C-Derivatization of Amino Acids.[1] Synthesis and Absolute Configuration of 3-Methylproline. *cis–trans* Isomers with Unusual Vicinal Proton Coupling

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Abstract: The concept of C-derivatization of amino acids *via* β- and γ-chloro amino acids, obtained by selective free-radical chlorination in strong acid solution, is now extended to the δ-chlorination of an amino acid and in turn to the first synthesis of the optically active isomers of 3-methylproline. The exceedingly convenient synthesis does not affect the two asymmetric centers of the isoleucines employed as starting material; therefore it delivers the isomeric 3-methylprolines in optically pure form directly, without resolution. Moreover it concurrently establishes the *absolute configuration* of each of the four isomers. The $J_{2,3}$ proton coupling values of the *cis–trans* isomers are practically identical.

The first synthesis and proof of the absolute configuration of 3-methylprolines is the main subject of this work; in addition, the unusual case of a *cis–trans* isomer pair with closely similar $J_{2,3}$ proton couplings will also be discussed.[2]

Although several syntheses of 3-methylproline have been described,[3] these syntheses invariably furnish a mixture of the racemates (*cis–trans*), the separation of which is quite laborious.[4] The optical resolution of the racemates has not been described and consequently the *absolute configuration* of the four diastereoisomers has not been assigned either.[5] We present now a two-step synthesis of the optically active 3-methylprolines which concurrently proves their absolute configuration.

Methods and Results

Whereas the above-mentioned syntheses of (racemic) 3-methylproline employed conventional methods of amino acid synthesis, the present method utilizes the recently described concept in amino acid chemistry, the *C-derivatization of amino acids*, the essence of which is *transformation of amino acids via their C-chloro derivatives.*[1] These extremely useful derivatives of amino acids became easily available with the discovery that the usually destructive character of the interaction of amino acids with chlorine[6] can be decisively modified by working in strong acid solution under free-radical-generating conditions.[7] In our first communication we described the preparation of several β- and γ-chloro amino acids; the key reaction of the present work is the δ-chlorination of isoleucine.

The isoleucine isomer chosen was chlorinated after dissolution in the selected strong acid (*e.g.*, ~6 moles of trifluoroacetic acid or 3 moles of 50% H_2SO_4 per mole of amino acid). In the dark, these systems are unreactive with chlorine, but when irradiated by an ultraviolet lamp, they react rapidly with chlorine, thus indicating the radical character of the reaction. The formation of dichlorinated products could be greatly diminished by interrupting the chlorinations after the uptake of 0.7–0.8 atom of Cl per mole of amino acid. The separation of the mixture of chloro amino acids was not attempted. Instead, the total reaction mixture was basified; after a few hours the amount of ionic chlorine present very closely approached the saponifiable Cl earlier assayed. The composition of the solution was then assayed by the Spinco–Beckman automatic amino acid analyzer (Spackman–Moore–Stein method, buffer pH, 3.25). A typical analysis indicated the presence of 25 mole % 3-methylproline and 25 mole % isoleucine. The yield of 3-methylproline, based on converted isoleucine, was 33% of the theoretical.[8]

For isolation of the 3-methylprolines, the mixture was treated with nitrous acid to form the N-nitrosoproline derivative; the primary amino acids simultaneously were deaminated to the hydroxycarboxylic acids. On acidic hydrolysis, 3-methylproline was reformed. It was desalted on an ion-exchange resin after

(1) Previous paper (part II): J. Kollonitsch, A. Rosegay, and G. A. Doldouras, *J. Am. Chem. Soc.*, **86**, 1857 (1964).

(2) (a) The 3-methylprolines have considerable biochemical interest; a mixture of the four isomers strongly inhibits the biosynthesis of actinomycin in the extremely low concentration of 0.1 μg/ml: 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. (b) It was claimed that an isomer of 3-methylproline was isolated from the hydrolysate of the peptide antibiotic, bottromycin A. On the basis of pmr and ORD data, it was designated the *cis*-L isomer: S. Nakamura, T. Chikaike, H. Yonehara, and H. Umezawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 599 (1965).

(3) (a) R. Adams and N. J. Leonard, *J. Am. Chem. Soc.*, **66**, 257 (1944); (b) T. Takahashi and K. Karyone, *Yakugaku Zashii*, **79**, 711 (1959); *Chem. Abstr.*, **53**, 21940 (1959); (c) D. A. Cox, A. W. Johnson, and A. B. Mauger, *J. Chem. Soc.*, 5024 (1964); (d) for a recent review of this field, see A. B. Mauger and B. Witkop, *Chem. Rev.*, **66**, 47 (1966).

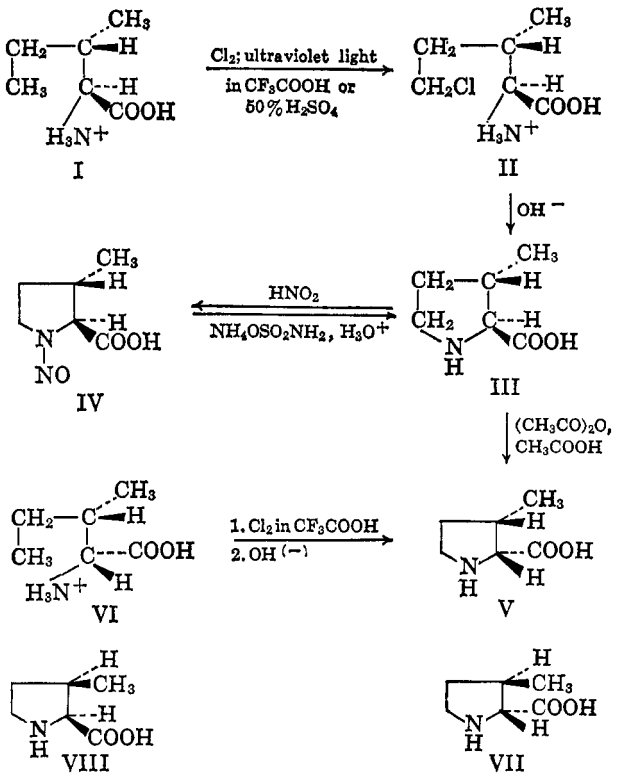
(4) (a) A. B. Mauger, F. Irreverre, and B. Witkop, *J. Am. Chem. Soc.*, **87**, 4975 (1965); (b) A. B. Mauger, F. Irreverre, and B. Witkop, *J. Am. Chem. Soc.*, **88**, 2019 (1966).

(5) Except the rather tentative assignment of the naturally occurring isomer; see ref 2b. The stereochemistry of the *racemates* has been elucidated; see ref 4a,b.

(6) The rapid degradation of leucine with chlorine was discussed as early as 1857: H. Schwanert, *Ann. Chem. Liebigs*, **102**, 221 (1857).

(7) The success of this approach is rather unexpected if one considers the extensive literature discussing complete destruction (deamination) or C-skeletal rearrangement of amino acids under free-radical-generating conditions, *e.g.*, J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, John Wiley and Sons, Inc., New York, N. Y., 1961, p 683; G. Ferrari and R. Cultrera, *Nature*, **190**, 326 (1961).

(8) In addition to 3-methylproline, the amino acid analyzer indicated the presence of two other amino acids (8 and 4 mole %), with chromatographic mobility as expected of hydroxyisoleucines. These amino acids disappeared from the chromatogram on acidification of the amino acid mixture, whereas after realkalization the chromatogram indicated their presence again, in the original amount. This behavior is expected of γ-hydroxyamino acids, which are known to lactonize easily in acid solution; the (strongly basic) lactones do not elute with buffer pH 3.25, employed for elution of neutral amino acids. We will return to these γ-hydroxyisoleucines in our forthcoming paper, where γ-chlorination of several other amino acids will be discussed in detail.



removal of small amounts of chlorine-containing impurities by catalytic hydrogenation.

Experimental Section

trans-**3-Methyl-L-proline from L-Isoleucine.** L-Isoleucine (I) (Calbiochem, A grade 39.3 g 0.3 mole) was dissolved in 120 ml of trifluoroacetic acid in a fused quartz flask and, while being irradiated by a 140-w quartz lamp (Hanovia No. 30620), chlorine gas was passed in for 30 min with vigorous stirring. Alkaline hydrolysis of a small sample indicated the uptake of *ca.* 0.7 atom of saponifiable chlorine per mole of amino acid. (When chlorinating in a Pyrex flask instead of quartz, 50 min was required to reach the same extent of reaction.) The solvent was evaporated *in vacuo*, and the residue was dissolved in 150 ml of water and slowly added to 500 ml of 2.5 *N* sodium hydroxide solution. After 16 hr,[9] hydrochloric acid was added in sufficient excess to make the solution *ca.* 1 *N* in acid (solution volume *ca.* 800 ml). The acidic solution was heated on the steam bath to 70–80°, then, while the hot solution was swirled, 45 g of $NaNO_2$ was added in several portions to form the N-nitroso derivative IV. The heating was continued for a total of 15 min. After cooling, the solution was adjusted to pH 1 and was extracted with ethyl acetate (six 50-ml portions). The extracts were dried and evaporated *in vacuo*. The residue, dissolved in 80 ml of 6 *N* HCl, was heated at 105–110° for 0.5 hr with 10 g of ammonium sulfamate. The hydrolyzed mixture was decolorized with activated charcoal and evaporated to dryness under vacuum to remove excess HCl. The residue was dissolved in water, adjusted to pH 7 with NH_4OH, and then subjected to hydrogenolysis for 24 hr at 150° with 5 g of 30% palladium–charcoal catalyst (2000 psi) in order to remove impurities containing chlorine. The hydrogenolyzed solution was passed through an ion-exchange column (Dowex 50, H^+ form), the column washed with water until the effluent became neutral, and III eluted with dilute NH_4OH. The NH_4OH effluent was evaporated *in vacuo* to give *trans*-3-methyl-L-proline (III) (yield after recrystallization from isopropyl alcohol 7.5 g, 19%); 100% pure by Spinco–Beckman analysis;[10] $[\alpha]^{24}D$ −30.0° (*c*, 1, H_2O); +4.6° (c, 1, 5 *N* HCl); prisms, which convert to needles at 180–200°, then melt at 240–248° dec (Kofler).

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.59; N, 10.85. Found: C, 55.96; H, 8.45; N, 10.78.

cis-**3-Methyl-D-proline (V) from Allo-D-isoleucine (VI).** Allo-D-isoleucine (VI, Calbiochem) was transformed in an analogous manner into *cis*-3-methyl-D-proline (V), prisms from isopropyl alcohol, which convert to needles at 170–220°, then melt at 227–230° dec (Kofler); $[\alpha]^{24}D$ +69.8° (*c* 1, H_2O); +36.4° (*c* 1, 5 *N* HCl).

Anal. Calcd for $C_6H_{11}NO_2$: C, 55.79; H, 8.59; N, 10.85. Found: C, 55.63; H, 8.43; N, 11.08.

The availability of both allo-L-isoleucine and D-isoleucine[11] allows the simple synthesis of *cis*-3-methyl-L-proline (VIII) and of *trans*-3-methyl-D-proline (VII) as well.

Epimerization of *trans*-3-Methyl-L-proline (III) into *cis*-3-Methyl-D-proline (V). The *trans* isomer III, 91 mg (0.7 mmole), was heated under reflux for 2 hr with 3.5 ml of glacial acetic acid and 1.5 ml of acetic anhydride. The solution then was evaporated to dryness *in vacuo*, 10 ml of 2 *N* HCl was added, and the resulting solution was boiled for 2 hr.

The automatic amino acid analyzer indicated the presence of 0.16 mmole (17%) of the epimerized product V, besides 0.41 mmole (58%) of III. Longer refluxing of the acetic anhydride–acetic acid solution caused little further change in the ratio of the isomers, indicating that the epimerization equilibrium strongly favors, as expected, the sterically less crowded *trans* diastereoisomer.

Proton Magnetic Resonance Spectra. Pmr spectra were recorded in D_2O solution, with external benzene standard at τ 3.5. The *cis* isomer V displayed a spectrum with a 2 H doublet at τ 5.9 indicating $J_{2,3}$ = 7.8 cps. The spectrum of the *trans* isomer III showed a 2 H doublet at τ 6.4 with $J_{2,3}$ = 7.8 cps.

Discussion

The *absolute configuration* of D-isoleucine hydrobromide was elucidated in the course of the classical X-ray diffraction studies of Bijvoet.[12] Since the asymmetric centers of isoleucine are unaffected by the reactions above described, the *absolute configurations of the 3-methylprolines synthesized are as depicted in formulas III and V.* Accordingly, their antimers, accessible from D-isoleucine and allo-L-isoleucine, respectively, have the configurations VII and VIII.

The pmr spectrum as well as the infrared spectrum of V was identical with those reported for the 3-methylproline isolated from the hydrolysate of bottromycin A, while its optical rotation was similar in magnitude, but opposite in sign. Thus the structure and absolute configuration of the natural isomer[2b] is now unambiguously established as *cis*-3-methyl-L-proline (VIII).

The Karplus relationship between the vicinal coupling constant and the HCCH dihedral angle is widely used to distinguish *cis–trans* isomers.[13] As shown above, III and V display practically identical $J_{2,3}$ coupling constants (*ca.* 7.8 cps) in their pmr spectra. Consequently, the correct assignment of the natural isomer[2b] as VIII on the basis of the Karplus equation must now be regarded as fortuitous. This is emphasized because it was recently claimed[4a] that the above *cis–trans* isomers can be correctly differentiated by comparison of their $J_{2,3}$ coupling constants. In fact, this claim can be ascribed only to a misunderstanding of the earlier, reported[3c] pmr data.[14] To our knowledge, no

(9) Analysis of this solution by the Spinco–Beckman automatic amino acid analyzer indicates a 33% yield of III, besides a considerable amount of the isomeric γ-hydroxyisoleucines. Employing citrate buffer (pH 3.25), III appears between glycine and alanine in the chromatogram, whereas V comes close to proline. The ninhydrin color constants for III and V at 440 mμ are each 65% of the constant for proline. The γ-hydroxyisoleucines appear between serine and glutamic acid, and between proline and glycine, respectively.

(10) Authors thank Mr. R. H. Redfield of these laboratories for performing several amino acid analyses.

(11) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 1–3, John Wiley and Sons, Inc., New York, N. Y., 1961.

(12) J. Trommel and J. M. Bijvoet, *Acta Cryst.*, 7, 703 (1954).

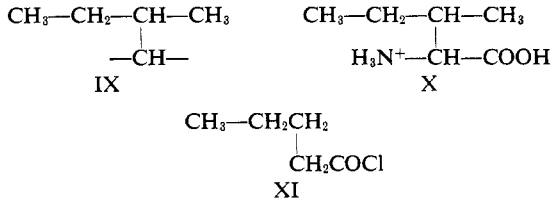
(13) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(14) This earlier work[3c] refers actually to the $J_{2,3}$ coupling constant of only one of the isomers (*cis*). Note: since submission of this manuscript, we learned from Dr. B. Witkop (National Institutes of Health) that he later reached the same results and conclusions.[4b]

other case of a *cis–trans* diastereoisomeric pair is known where the Karplus relationship is similarly indiscriminative. This lack of differentiation can perhaps be a consequence of flexing of the pyrrolidine ring. In a Dreiding model of the five-membered ring of 3-methylproline, flexing can introduce deviations in the HC_2C_3H dihedral angle of nearly $\pm 30°$ from the eclipsed form, *i.e.*, *cis* vicinal bond angle can twist *ca.* $\pm 30°$ around 0°, while the *trans* angle can twist between *ca.* 90–150° (around 120°).

Applying the Karplus equation to these angle ranges, one predicts a large J value, *ca.* 6–8 cps between the *cis* protons. The experimental $J_{2,3}$ for V falls within this range. For the *trans* coupling constant, one calculates a small J value (*ca.* 2 cps) for the average angle of 120°, but a wide range of J values between 0 and 7 cps for the angles 90 and 150°, respectively. For *trans*-3-methylproline the experimental $J_{2,3}$ of 7.8 cps, although much larger than 2 cps, is not far from the value calculated for the angle 150°. The large deviation of the *trans* $J_{2,3}$ from theory thus could be due in part to small perturbations in the *trans* dihedral angle. It cannot be excluded that this discrepancy is caused by other factors; Karplus[15] has emphasized that vicinal J values may be influenced by factors other than dihedral angle. The concidence of $J_{2,3}$ values in the 3-methylprolines reported here reinforces the need for caution in deducing *cis–trans* stereochemistry from coupling constants alone.

A detailed discussion of the *orientation rules* in radical chlorination of amino acids will be included in our subsequent paper, where comparative results will be presented for several aliphatic amino acids. Therefore, a few remarks should suffice here. The relative selectivity in radical chlorine attack on aliphatic hydrocarbons is usually given as 1:3.7:5 for primary, secondary, and tertiary hydrogens, respectively.

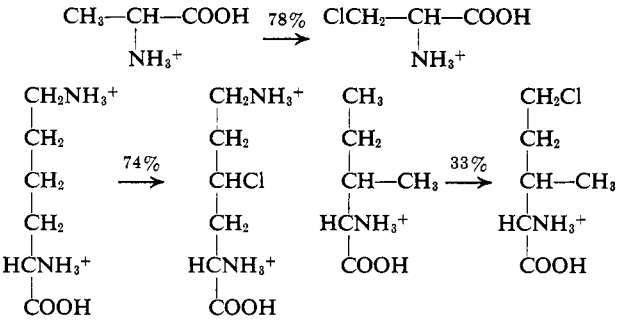


IX X

XI

On this basis, for the hypothetical "parent hydrocarbon" of isoleucine (IX) the expected amount of δ-chlorination product is calculated as 13%; since minimally 33% of the δ-chloro derivative was obtained from isoleucine, there must exist a powerful inhibition by the monoprotonated amino acid grouping[16] against attack of chlorine on the α-, β-, and γ-hydrogens. Its effect is comparable thus to the influence of the carboxylic acid chloride group, as valeryl chloride gives about 32% of δ-chloro derivative.[17]

Considering our earlier results,[1] the following illustrative cases are presented.



It appears that predominantly β-, γ-, or δ-chloro amino acids are formed in these reactions; furthermore, in every case the C–H bonds furthermost from the protonated amino acid moiety are attacked in preference. The main factor behind this preference is probably the powerful inductive (electron-withdrawing) effect of the aminium group, accentuated by the similar effect of the carboxyl group. Both effects should diminish the electron density along the hydrocarbon chain. The magnitude of this inductive effect obviously decreases with increasing distance from the α carbon; consequently, the similarly decreased repulsion between the electron-deficient C–H bonds and the electrophilic chlorine atoms[18] will allow the preferential substitution of the hydrogens farthest from the α carbon. These effects are probably further potentiated by the repulsion between the (electrophilic) chlorine atom and the positively charged aminium radical.[19]

The above-discussed structure dependence of these chlorinations allows the exclusion of any *intramolecular* mechanism similar to that of the Hofmann–Loeffler–Freytag (HLF) reaction. It has been shown that β- or γ-chloro amines are not formed in the HLF reaction;[20] conversely, only δ-chloro amines are formed in this reaction, even in cases where this imposes severe steric strain.[21]

Acknowledgment. The authors wish to thank Mr. B. Arison and Dr. N. Trenner for the pmr spectra and Mr. R. N. Boos and his associates for the microanalysis.

(15) M. Karplus, *J. Am. Chem. Soc.*, **85**, 2870 (1963).

(16) α-Amino acids are protonated mainly on the amino group, the protonation of the carboxyl group is slight (~20%), probably because of the electrostatic repulsion of the positively charged aminium group inhibits the formation of a second positive charge in its proximity: J. L. O'Brien and L. Nieman, *ibid.*, **73**, 4264 (1951).

(17) H. Singh and J. M. Tedder, *Chem. Commun.*, 5 (1965).

(18) G. A. Russell, *Tetrahedron*, **8**, 101 (1960).

(19) For discussion of the electrostatic effect as a selectivity determining factor in radical reactions see J. M. Tedder, *Quart. Rev.* (London), **14**, 336 (1960); H. Singh and J. M. Tedder, *J. Chem. Soc.*, 4737 (1964).

(20) E. J. Corey and W. R. Hertler, *J. Am. Chem. Soc.*, **82**, 1657 (1960).

(21) P. G. Gassman and D. C. Heckert, *Tetrahedron*, **21**, 2725 (1965).