3-methylproline which have been prepared (Table I). By analogy with the case of 3-hydroxyproline,[5,6] this alone gave a tentative basis for stereochemical assignments. However, comparison of J_{23} for the *free* amino acids was difficult, because recognition of the 2-proton signal in *trans*-3-methylproline was obscured by the signals for the 5 protons. In *cis*-3-methylproline, the 2 proton gives a doublet at lower field. This problem was resolved by preparing 4,5-dideuterio-*trans*-3-methylproline as described below. Examination of its nmr spectrum and that of the unlabeled substance shows a J_{23} comparable in magnitude with that of the *cis* compound (Table I). This could be explained by a degree of flexibility of the ring in the free amino acids which is higher than in their derivatives carrying substituents on nitrogen. Rapid fluctuation of dihedral angle between the 2 and 3 protons in both the *cis* and *trans* isomers would give observed coupling constants which are an "average" of maximum and minimum values. The two spectra show different chemical shifts for both the 2 proton and 3-methyl protons, and this can be accounted for in terms of steric hindrance to rotation of the carboxyl (or carboxylate anion) function in the *cis* isomer. Space-

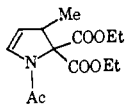
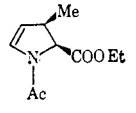
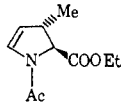
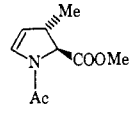
(1) T. Yoshida, A. B. Mauger, B. Witkop, and E. Katz, 148th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1964, Abstracts, p 40C; also in press.
(2) D. A. Cox, A. W. Johnson, and A. B. Mauger, *J. Chem. Soc.*, 5024 (1964).
(3) The coupling constants J_{23} given in ref 4 refer to the N-*p*-toluenesulfonyl derivatives, not the free amino acids as stated.
(4) A. B. Mauger, F. Irreverre, and B. Witkop, *J. Am. Chem. Soc.*, 87, 4975 (1965).
(5) F. Irreverre, K. Morita, A. V. Robertson, and B. Witkop, *ibid.*, 86, 8293 (1964).
(6) J. Blake, C. D. Willson, and H. Rapoport, *ibid.*, 86, 5293 (1964).

Table I. Nmr Spectra[a] of 3-Methylprolines and Their Derivatives

Compd	Structure	Data	3-Methyl	N-Acetyl	Tosyl CH_3	Ester CH_2	Ester CH_3	2 proton
II		δ, ppm	1.00	...	...	...	...	4.08
		J, cps	d, 6.9	...	...	...	...	d, 7.2
I		δ, ppm	1.22	...	...	...	...	3.62
		J, cps	d, 6.5	...	...	...	...	d, 7.7
XIII		δ, ppm	1.05	2.05	...	4.19	1.30	4.41
		J, cps	d, 6.9	s	...	q, 7.4	t, 7.4	d, 7.9
XIV		δ, ppm	1.18	2.08	...	...	3.74	3.96
		J, cps	d, 6.8	s	...	...	s	d, 5.1
III		δ, ppm	0.86	...	2.36	...	3.60	4.17
		J, cps	d, 6.2	...	s	...	s	d, 8.0
VI		δ, ppm	0.86	...	2.36	...	3.65	Obscured by ester CH_3
		J, cps	d, 6.2	...	s	...	s	

[a] Free methylproline in D_2O–TMSP; derivatives in $CDCl_3$–TMS.

Table II. Nmr Spectra[a] of N-Acetyl-Δ^2-pyrrolines

Compd	Structure	Data	3-Methyl	N-Acetyl	Ester CH_2	Ester CH_3	2 proton	3 proton	4 proton	5 proton
VII		δ, ppm	1.16	2.15	4.23 4.25	1.27 1.27	...	3.47	5.03	6.52
		J, cps	d, 7.1	s	q, 7.1 q, 7.1	t, 7.1 t, 7.1	...	m	d, 4.4/ d, 2.6	d, 4.4/ d, 2.0
XI		δ, ppm	1.11	2.17	4.26	1.30	4.82	~3.4	5.10	6.53
		J, cps	d, 7.6	s	q, 6.9	t, 6.9	d, 11.4	m	d, 4.1/ d, 2.1	d, 4.1/ d, 2.1
X		δ, ppm	1.23	2.18	4.24	1.29	4.37	~3.0	5.14	6.52
		J, cps	d, 7.0	s	q, 7.0	t, 7.0	d, 5.0	m	d, 4.3/ d, 2.7	d, 4.3/ d, 1.9
XII		δ, ppm	1.24	2.19	...	3.77	4.37	~3.0	5.15	6.53
		J, cps	d, 7.0	s	...	s	d, 4.5	m	d, 4.3/ d, 2.9	d, 4.3/ d, 1.9

[a] In $CDCl_3$–TMS; m = ill-defined multiplet; d/d = doublet-split doublet.

filling models show that in the *cis* isomer the 2 proton is held in the plane of the carboxyl group, is thereby deshielded, and gives a signal at lower field (by 0.46 ppm) than is the case in the *trans* isomer, where rotation can occur. Conversely, the 3-methyl protons in the *cis* isomer appear at higher field, because they are above the plane of the carboxyl group, and therefore shielded relative to those of the *trans* isomer, in which the carboxyl group can rotate. These and other aspects of the nmr spectra recorded here are under further study.

Final proof of the stereochemistry of the two racemates was obtained by correlation of *cis*- and *trans*-3-methylprolines with alloisoleucine and isoleucine, respectively. For this purpose an intermediate was required which could be separated into diastereoisomers, each convertible to the cyclic and open-chain amino acids, without disturbing the relative configuration at C-2 and C-3. N-Acetyl-4,5-dehydro-3-methylproline ethyl ester readily fulfilled these requirements.

The first compound in this series was the dicarboxylic diester VII which was prepared from the cyclic[2] crotonaldehyde–diethyl acetamidomalonate addition product VIII.[7] The enamide VII was first obtained by thermolysis of the urethan IX by an elimination reaction with formation of 1-naphthylamine. More conveniently, VIII in boiling toluene containing a catalytic amount of *p*-toluenesulfonic acid rapidly furnished VII in high yield. The structure of VII followed from its molecular formula and infrared, nmr, and mass spectra. The mass spectrum showed a parent ion peak and other peaks corresponding to loss of $COCH_2$ and $COOC_2H_5$ fragments. In the nmr spectra this and re-

(7) D. T. Warner and O. A. Moe, *J. Am. Chem. Soc.*, 71, 2586 (1949).

lated compounds (Table II) showed vinyl proton signals consistent with the structure and with chemical shifts similar to those (5.3 and 6.9 ppm from TMS) reported[8] for N-ethoxycarbonyl-Δ^4-pyrrolin-2-one.

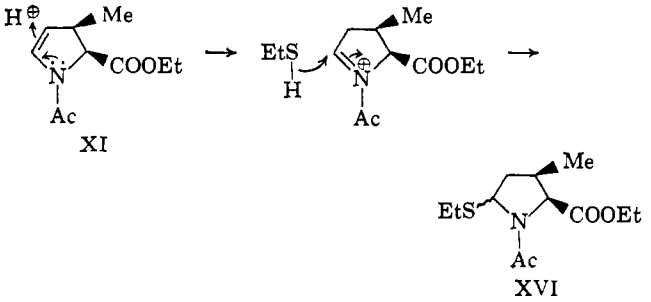
Partial saponification of VIII followed, without isolation of the half-ester, by decarboxylation and dehydration in boiling toluene afforded N-acetyl-4,5-dehydro-3-methylproline ethyl ester in good yield. Separation of this intermediate into *cis* and *trans* forms by silica gel chromatography was only partly successful; some crystalline *trans* isomer X was isolated by this method. Preferential saponification was superior and gave the *cis* ester XI and the *trans* acid. The latter was not isolated but converted by means of diazomethane to its crystalline methyl ester XII.

Initially[4] X and XI were related to the epimeric 3-methylprolines by hydrogenation followed by glpc comparison with derivatives prepared from I and II on a small scale. Recently we have used XI and XII for a convenient synthesis of *cis*- and *trans*-3-methylprolines. Catalytic hydrogenation gave XIII and XIV, respectively, and after hydrolysis afforded *cis*- and *trans*-3-methylprolines identical with samples prepared by the other route. This synthesis has the advantage that the intermediates are easily separated into isomers by preferential saponification, and that catalytic tritiation of the dehydro intermediates opens a route to radioactive 3-methylprolines, required for biochemical investigations. Catalytic addition of deuterium to XII gave 4,5-dideuterio-XIV, and after hydrolysis 4,5-dideuterio-*trans*-3-methylproline.

The conversion of X and XI to the N-acetyl ethyl esters of isoleucine and alloisoleucine was achieved *via* reaction with ethyl mercaptan. The final product of the hydrogen chloride catalyzed reaction at room temperature was a mercaptal of 3-methylglutamic-5-semialdehyde, *e.g.*, XV. When the reaction was carried out at $-8°$, an intermediate "hemimercaptal" XVI could be isolated.

Raney nickel desulfurization of XV gave N-acetyl-DL-alloisoleucine ethyl ester (XVII), identified by nmr and glpc.[4] Similarly, the other diastereoisomer, X, gave a mercaptal XVIII which could be desulfurized to N-acetylisoleucine ethyl ester (XIX). For the purpose of nmr and glpc comparison the authentic derivatives of L-isoleucine and D-alloisoleucine were prepared. These interconversions provide unambiguous confirmation of the stereochemical assignments based on saponification rates.

The conversion of XI to XVI is interpreted as proceeding *via* C-4 protonation. Further reaction of XVI with ethyl mercaptan–hydrogen chloride at room temperature was shown to give the mercaptal XV.

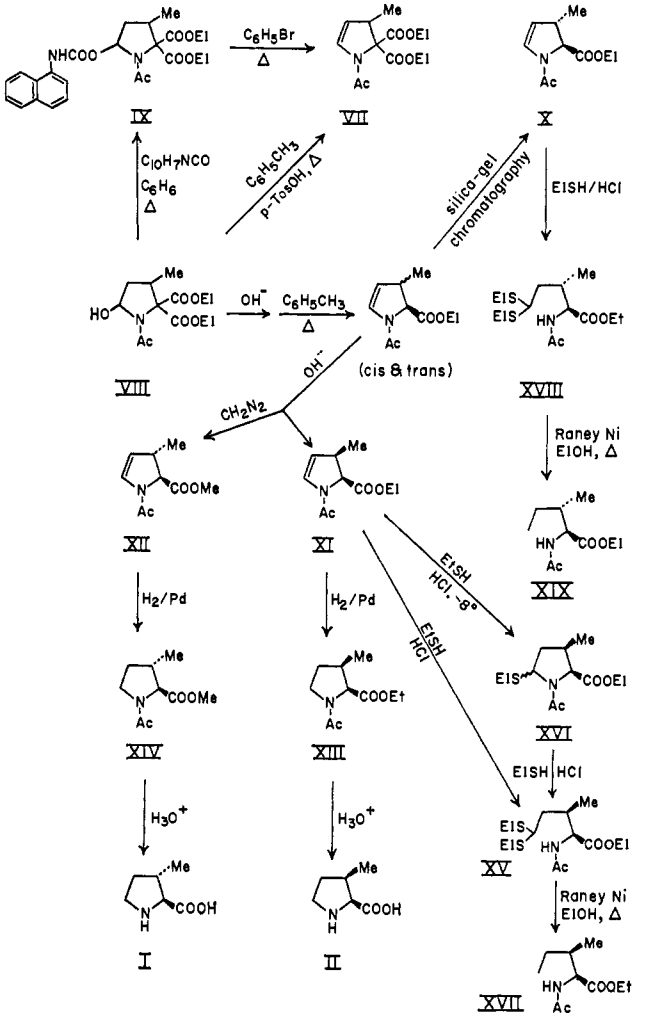


(8) J. Border and H. Rapoport, *J. Org. Chem.*, **30**, 3824 (1965).

The mass spectrum of the mercaptal XVIII, in addition to a parent ion peak and one at *m/e* M − 29 (loss of C_2H_5), contained a peak corresponding to $C_{12}H_{21}NO_3S$, *i.e.*, the "hemimercaptal." This is assumed to result from thermal ring closure with loss of ethyl mercaptan. All the peaks of lower mass in the spectrum of XVIII were also present in that of XVI (see the Experimental Section) and correspond to loss of C_2H_5S, $COCH_2$, and $CO_2C_2H_5$ fragments in turn. The spectrum of XVI also includes peaks at *m/e* 197 and 155, caused by thermal elimination of ethyl mercaptan to give XI, and subsequent loss of ketene.

The recent claim[9] that 3-methylproline occurs in its L-*cis* form in the peptide antibiotic bottromycin A was not originally substantiated by direct comparison with synthetic *cis*- and *trans*-3-methylprolines. The *cis* configuration was suggested on the basis of the coupling constant J_{23}. However, the J_{23} for the *trans* isomer is very similar in magnitude (Table I) and additional evidence would be required to establish the stereochemistry of the natural product. Accordingly, we have compared the published nmr spectrum for the 3-methylproline from bottromycin A with those of our synthetic products, and confirmed its identity with *cis*-3-

Scheme I



(9) S. Nakamura, T. Chikaike, K. Karasawa, H. Honehara, and H. Umezawa, *J. Antibiotics* (Tokyo), **A18**, 47 (1965); S. Nakamura, T. Chikaike, and H. Umezawa, *ibid.*, **A18**, 60 (1965); S. Nakamura, T. Chikaike, H. Yonehara, and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 599 (1965).

methylproline. (See Scheme I, where L forms represent racemates throughout.)

Experimental Section

Infrared spectra were obtained on a Perkin-Elmer grating spectrophotometer Model 237-B, nmr spectra on a Varian A-60, and mass spectra on an AEI MS9 (direct inlet system) at 70 ev. Thin layer chromatography (tlc) was carried out on silica gel G (Merck). "Silica gel" applied to column chromatography refers to silica gel 0.05–0.20 mm (Merck). The columns used for gas–liquid partition chromatography (glpc) were as follows: column A, 1% SE30 on Gaschrom P (6 ft); column B, 3% NGS on Gaschrom Z (6 ft); column C, 3% SE52 on Gaschrom A (6 ft); column D, 3% SE30 on Chrom WAW (3 ft).

Separation of *cis*- and *trans*-3-Methylprolines by Ion-Exchange Chromatography. On the automatic amino acid analyzer (Phoenix Precision Instrument Co., Philadelphia, Pa.) elution from a 150-cm column of Amberlite IR-120 at 50° with 0.2 *N* sodium citrate (pH 3.25 and 4.25) gave peaks for 3-methylproline at 187 and 233 ml. Other amino acids in the vicinity were proline (173 ml), glycine (216 ml), and alanine (230 ml).

Preparative separation was effected as follows. A column (91 × 5.1 cm) of Amberlite IR-120, particle size 47–65 μ, was employed; the volume of the resin was 1725 ml. Sodium citrate buffer (0.2 *N*, pH 3.25) was used for elution and was pumped into the column with a peristaltic pump (Sigmamotor, Middleport, N. Y.). The mixture (106 mg) was dissolved in citrate buffer (pH 2.28) and brought onto the column, and the pump was adjusted to elute at 230 ml/hr. After the first 1500 ml of effluent was discarded, fractions (18 ml) were collected. Aliquots (10 μl) of each fraction were assayed on filter paper by dipping in ninhydrin solution (0.25% in acetone) followed by exposure to steam for 2 min. The spots exhibited a reddish fluorescence under ultraviolet light. The first isomer was located in fractions 78–113 and the second in 155–190.

The tubes containing the first peak were pooled and desalted on a column of Dowex 50-W by elution with 7.0 *N* ammonium hydroxide and the product was obtained by evaporation *in vacuo*. The combined material from three such separations (representing 318 mg of mixture) was decolorized in aqueous solution with charcoal and again evaporated *in vacuo*. The residue was recrystallized from ethanol–ether and obtained as prisms, mp 210–211°, identified by nmr spectroscopy with *cis*-3-methylproline; yield 78 mg.

The tubes containing the second peak were processed in the same manner, except that the dried residue was dissolved in water (0.5 ml) and recrystallized by refrigeration after addition of ethanol (10 ml), forming needles, mp 217–219°, identified by nmr spectroscopy with *trans*-3-methylproline; yield 134 mg.

Separation of *cis*- and *trans*-N-*p*-Toluenesulfonyl-3-methylprolines *via* Preferential Saponification. The isomeric mixture of 3-methylprolines (5.83 g) and sodium bicarbonate (12.5 g) in water (230 ml) was stirred briskly during addition of *p*-toluenesulfonyl chloride (12.0 g) in acetone (120 ml). After stirring for 3 hr at room temperature, the solution was diluted with water (200 ml), washed with ethyl acetate (three 200-ml portions), acidified with hydrochloric acid, and extracted with ethyl acetate (200-ml portion and two 100-ml portions). The pooled extracts were washed with water (200 ml), dried (Na_2SO_4), concentrated to 200 ml, and treated with ethereal diazomethane until yellow. The color was discharged with acetic acid and the resulting solution was filtered through a column (6 × 4 cm) of acid-washed alumina (Merck) which was washed with ethyl acetate. Filtrate and washings were combined and the solvent was removed to afford a mixture of III and VI as a colorless gum (11.16 g); infrared absorption (Nujol) at 1750 (ester C=O) cm^{-1}; nmr spectrum identical with superimposed spectrum of separate isomers. On glpc (column A at 186°) two peaks appeared at 8.7 (54%) and 9.2 (46%) min. This mixture (11.0 g) in methanol (650 ml) was mixed with 2 *N* sodium hydroxide (90 ml) and kept at 32°. The isomeric ratio was followed during saponification by glpc; the peak at 8.7 min (*trans*) diminished until at 55 min the *trans* isomer amounted to only 5% of the mixture. Then, concentrated hydrochloric acid (20 ml) was added and the mixture was concentrated to 200 ml under reduced pressure, diluted with water (400 ml), and extracted with ethyl acetate (two 100-ml portions). The extract was washed with water and extracted with aqueous sodium bicarbonate (three 100-ml portions). The bicarbonate extract was washed with ethyl acetate (three 100-ml portions), acidified with hydrochloric acid, and extracted with ethyl acetate (two 100-ml portions). The extract was washed with water (100 ml) and dried (Na_2SO_4), and the solvent was removed. The crystalline residue (5.27 g) of N-*p*-toluenesulfonyl-3-methylproline was 95% *trans* as shown by glpc after reesterification of an aliquot with diazomethane. Recrystallization from ether–petroleum ether (bp 30–40°) gave IV as white needles, mp 113–115°,[2] yield 4.41 g.

The original ethyl acetate extract was washed with water (100 ml) and dried (Na_2SO_4), and the solvent was removed to give N-*p*-toluenesulfonyl-3-methylproline methyl ester (95% *cis*) as a colorless oil (4.63 g). An aliquot (3.37 g) was dissolved in acetic acid (25 ml), mixed with concentrated hydrochloric acid (25 ml), and heated under reflux for 80 min. Evaporation *in vacuo* gave a crystalline residue which was washed with water (25 ml), dried, and recrystallized from ethyl acetate to afford V as colorless rhombs, mp 183–185°.[2] Glpc of an aliquot after reesterification with diazomethane gave only one peak at 9.2 min.

N-*p*-Toluenesulfonyl-*cis*-3-methyl-DL-proline Methyl Ester (III). Ethereal diazomethane was added to a solution of V (0.90 g) in methanol (18 ml) until a persistent yellow color appeared. The color was discharged with acetic acid, and after dilution with ether (60 ml) the solution was washed with aqueous sodium bicarbonate (two 100-ml portions) and water (60 ml) and dried (Na_2SO_4). Removal of the solvent gave a colorless oil which slowly crystallized, yield 0.96 g. After sublimation at 150° (0.05 mm) and recrystallization from ethyl acetate–petroleum ether (bp 30–60°), the product formed white needles, mp 73–74° (for the nmr spectrum see Table I).

Anal. Calcd for $C_{14}H_{19}NO_4S$: C, 56.53; H, 6.44; N, 4.71; S, 10.77. Found: C, 56.36; H, 6.33; N, 4.80; S, 10.59.

N-Acetyl-2,2-diethoxycarbonyl-3-methyl-5-[1′-naphthylcarbamoyloxy]pyrrolidine (IX). 1-Naphthyl isocyanate (19.5 ml) and VIII (16.05 g) in benzene (200 ml) were heated under reflux for 1 hr. After cooling, 20–40° petroleum ether (500 ml) was added, the mixture was refrigerated for 3 hr, the solid precipitate was separated, extracted with hot ethyl acetate, and filtered, and the filtrate was evaporated. The residue upon recrystallization from ethyl acetate–petroleum ether (bp 60–70°) gave the urethan as needles, mp 133–134°, yield 16.87 g; infrared absorption (Nujol) at 1750 and 1730 (ester C=O), 1680 (urethan C=O), and 1670 (amide C=O) cm^{-1}.

Anal. Calcd for $C_{24}H_{28}N_2O_7$: C, 63.14; H, 6.18; N, 6.14. Found: C, 62.80; H, 6.10; N, 5.84.

Thermal Fragmentation of Foregoing Urethan. A solution of IX (11.74 g) in bromobenzene (60 ml) was heated under reflux for 10 min. On cooling, a crystalline precipitate identified as di-1-naphthylurea (0.25 g) separated. Benzene (100 ml) was added, affording a second crystalline precipitate tentatively identified as 1,3-di[-1′-naphthyl]uretidione (3.08 g) by infrared and nmr spectroscopy. The filtrate was brought onto a column (45 × 6 cm) of silicic acid (Mallinckrodt, 100 mesh) and elution was commenced with benzene. Fractions (100 ml) were collected and examined by tlc in benzene–ethyl acetate (1:1). The solvent was changed to benzene–ethyl acetate 3:1, 2:1, and 1:1 at fractions 16, 28, and 61, respectively. Fractions 24–30 contained a crystalline product (1.90 g) identified as 1-naphthylamine. Fractions 34–43 contained an unidentified product (1.09 g). Fractions 53–71 upon evaporation gave an oil which was distilled at 0.025 mm; the fraction of bp 127–130° was collected to afford VII (2.94 g) as a colorless oil; infrared absorption (liquid film) at 1750 (ester C=O), 1665 (amide C=O), and 1620 (C=C) cm^{-1}; for the nmr spectrum see Table II; mass spectrum showed principal peaks at *m/e* 269.122 (calcd for $C_{13}H_{19}NO_5$, 269.126), 227, 196, and 154.

Anal. Calcd for $C_{13}H_{19}NO_5$: C, 57.98; H, 7.11; N, 5.20. Found: C, 58.06; H, 7.04; N, 5.10.

N-Acetyl-4,5-dehydro-2,2-diethoxycarbonyl-3-methylpyrrolidine (VII). A solution of VIII (6.07 g) in toluene (105 ml) containing *p*-toluenesulfonic acid (100 mg) was heated under reflux in a Dean–Stark apparatus. After 15 min, elimination of water appeared complete and tlc in ethyl acetate indicated that starting material (R_f 0.25) had been entirely converted to product (R_f 0.51). After cooling the solution was diluted with benzene (80 ml), washed with aqueous sodium bicarbonate (150 ml) and water (100 ml), and dried (Na_2SO_4). The solvent was removed and the residual oil distilled at 0.025 mm. The fraction of bp 128–130° was collected to afford VII as a colorless oil, $n^{22}D$ 1.4862, yield 4.63 g. This product was identical with that of the foregoing experiment in its infrared and nmr spectra and glpc behavior (retention time 1.7 min on column B at 175°).

N-Acetyl-4,5-dehydro-3-methyl-DL-proline Ethyl Ester (*cis* + *trans*). A solution of VIII (36.29 g) in 1.5 *N* sodium hydroxide (360 ml) was kept at room temperature for 2 hr. An equivalent amount (120 ml) of 4.5 *N* hydrochloric acid was added and the

water was removed under reduced pressure; final traces were removed by azeotropic distillation with benzene. Toluene (500 ml) was added and the mixture was heated under reflux in a Dean–Stark apparatus for 45 min. The solution was filtered, washed with aqueous sodium bicarbonate (300 ml) and water (250 ml), and dried (Na_2SO_4), and the solvent was removed. The residual oil was distilled at 0.1 mm and the fraction of bp 92–105° was collected as a colorless oil, yield 17.82 g. Glpc on column B at 195° gave two peaks: *trans*, 5.0 min (69%) and *cis*, 6.7 min.

Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 60.93; H, 7.44; N, 6.99.

N-Acetyl-4,5-dehydro-*cis*-3-methyl-DL-proline Ethyl Ester (XI) and N-Acetyl-4,5-dehydro-*trans*-3-methyl-DL-proline Methyl Ester (XII). The foregoing isomeric mixture (17.00 g) was dissolved in methanol (400 ml) containing 4 *N* sodium hydroxide (74 ml) and kept at room temperature. After 50 min, glpc showed that only the *cis* ester remained; acetic acid (20 ml) was added and the solution was concentrated to 100 ml under reduced pressure. Water (300 ml) and sodium bicarbonate (20 g) were added and the mixture was extracted with ethyl acetate (two 150-ml portions); the latter extracts were washed with water (250 ml), dried (Na_2SO_4), and evaporated to a residual oil which was distilled at 0.05 mm. The fraction of bp 89–92° was collected to afford XI as a colorless oil, $n^{22}D$ 1.4935, yield 5.45 g; glpc (column B at 190°) gave one peak at 7.5 min; infrared absorption (liquid film) at 1740 (ester C=O), 1660 (amide C=O), and 1615 (C=C) cm^{-1}; for the nmr spectrum see Table II.

Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 60.73; H, 7.40; N, 7.10.

The aqueous phase from the above extraction was acidified with hydrochloric acid and extracted with *t*-amyl alcohol (two 250-ml portions). The latter extracts were washed with water and ethereal diazomethane was added until a persistent yellow color appeared. The color was discharged with acetic acid and the solvent was removed under reduced pressure. The yellow crystalline residue was passed, in ethyl acetate, through a column (20 × 3.7 cm) of silica gel and the solvent removed to give a pale yellow solid (3.19 g) which upon glpc (column B at 190°) gave a major peak (97.8%) at 5.5 min and a minor peak (2.2%) at 7.7 min. After recrystallization from ethyl acetate–petroleum ether (bp 30–60°) XII was obtained as long white needles, mp 60–62°, which on glcp gave only the 5.5-min peak, yield 2.72 g; infrared absorption (Nujol) at 1740 (ester C=O), 1640 (amide C=O), and 1615 (C=C) cm^{-1}; for the nmr spectrum see Table II.

Anal. Calcd for $C_9H_{13}NO_3$: C, 59.00; H, 7.15; N, 7.65. Found: C, 59.27; H, 6.98; N, 7.51.

N-Acetyl-4,5-dehydro-*trans*-3-methyl-DL-proline Ethyl Ester (X). The crude *cis–trans* mixture (13.60 g) was subjected to chromatography on a column (52 × 5.8 cm) of silica gel in benzene–ethyl acetate (1:1). Fractions (12 ml) were collected and assayed on glpc on column C at 175°. Fractions 157–354 contained a mixture (6.82 g) of X and XI (peaks at 2.3 and 3.1 min) whereas fractions 125–156 gave one peak at 2.3 min. The latter fractions were pooled and evaporated and the residue on sublimation at 75° (0.1 mm) gave X as needles, mp 49–51°, after recrystallization from ethyl acetate–petroleum ether (bp 30–60°), yield 1.82 g; infrared absorption (KBr disk) at 1755 (ester C=O), 1670 (amide C=O), and 1625 (C=C) cm^{-1}; for the nmr spectrum see Table II.

Anal. Calcd for $C_{10}H_{15}NO_3$: C, 60.89; H, 7.67; N, 7.10. Found: C, 61.11; H, 7.49; N, 7.12.

N-Acetyl-*cis*-3-methyl-DL-proline Ethyl Ester (XIII). A solution of XI (4.69 g) in ethanol (80 ml) was shaken with 10% palladium–charcoal (0.5 g) under hydrogen (1 atm) at room temperature until hydrogen uptake ceased (less than 30 min). The mixture was filtered through Hyflo Super Cel and the filtrate was evaporated. The residual oil was distilled at 0.02 mm. The fraction bp 90–92° was collected to afford XIII as a colorless oil, $n^{22}D$ 1.4689, which gave a single peak (8.9 min) on glpc on column B at 190° (XI at 7.5 min), and which cochromatographed with a sample of the corresponding derivative prepared from II, but not from I; yield 3.57 g; infrared absorption (liquid film) at 1735 (ester C=O) at 1650 (amide C=O) cm^{-1}; for the nmr spectrum see Table II.

Anal. Calcd for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.02; H, 8.58; N, 6.75.

N-Acetyl-*trans*-3-methyl-DL-proline Methyl Ester (XIV). A solution of XII (2.29 g) in ethanol (80 ml) was shaken with 10% palladium–charcoal (0.4 g) under hydrogen (1 atm) at room temperature until hydrogen uptake ceased (within 20 min). The mixture was filtered through Hyflo Super Cel and the filtrate was evaporated. The residual oil was distilled at 0.02 mm. The fraction bp 86–88° was collected to afford XIV as a colorless oil, $n^{22}D$ 1.4722, which gave a single peak (7.4 min) on glpc on column B at 190° (XII at 5.5 min), yield 2.00 g; infrared absorption (liquid film) at 1735 (ester carbonyl) and 1650 (amide carbonyl) cm^{-1}; for the nmr spectrum see Table I.

Anal. Calcd for $C_9H_{15}NO_3$: C, 58.36; H, 8.16; N, 7.56. Found: C, 58.67; H, 8.07; N, 7.33.

***cis*-3-Methyl-DL-proline (II).** Hydrochloric acid (7 *N*, 15 ml) was added to a solution of XIII (1.38 g) in acetic acid (10 ml) and the solution was heated under reflux for 3 hr, then evaporated *in vacuo*. The residue was dissolved in water and brought onto a column (10 × 1.4 cm) of Biorad AG 50W-X2 resin which was washed until the washings were neutral. The product was eluted in 2 *N* ammonium hydroxide; after evaporation the solid residue was recrystallized from ethanol–ether as prisms, mp 210–211°, yield 0.59 g; for the nmr spectrum see Table I.

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.58; N, 10.85. Found: C, 55.71; H, 8.35; N, 10.65.

***trans*-3-Methyl-DL-proline (I).** Hydrochloric acid (15 ml; 7 *N*) was added to a solution of XIV (1.11 g) in acetic acid (10 ml), and the solution was heated under reflux for 2 hr, then evaporated and the product was isolated as described for the *cis* isomer; needles from ethanol–ether, mp 217–219°, yield 0.65 g; for the nmr spectrum see Table I.

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.58; N, 10.85. Found: C, 56.03; H, 8.41; N, 10.53.

Reaction of N-Acetyl-4,5-dehydro-*cis*-3-methyl-DL-proline Ethyl Ester (XI) with Ethyl Mercaptan. Hydrogen chloride (3 *N*) in dioxane (2.5 ml) was added to a solution of XI (0.739 g) in ethyl mercaptan (4.5 ml). After 24 hr at room temperature the presence of a single product was indicated by tlc and glpc. The solution was evaporated *in vacuo* and the residue subjected to chromatography on a column (20 × 3.6 cm) of silica gel in benzene–ethyl acetate (1:1). Fractions (20 ml) were collected and assayed by tlc. Fractions 13–19 contained the product; these were pooled and evaporated, and the residual oil, after molecular distillation at 165° (0.1 mm), was still yellow. This oil was passed in ethyl acetate through a column (11 × 1.8 cm) of alumina and the eluate was evaporated. The residual oil on molecular distillation as before gave XV as a colorless oil, yield 0.925 g; for the nmr spectrum see Table III.

Anal. Calcd for $C_{14}H_{27}NO_3S_2$: C, 52.30; H, 8.47; N, 4.36; S, 19.95. Found: C, 52.56; H, 8.22; N, 4.29; S, 20.12.

Desulfurization of XV. A suspension (6 ml) of W2 Raney nickel (3.5 g) in ethanol was added to a solution of XV (225 mg) in ethanol (4 ml). After heating under reflux for 30 min, glpc (column D at 175°) showed two peaks at 0.8 and 2.4 min (starting material at 5.3 min). Additional Raney nickel (3.5 g) in ethanol was added and the mixture again was heated for 30 min, when only the glpc peak at 0.8 min remained. The mixture was filtered, the filtrate was evaporated, and the residual oil was purified by molecular distillation at 100° (0.1 mm) to give XVII as a colorless oil, yield 127 mg. Infrared and nmr spectra were identical with those of N-acetyl-D-alloisoleucine ethyl ester. Glpc on column B at 158° gave a single peak at 11.0 min, unchanged on admixture with N-acetyl-D-alloisoleucine ethyl ester (11.0 min), but a double peak on admixture with N-acetyl-L-isoleucine ethyl ester (11.7 min).

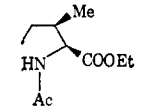
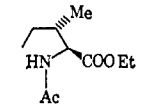
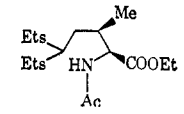
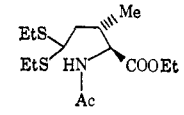
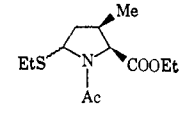
Anal. Calcd for $C_{10}H_{10}NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.90; H, 9.35; N, 6.74.

Reaction of N-Acetyl-4,5-dehydro-*trans*-3-methyl-DL-proline Ethyl Ester (X) with Ethyl Mercaptan. Hydrogen chloride (3 *N*) in dioxane (2 ml) was added to a solution of X (1.00 g) in ethyl mercaptan (5 ml). After 48 hr at room temperature, the mixture was evaporated *in vacuo* and the residue was subjected to chromatography on a column (20 × 3.6 cm) of silica gel in benzene–ethyl acetate (1:1). Fractions (12 ml) were collected and the product was located in tubes 16–29 by tlc. These fractions were pooled and evaporated and the residual oil was purified by molecular distillation at 150° (0.1 mm) to give XVIII as a colorless syrup, yield 1.24 g; for the nmr spectrum see Table III; mass spectrum principal peaks at *m/e* 321, 292, 259.1227 (calcd for $C_{12}H_{21}NO_3S$, 259.1241), 198, 156.1017 (calcd for $C_8H_{14}NO_2$, 156.1024), and 82.

Anal. Calcd for $C_{14}H_{27}NO_3S_2$: C, 52.30; H, 8.47; N, 4.36; S, 19.95. Found: C, 52.29; H, 8.40; N, 4.23; S, 20.33.

Desulfurization of XVIII. A suspension (20 ml) of W2 Raney nickel (12 g) in ethanol was added to a solution of XVIII (380 mg) in ethanol (5 ml) and the mixture was heated under reflux. During the reaction glpc on column D at 180° gave two new peaks (XVIII at 4.2 min) at 0.6 and 2.0 min. After 30 min only the 0.6-min peak remained; the mixture was filtered, the filtrate was evaporated, and the residual oil was purified by molecular distillation at 110°

Table III. Nmr Spectra[a] of Isoleucine Derivatives and Related Mercaptals

Compd	Structure	Data	3-Methyl	N-Acetyl	Ester CH_2	Ester CH_3	5 proton	S-Ethyl CH_2	S-Ethyl CH_3	2 proton
XVII		δ, ppm	0.90	2.09	4.27	1.31	Obscure	...	...	4.78
		J, cps	d, 7.0	s	q, 7.0	t, 7.0	Obscure	...	...	d, 9.2/ d, 4.0
XIX		δ, ppm	0.93	2.06	4.28	1.30	Obscure	...	...	4.68
		J, cps	d, 6.9	s	q, 7.0	t, 7.0	Obscure	...	...	d, 8.9/ d, 4.8
XV		δ, ppm	0.89	2.05	4.23	1.26	4.0	2.66	1.30	4.64
		J, cps	d, 6.9	s	q, 7.5	t, 7.5	t, 8.0	2q, 7.0	2t, 7.0	d, 9.0/ d, 3.8
XVIII		δ, ppm	0.98	2.05	4.24	1.31	3.87	2.67	1.27	4.64
		J, cps	d, 6.6	s	q, 7.2	t, 7.2	d, 8.4/ d, 6.8	2q, 7.3	2t, 7.3	d, 8.8/ d, 4.5
XVI		δ, ppm	1.06	2.30	4.20	1.28[b]	4.98	2.61	1.30[b]	4.37
		J, cps	d, 6.8	s	q, 7.5	t, 7.5	m	q, 7.5	t, 7.5	d, 8.0

[a] In $CDCl_3$–TMS. [b] These chemical shifts may be interchangeable.

(0.05 mm) to give XIX as a colorless oil, yield, 112 mg; infrared and nmr spectra identical with those of N-acetyl-L-isoleucine ethyl ester. Glpc on column B at 158° gave a single peak at 11.7 min unchanged on admixture with N-acetyl-L-isoleucine ethyl ester (11.7 min), but a double peak on admixture with N-acetyl-D-allo-isoleucine ethyl ester (11.0 min).

Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.47; H, 9.30; N, 6.93.

N-Acetyl-D-alloisoleucine Ethyl Ester. N-Acetyl-D-alloisoleucine (0.30 g) in 7 *N* ethanolic hydrogen chloride (4 ml) was kept at 50° in a sealed tube for 1 hr. The solution was evaporated under reduced pressure and the residue was dissolved in ether (30 ml) and washed with water (25 ml), aqueous sodium bicarbonate (35 ml), and water (20 ml), and dried (Na_2SO_4). After evaporation the residual oil was purified by molecular distillation at 110° (0.1 mm) to give the ester as a colorless oil, $n^{22}D$ 1.4531, yield, 0.25 g; infrared absorption (liquid film) at 1740 (ester C=O) and 1650 (amide C=O) cm^{-1}; for the nmr spectrum see Table III.

Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 60.13; H, 9.48; N, 7.14.

N-Acetyl-L-isoleucine Ethyl Ester. N-Acetyl-L-isoleucine (0.30 g) in 7 *N* ethanolic hydrogen chloride (4 ml) was kept in a sealed tube at 50° for 1 hr. The product was worked up as described above for the D-allo isomer, purified by molecular distillation at 110° (0.1 mm), and obtained as a colorless oil, $n^{22}D$ 1.4528, yield 0.19 g; infrared absorption (liquid film) at 1740 (ester C=O) and 1650 (amide C=O) cm^{-1}; for the nmr spectrum see Table III.

Anal. Calcd for $C_{10}H_{19}NO_3$: C, 59.67; H, 9.52; N, 6.96. Found: C, 59.64; H, 9.71; N, 6.81.

N-Acetyl-5-ethylmercapto-*cis*-3-methyl-DL-proline Ethyl Ester (XVI). A solution of XI (207 mg) in ethyl mercaptan (2.0 ml) was mixed with *N* hydrogen chloride in dioxane (0.2 ml) and the solution was kept at −8° and assayed at intervals by glpc on column D at 175°. After 43 hr there were two peaks at 0.7 (XI) and 1.9 min (XVI). Further 3 *N* hydrogen chloride in dioxane (0.2 ml) was added and the solution again was kept at −8°. After a total of 3 days no peak at 0.7 min in the glpc remained, and a new peak at 5.1 min (XV) was beginning to appear. The solution was evaporated *in vacuo*, and the residue was dissolved in ethyl acetate (30 ml), washed with aqueous sodium bicarbonate (30 ml) and water (20 ml), and dried (Na_2SO_4). Removal of the solvent gave a residue which was dissolved in benzene–ethyl acetate (2:1, 4 ml), brought onto a column (13 × 1.5 cm) of silica gel, and eluted with the same solvent mixture. Fractions (4 ml) were collected and assayed by glpc. The product was located in fractions 8–18 which were pooled and evaporated giving a solid residue which crystallized from ethyl acetate–petroleum ether (bp 30–60°) as prisms, mp 96.5–97°, yield 113 mg; infrared absorption (Nujol) at 1730 (ester C=O) and 1655 (amide C=O) cm^{-1}; for the nmr spectrum see Table III; mass spectrum principal peaks at *m/e* 259, 198, 197, 156, 155, 124, and 82.

Anal. Calcd for $C_{12}H_{21}NO_3$: C, 55.55; H, 8.16; N, 5.40; S, 12.36. Found: C, 55.80; H, 8.04; N, 5.33; S, 12.38.

Further Reaction of Foregoing XVI with Ethyl Mercaptan. XVI (45 mg) in ethyl mercaptan (0.5 ml) was treated with 3 *N* hydrogen chloride in dioxane (0.25 ml) and the solution was kept at room temperature for 24 hr. After evaporation *in vacuo* the residue was subjected to molecular distillation at 150° (0.5 min), to give a product identical with XVII in its infrared and nmr spectra and upon glpc (column D at 175°).

N-Acetyl-4,5-dideuterio-*trans*-3-methyl-DL-proline Methyl Ester. A solution of XII (250 mg) in ethyl acetate (5 ml) was shaken with 10% palladium–charcoal under deuterium (1 atm) until uptake ceased. After filtration and evaporation the residue was subjected to molecular distillation at 90° (0.3 mm) to give 4,5-dideuterio-XIV as a colorless oil (238 mg), identical in glpc behavior with XIV; infrared absorption (liquid film) at 1735 (ester C=O) and 1650 (amide C=O) cm^{-1}. The nmr spectrum ($CDCl_3$) showed signals identical with those of XIV for the 2-proton and methyl groups.

4,5-Dideuterio-*trans*-3-methyl-DL-proline. 4,5-Dideuterio-XIV (140 mg) in acetic acid (5 ml) with 7 *N* hydrochloric acid (8 ml) was heated under reflux for 1.2 hr. The solution was evaporated and the product was worked up as described for I. After recrystallization from ethanol–ether, 4,5-dideuterio-I formed needles, mp 216–218°. The infrared spectrum (Nujol mull) was somewhat similar but not identical with that of I, with carbonyl absorption at 1600 cm^{-1}. In the nmr spectrum (D_2O) a doublet at δ = 1.22 ppm (J = 6.6 cps) was assigned to the 3-methyl protons and one at δ = 3.62 ppm (J = 7.7 cps) to the 2 proton; both were present also in the spectrum of I. Other signals (5 protons) in the region δ = 3.2–3.6 ppm were blurred and diminished in intensity compared with the spectrum of I.

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