flask was placed in a constant temperature bath and 0.05 mole of benzyl chloride dissolved in 20 g. of nitromethane was added dropwise into the vigorously stirred aromatic, $AlCl_3$, nitromethane solution. The usual reaction time was 15 minutes. After the addition of the benzyl chloride the mixture was stirred for another 5 minutes, then washed with 100 ml. of 5% HCl water solution, followed by washing twice with 50 ml. of water. The organic layer was dried over $CaCl_2$ and a small amount of K_2CO_3 and analyzed by gas–liquid chromatography.

Analytical Procedure.—The analyses were carried out on a Perkin–Elmer model 154-D vapor fractometer equipped with a 150′ Golay capillary column and a hydrogen flame ionization detector. Peak areas were obtained with a Perkin–Elmer model 194 electronic printing integrator.

The capillary column used was obtained from Perkin–Elmer Corp. designated "R-polypropylene glycol" and was 150′ in length. The operating parameters differed for the various halodiphenylmethanes and are listed with the retention times in Table IV.

Response data were determined by running solutions of the respective pure halodiphenylmethane isomers and diphenylmethane in benzene in the approximate ratios in which they occurred in the reaction mixtures.

[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

Preparation, Resolution and Optical Stability of 3,4-Dehydroproline and 3,4-Dehydroprolinamide

BY ALEXANDER V. ROBERTSON[1] AND BERNHARD WITKOP

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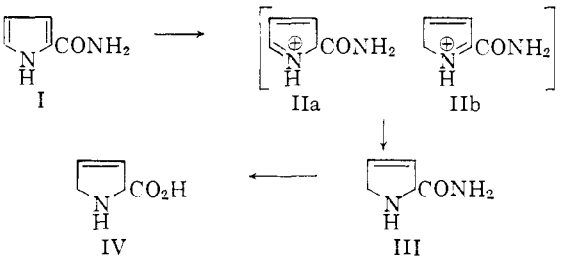
The reduction of pyrrole-2-carboxamide with phosphonium iodide in fuming hydriodic acid under special conditions leads to 70% of 3,4-dehydro-DL-prolinamide (III) and 10% of 3,4-dehydro-DL-proline (IV) which were isolated and purified by ion exchange technique. The structure of III and IV was proved by catalytic hydrogenation to proline and prolinamide, by nuclear magnetic resonance spectroscopy and by resolution. Chemical resolution yielded the optically labile 3,4-dehydro-D-prolinamide, $[\alpha]^{20}D > +320°$, $t_{1/2} \sim 48$ hours. Combination of enzymatic hydrolysis of III with its asymmetric transformation yielded 75% of 3,4-dehydroproline containing at least 90% of the L-configuration. 3,4-Dehydro-L-proline in aqueous solution is comparatively stable at room temperature, but has a half-life time of six days at 90°. The implication of the principle of 100% conversion of a racemate into one natural optical isomer is discussed with regard to the origin of asymmetry in nature.

The 3,4-dehydroproline system is of interest because the double bond in the allylic position to the asymmetric center will make the antipodes optically labile and, in addition, the class will lend itself to the preparation of novel functional derivatives of proline and hydroxyproline which are needed in current metabolic studies. This communication reports on the aspects of optical lability.[2]

Extensive efforts to convert hydroxyproline to 3,4-dehydroproline were of little success[3a]; a better approach is the method of Fischer and Gerlach[3b] who reduced pyrrole-2-carboxamide (I) with phosphonium iodide in fuming hydriodic acid and obtained a 25% yield of a compound which was presumed but not proved to be 3,4-dehydroproline. Modification of the reaction conditions together with application of modern isolation techniques afford ready access to the 3,4-dehydroproline oxidation level in high yield. The product of reduction is 3,4-dehydroprolinamide (III) which then slowly hydrolyzes to the amino acid IV in the strongly acidic reaction mixture. The concentration of fuming hydriodic acid is critical. The reduction is complete in 2 hours with acid saturated at −20°, but takes 8 hours with acid saturated at 0° and the yields are lower. No reduction occurs with phosphonium iodide and constant boiling acid. Much iodine was liberated when pyrrole-2-carboxamide was treated with fuming hydriodic acid alone, and starting material was the only homogeneous compound isolable. These facts can best be accommodated by assuming that the reaction proceeds *via* the pyrroleninium ions (IIa, IIb) which are stabilized in the very strong acid. Reduction of the immonium double bond leads to III in which the Δ^3-double bond is isolated and resistant to further reduction. Phosphonium iodide removes free iodine as it is formed, thus avoiding electrophilic attack on unreacted starting material.



The combined yield of III and IV is 70–80%, and the ratio of the compounds depends on the speed and temperature of isolation from the strongly acidic reaction mixture. Under optimum conditions 70% of the amide and 10% of the amino acid were obtained. Differences in their solubilities and in their adsorption on ion exchange resin made their separation and purification easy.

Dehydroprolinamide gave positive tests for olefin and amide functions. The formation of 3,4-dehydroproline on acid hydrolysis showed that the double bond position in III and IV was identical. The amide absorbed one mole of hydrogen over platinum, yielding DL-prolinamide.

Dehydroproline gave positive tests for unsaturation, and a yellow ninhydrin color. It was quantitatively reduced by platinum and one mole of

(1) Visiting Scientist of the USPHS, 1959–1961.

(2) *Cf.* A. V. Robertson and B. Witkop, *J. Am. Chem. Soc.*, **82**, 5008 (1960).

(3) (a) J. E. Francis and B. Witkop, unpublished; (b) E. Fischer and F. Gerlach, *Ber.*, **45**, 2453 (1912).

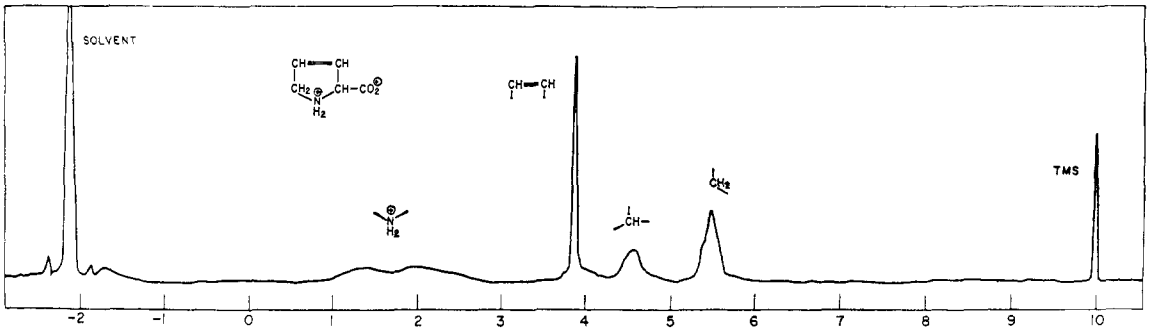
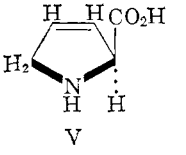


Fig. 1.—Proton magnetic resonance spectrum of 3,4-dehydroproline in trifluoroacetic acid solution with tetramethylsilane as internal reference. The scale is in τ-values.

hydrogen to DL-proline. These facts and the analyses prove that the compound was one of the isomeric pyrroline-2-carboxylic acids. Nuclear magnetic resonance spectroscopy provided decisive evidence for the Δ^3-position of the double bond (Fig. 1). The Δ^1-, Δ^2- and Δ^5-structures are excluded by a peak for two olefinic protons at τ 3.88. The Δ^4-structure is excluded by the peak at τ 5.49 for two protons adjacent to nitrogen. These two protons and the single proton alpha to the carboxyl (peak at τ 4.66) are allylic to the Δ^3-olefin and their signals thus appear at lower field than the corresponding proton peaks in proline (CH_2–N, 6.30; α-H, 5.26[4]). The n.m.r. spectrum of 3,4-dehydroprolinamide has signals almost identical with those in dehydroproline for protons attached to carbon.

The optical enantiomorphs of 3,4-dehydroproline were needed for our chemical and biochemical program. The most convenient method of resolution appeared to be asymmetric enzymatic hydrolysis[5] of dehydroprolinamide. Hog kidney amidase[6] slowly hydrolyzed 3,4-dehydro-DL-prolinamide, but in contrast to all previous examples of enzymatic resolution hydrolysis did not stop at 50% of total racemate. The best conditions used for resolution of prolinamide[7] were also optimal for the dehydro compound, except that activation of the enzyme with magnesium gave cleaner products than with manganese. With fresh enzyme 50% of racemate was cleaved in about 30 hours. From various experiments in which the reaction mixture was worked up after 40–60% hydrolysis (1–2 days' reaction time), dehydroproline was isolated with a range of $[\alpha]^{20}D$ $-370°$ to $-385°$ in water. When the reaction was left for four days with addition of further enzyme on the third day, 75% of the weight of starting material was isolated as dehydroproline with $[\alpha]^{20}D$ $-362°$. Dehydroprolinamide recovered from all runs was either optically inactive or had small dextrorotation ($< +30°$). The best rationalization of these results was that excess of D-amide remaining after specific hydrolysis of some L-form was optically labile, and racemized on standing to provide additional L-amide as substrate for the enzyme. This assumption was confirmed by the chemical resolution of 3,4-dehydro-DL-prolinamide through the (+)-α-bromocamphor-π-sulfonate salt. The dextrorotatory enantiomorph of the amide formed the less soluble diastereoisomer and decomposition of the salt gave dehydroprolinamide of varying optical activity, in some experiments as high as $[\alpha]^{20}D$ $+320°$ in water. At 20° the rotation of these aqueous solutions (*p*H 9.5) decreased with a half-life time of 48 hours. At 100° the rotation was zero in 30 minutes. From such experiments racemic 3,4-dehydroprolinamide was recovered quantitatively. This excludes double bond migration. The hydrochloride and bromocamphorsulfonate of (+)-dehydroprolinamide are optically stable. The easy racemization of the (+)-amide is independent evidence for the position of the double bond: only the Δ^3-structure could be both optically active and optically unstable, racemization occurring by base-catalyzed abstraction of the labile allylic hydrogen alpha to the amide carbonyl.

3,4-Dehydroproline having $[\alpha]^{20}D$ $-378°$ gave L-proline with $[\alpha]^{20}D$ $-82°$ on catalytic hydrogenation indicating an optical purity of 95–96%; optically pure L-proline has $[\alpha]D$ $-86°$.[7,8] Formation of L-proline confirms the absolute configuration V for 3,4-dehydro-L-proline already anticipated on the basis of enzyme specificity. The Lutz–Jirgen-



V

sons[9] rule applies to dehydro-L-proline, since a specimen having $[\alpha]D$ $-385°$ in water had $[\alpha]D$ $-257°$ in 5 *N* hydrochloric acid. Assay of this sample with D-amino acid oxidase[10] showed the presence of 3% of dehydro-D-proline. Attempts to obtain optically pure dehydro-L-proline by removal of the trace of D-form through fractional

(4) F. A. Bovey and G. V. D. Tiers, *J. Am. Chem. Soc.*, **81**, 2870 (1959).

(5) J. P. Greenstein, *Adv. Protein Chem.*, **9**, 174 (1954).

(6) S. M. Birnbaum, "Methods in Enzymology," Vol. II, Academic Press, Inc., New York, N. Y., 1955, p. 397.

(7) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951).

(8) Catalytic reduction of 3,4-dehydroproline in the presence of gaseous deuterium leads to randomization of deuterium in the proline so formed, as evidenced by mass-spectrophotometric analysis, obtained through the courtesy of Dr. K. Biemann, Mass. Institute of Technology. Conditions are being studied to avoid such randomization in order to make available selectively tritiated L-proline (*cf.* ref. 2).

(9) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931).

(10) A. Meister, L. Levintow, R. B. Kingsley and J. P. Greenstein, *J. Biol. Chem.*, **192**, 535 (1951).

crystallization have so far been unsuccessful, because even the amino acid is noticeably unstable optically and the racemate is less soluble than the enantiomorph. The optical activity of solutions of dehydro-L-proline in water (*p*H 6) at 20° was unchanged after a week, but at 90° the rotation decreased by 10% per day. Racemization is base-catalyzed, the half-life time at 20° being 17 days at *p*H 11.5, and 9.5 days at *p*H 14. Recovery of racemic dehydroproline after heating the (−)-compound in alkali showed that the double bond did not migrate under these conditions. At 38° and *p*H 8 the optical activity decreased by 5% in 2 days and 10% in 4 days, which accounts exactly for the presence and quantity of dehydro-D-proline in the various specimens from enzymatic resolution. Attempts to obtain pure dehydro-L-proline by destroying the small contamination of D-form with D-amino acid oxidase[11] failed. Pyrrole-2-carboxylic acid is formed and about 3% of dehydro-D-proline can be oxidized before enzyme action ceases due to the powerful inhibition by the product.[11] However, the optical rotation of the (−)-dehydroproline did not increase because racemization during the enzymatic reaction and subsequent isolation offset any possible gain in purity due to enzymatic removal of the D-antipode.

(+)-3,4-Dehydroprolinamide, $[\alpha]_D$ +300°, was reduced in acid solution to prolinamide hydrochloride, $[\alpha]_D$ +59°, corresponding to an optical purity of 84%. This confirms the D-configuration of dextrorotatory dehydroprolinamide; optically pure D-prolinamide has $[\alpha]_D$ +70°.[7] The chemical resolution is not efficient because the solubility difference of the diastereoisomers is not great. Resolution of dehydroprolinamide with tartaric acid was not satisfactory.

Snake venom L-amino acid oxidase converts 3,4-dehydro-L-proline quantitatively to pyrrole-2-carboxylic acid. This process can be used for a spectrophotometric assay of the enzyme which is much faster than previous manometric methods.[12] The reaction is noteworthy because dehydro-L-proline is the first example of an imino acid or N-alkyl amino acid to be oxidized by this enzyme. Failure of molecules such as proline to be attacked must therefore depend on reasons more subtle than simple inability to be accommodated on the enzyme surface.

The present work is the first example of enzymatic resolution combined with asymmetric transformation[13] of an optically labile substrate. The slight optical instability of dehydro-L-proline, formation of gummy side products and denaturation of the crude amidase during the long reaction time conspire to prevent total conversion of racemic dehydroprolinamide to optically pure dehydro-L-proline. The rate of asymmetric enzymatic hydrolysis of amides of the proline type is very slow.[6] It may be possible to find systems in which the optical lability of the substrate, its rate of hydrolysis and the optical stability of the product are so balanced as to afford a pure enantiomorph quantitatively from a racemic derivative.

Discussions of dissymmetry in connection with the origin of life[14] have largely avoided the question of what happens to the "unnatural" isomers which would accumulate as a result of the various proposed resolution procedures. In many living systems the presence of specific destructive enzymes (*e.g.*, D-amino acid oxidases) may serve this particular purpose.[15] But in a general sense the complete conversion of the racemate of an optically labile substance to a single isomer of an optically more stable compound by an asymmetric force (not necessarily an enzyme) is an idea of considerable theoretical interest. The dehydroproline case provides an experimental precedent, although for reasons stated above the conversion is not quantitative.

The reducing system phosphonium iodide and hydriodic acid used to be a standard method in classical organic chemistry.[16] It has not found much use in recent times, possibly because the reagents are somewhat unpleasant to prepare and to handle. Nevertheless, the reduction reaction is straightforward and requires no complex apparatus. The selective, clean and efficient conversion of pyrrole-2-carboxamide to the 2,5-dihydro derivative warrants a revival of interest in phosphonium iodide and hydriodic acid and encourages further application.[17]

Antimetabolic Effects.—3,4-Dehydro-L-proline at a concentration of 0.5 part per million still causes noticeable inhibition of growth in growing carrot phloem explant tissue cultures,[18] and in this respect surpasses hydroxy-L-proline by a factor of ten.[19] The reversible competitive inhibition is counteracted by the addition of L-proline. 3,4-Dehydroproline is a powerful proline antimetabolite for several microörganisms.[20]

As Dr. E. Katz found in these Institutes 3,4-dehydro-L-proline, added to the culture medium of *Streptomyces antibioticus*, significantly changed the normal composition[21] of the actinomycin complex in such a way that the biosynthesis of actinomycine II (containing *inter alia* 4 sarcosines and *no* proline) and III (containing 3 sarcosines and one proline) was favored. This would again point to dehydroproline as an inhibitor of the incorporation of proline, an aspect which is under investigation in mammalian systems at the Institutes.

(11) J. R. Parikh, J. P. Greenstein, M. Winitz and S. M. Birnbaum, *J. Am. Chem. Soc.*, **80**, 953 (1958).

(12) H. H. Weissbach, A. V. Robertson, B. Witkop and S. Udenfriend, *Anal. Biochem.*, **1**, 286 (1960).

(13) *Cf.* M. M. Harris, "Progress in Stereochemistry," Vol. 2, Academic Press, Inc., New York, N. Y., 1958, p. 157.

(14) *E.g.*, "The Origin of Life on Earth," Proceedings of Moscow Symposium, 1957. English edition edited by F. Clark and R. L. M. Synge, Pergamon Press, New York, N. Y., 1959, pp. 95, 154.

(15) W. Kuhn, *Adv. in Enzymol.*, **20**, 1 (1958).

(16) *E.g.*, J. Houben, "Die Methoden der Organischen Chemie," Vol. 2, 3rd edition, Georg Thieme, Leipzig, 1925, p. 456.

(17) For example, the method may facilitate the preparation of the notoriously unstable porphyrinogens (H. Fischer and A. Stern, "Die Chemie des Pyrrols," Vol. II, pt. 2, Leipzig, 1940, p. 420) which have recently been shown to be intermediates in the biosynthesis of heme and chlorophyll (D. Mauzerall and S. Granick, *J. Biol. Chem.*, **232**, 1141 (1958)).

(18) F. C. Steward, Department of Botany, Cornell University, personal communication.

(19) F. C. Steward, J. K. Pollard, A. A. Patchett and B. Witkop, *Biochim. Biophys. Acta*, **28**, 308 (1958).

(20) C. G. Skinner, Department of Chemistry, University of Texas, personal communication.

(21) *Cf.* E. Katz and W. A. Goss, *Nature*, **182**, 1668 (1958).

Whether it is incorporated into the peptide part of the antibiotic remains to be seen.[22]

Acknowledgment.—We are greatly indebted to Dr. S. M. Birnbaum for his valuable assistance with the enzymatic resolution and assays. We thank Mr. Robert Bradley for taking the n.m.r. spectra.

Experimental[23]

Synthesis of 3,4-Dehydro-DL-proline and 3,4-Dehydro-DL-prolinamide.—Pyrrole-2-carboxylic acid[24] was converted to pyrrole-2-carboxamide[25] *via* the acid chloride. An ellipsoidal Teflon-covered magnet (0.75 × 1.5″) and 250 ml. of 57% hydriodic acid were placed in a 1-l. round-bottom flask with a 24/40 ground glass neck and the solution was saturated with hydrogen iodide[26] at −20° (acetone–Dry Ice cooling bath). The flask was supported above a powerful magnetic stirrer (no cooling bath), stirring was started and quickly crushed phosphonium iodide[27] (25 g.) and finely powdered pyrrole-2-carboxamide (45 g.) were added. A greased heavy glass stopper was wired on tightly (Corning stopper No. 7620 was the most convenient). These operations were done as rapidly as possible to prevent loss of hydrogen iodide. The solids are not appreciably soluble and a reaction complex develops which is thickest after about 30 min. and which then gradually dissolves within about 2 hr. leaving a deep brown solution. Reduction is complete when the complex has dissolved; a few undissolved crystals of cubic shape are phosphonium iodide. The pressure of hydrogen iodide which is generated as the reaction mixture slowly warms up to room temperature must be maintained. If gas escapes, the reaction time is longer, the over-all yield is lower and more amide is hydrolyzed. When the reaction complex had dissolved, the flask was quickly cooled below 0°, the stopper was cautiously removed and the contents were poured onto an equal volume of a mixture of ice and water. The solution was evaporated as rapidly as possible at 1 mm. and 45° (bath) on a rotary evaporator with a train of Dry Ice–acetone condensing traps. The resulting semi-crystalline yellow paste was dissolved in water (500 ml.) and chromatographed on Dowex 50W-X8 resin in the hydrogen ion form (600 ml., 50–100 mesh). The column was washed with water until the eluate was colorless, neutral and free of iodide ion. Dehydroproline and its amide were then eluted with 2 *N* ammonium hydroxide as a slightly yellow fraction which was collected from the time the eluate became alkaline until it was colorless (*ca.* 700 ml.). Removal of solvent *in vacuo* left a light-yellow crystalline mass (36 g., 80%) of the amino acid and its amide which was dried overnight in a desiccator. The mixture was extracted with three lots of boiling absolute ethanol containing 10% of benzene. **3,4-Dehydro-DL-proline** (4 g.) remained as a white crystalline powder. Recrystallization from water (2 parts) and ethanol (at least 10 parts) yielded beautiful prisms, m.p. 236–237°.

Anal. Calcd. for $C_5H_7NO_2$: C, 53.06; H, 6.24; N, 12.39. Found: C, 53.43; H, 6.52; N, 11.83.

The combined ethanol–benzene extracts were evaporated to dryness leaving **3,4-dehydro-DL-prolinamide** (32 g.) of high purity. Traces of dehydroproline were removed by chromatographing the amide in aqueous solution on Dowex 1-X8 in the hydroxide ion form (200 ml., 50–100 mesh). The amide was washed through with water, collecting eluate from the time it became alkaline until it was again neutral. After evaporation of solvent and recrystallization from benzene, 3,4-dehydroprolinamide was obtained as white needles, m.p. 95–96°. The compound slowly becomes yellow on standing.

Anal. Calcd. for $C_5H_8N_2O$: C, 53.55; H, 7.19; N, 24.99. Found: C, 53.82; H, 7.01; N, 24.74.

A small quantity of dehydroproline was recovered from the Dowex 1 column by elution with 2 *N* acetic acid.

Dehydroproline and its amide decolorized bromine water instantly. The amino acid gave a yellow ninhydrin color and the amide liberated ammonia in hot aqueous sodium hydroxide. In water the ultraviolet spectrum of both compounds showed only end absorption.

N.m.r. Spectra.—The 60 Mc./sec. spectrum of 3,4-dehydroproline in trifluoroacetic acid with internal tetramethylsilane as reference showed peaks for protons on carbon at τ 3.88 (2 protons), 4.66 (1 proton) and 5.49 (2 protons) p.p.m. The amide spectrum had peaks of similar relative area at τ 3.87, 4.66 and 5.50 p.p.m.

3,4-Dehydro-DL-prolinamide hydrochloride crystallized when 10 *N* hydrochloric acid (1 ml.) was added to the amide (1 g.) in cold ethanol (10 ml.). Recrystallization from ethanol gave colorless needles, m.p. 190–192° dec.

Anal. Calcd. for $C_5H_8N_2O \cdot HCl$: C, 40.40; H, 6.10; Cl, 23.85; N, 18.85. Found: C, 40.41; H, 6.25; Cl (ionic), 23.54; N, 18.37.

Hydrolysis of 3,4-Dehydroprolinamide.—Dehydroprolinamide (100 mg.) was heated on the steam-bath (2 hr.) in 5 *N* hydrochloric acid (5 ml.). The solvent was removed *in vacuo* and the residue was chromatographed on Dowex 50W in the hydrogen ion form (5 ml.). The resin was washed with water until free of chloride ion and then eluted with 2 *N* ammonium hydroxide (20 ml.). Evaporation of solvent left a crystalline residue which had m.p. 236–237° after recrystallization from water–ethanol. It was identified as 3,4-dehydroproline by mixed m.p., infrared spectrum (Nujol) and paper chromatography.

Catalytic Reduction of 3,4-Dehydroproline and 3,4-Dehydroprolinamide.—One molar equivalent of hydrogen was rapidly absorbed when 3,4-dehydroproline (100 mg.) in 2 *N* hydrochloric acid (2 ml.) was shaken with Adams catalyst (20 mg.). The reaction mixture was filtered and percolated through Dowex 50W in the hydrogen ion form (5 ml.). The column was washed with water to remove chloride ion completely, and the amino acid was eluted with 2 *N* ammonium hydroxide. The residue obtained by removal of solvent was recrystallized from methanol–ether and identified as **DL-proline**[28] by paper chromatography and infrared spectrum (Nujol). The reduction of 3,4-dehydroproline in the presence of deuterium or tritium under these conditions leads to proline samples in which there is considerable randomization of H^2 and H^3. Experiments in progress aim at the synthesis of selectively deuterated and tritiated prolines.

Hydrogenation of 3,4-dehydroprolinamide (50 mg.) in ethanol (5 ml.) in the presence of Adams catalyst (10 mg.) was rapid and quantitative. The reaction mixture was filtered and the residue obtained by evaporating the solvent was recrystallized from benzene–cyclohexane; m.p. 99°. Its infrared spectrum (Nujol) was identical with that of authentic **DL-prolinamide.**[28]

Anal. Calcd. for $C_5H_{10}N_2O$: C, 52.61; H, 8.83; N, 24.54. Found: C, 52.27; H, 9.14; N, 24.52.

Enzymatic Hydrolysis of 3,4-Dehydro-DL-prolinamide.—The amidase fraction from hog kidney was prepared as described by Birnbaum.[7] In preliminary experiments the conditions of *p*H and concentration used for resolution of prolinamide[8] were found suitable for dehydroprolinamide. Hydrolysis in the absence of metal ion activation was very slow and small yields of dehydroproline with optical activity in the range $[\alpha]^{20}D$ −250 to −350° were obtained in various experiments depending on the reaction time. Enzyme activation with *manganese* ions resulted in much faster hydrolysis, but the products were difficult to separate from colored impurities formed by general decomposition. Activation with *magnesium* ions was satisfactory.

In a typical experiment, 3,4-dehydro-DL-prolinamide (28 g., 0.25 *M*) was dissolved in water (*ca.* 500 ml.) and brought to *p*H 8.0 by addition of glacial acetic acid. Meanwhile the lyophilized enzyme preparation derived from 2 kg. of hog kidney was dissolved in water (*ca.* 300 ml.) containing magnesium chloride hexahydrate (2.0 g., 0.01 *M*) and incubated at 38° (1 hr.). The amide and enzyme solutions

(22) E. Katz, A. V. Robertson, S. Udenfriend and B. Witkop, unpublished.

(23) Melting points are corrected. 1-Dm. polarimeter tubes were used unless stated otherwise.

(24) E. E. Smissman, M. B. Graber and R. J. Winzler, *J. Amer. Pharm. Assoc.*, **45**, 509 (1956).

(25) E. Fischer and D. D. Van Slyke, *Ber.*, **44**, 3166 (1911).

(26) Hydrogen iodide was generated as described by A. I. Vogel, "Practical Organic Chemistry," Longsmans, Green and Co., 2nd edition, 1951, p. 178; 150 g. of red phosphorus was used and about 450 ml. of a solution of iodine in 57% hydriodic acid (2:1, w./w.) was required.

(27) J. B. Work, *Inorg. Syntheses*, **2**, 141 (1946).

(28) V. E. Price, L. Levintow, J. P. Greenstein and R. B. Kingsley, *Arch. Biochem.*, **26**, 92 (1950).

were mixed, made up to 1 l. with water and incubated. The progress of the hydrolysis was followed on aliquots by titration of ammonia produced.[7] Acetic acid was added from time to time to adjust the *p*H to 8.0. The amide was 50% hydrolyzed after 25 hr. and 60% after 43 hr. The rate of hydrolysis is much slower with enzyme more than a week old. The solution was adjusted to *p*H 5 with acetic acid in order to precipitate most of the protein, stirred with charcoal and filtered. The filtrate was chromatographed on Dowex 50W in the hydrogen ion form (300 ml.), and the column was washed with water until the washings were neutral and colorless. The products were removed by eluting with 2 *N* ammonium hydroxide and after removal of solvent the residue was dissolved in water and chromatographed on Dowex 1 in the hydroxide ion form (300 ml.). Optically inactive 3,4-dehydroprolinamide (6 g.) was recovered by washing the column with water. Elution with 2 *N* acetic acid then gave 3,4-dehydroproline (17 g.), m.p. 236–237°, $[\alpha]^{20}D$ −375° (*c* 2, H_2O).

Anal. Calcd. for $C_5H_7NO_2$: C, 53.06; H, 6.24; N, 12.39. Found: C, 53.34; H, 6.51; N, 12.39.

Attempts to increase the specific rotation of samples with $[\alpha]D > -370°$ by recrystallization from water–ethanol failed. Fractional crystallization of dehydroproline with $[\alpha]^{20}D$ −250° gave racemic dehydroproline as the first crop and material in the filtrate with $[\alpha]^{20}D$ −370°. Further examination of recrystallization filtrates becomes impractical because of the accumulation of colored impurities due to decomposition.

3,4-Dehydroproline (226 mg., 2 mmole) with $[\alpha]D$ −378° was hydrogenated as for the racemic compound. Evaporation of the ammonium hydroxide eluate gave proline, $[\alpha]^{20}D$ −82° (*c* 2, H_2O).

Dehydroproline having $[\alpha]^{20}D$ −385° (*c* 1, H_2O, 2-dm. tube), $[\alpha]^{20}D$ −257° (*c* 1, 5 *N* HCl, 2-dm. tube) contained 3% of 3,4-dehydro-D-proline by manometric assay with D-amino acid oxidase.[29] Pyrrole-2-carboxylic acid was identified as the product of oxidation by its ultraviolet absorption, λ_{max} 263 mμ, after deproteinizing the reaction mixture at *p*H 5. It was formed quantitatively.

Optical Stability of 3,4-Dehydro-L-proline.—Solutions of dehydroproline (2%) in water (*p*H 6) were unchanged after a week at 20°, but at 20° in 2 *N* ammonium hydroxide (*p*H 11.5) and 1 *N* sodium hydroxide (*p*H 14) rotations decreased with a half-life time of 17 and 9.5 days, respectively. Stoppered all-glass tubes were used for the experiments at higher temperatures and measurements were taken at 20° after cooling the sample momentarily to enable solvent loss by evaporation to be replaced. At 90° in water the rotation decreased by *ca.* 10%/day ($t_{1/2}$ = 6 days) and at 38° in ammonium acetate buffer of *p*H 8.0 the decrease was 5% in 2 days and 10% in 4 days.

(29) Assayed by Dr. S. M. Birnbaum.

A solution of 3,4-dehydroproline (165 mg., $[\alpha]D$ −256° in water) in 1 *N* sodium hydroxide (8 ml.) was optically inactive after being heated on the steam-bath for 2 hr. The solution was percolated through Dowex 1 in the hydroxide ion form (10 ml.). The column was rinsed with water until the washings were neutral and the amino acid was eluted with 2 *N* acetic acid. Removal of solvent left a crystalline, slightly yellow residue, m.p. 234–236° after crystallization from ethanol–water. The infrared spectrum (Nujol) was identical with that of authentic **3,4-dehydro-DL-proline**.

Chemical Resolution, Optical Stability and Optical Purity of 3,4-Dehydroprolinamide.—Ammonium (+)-α-bromocamphor-π-sulfonate (21.2 g., 65 mmole) and 3,4-dehydro-DL-prolinamide (7.3 g., 65 mmole) were dissolved in a small volume of water and evaporated several times *in vacuo* with small volumes of methanol to remove ammonia completely. The residue was dissolved in 200 ml. of hot ethanol and 200 ml. of hot benzene was added. The mixture was concentrated to half the volume by boiling and was left overnight. The crystals of (+)-3,4-dehydroprolinamide α-bromocamphor-π-sulfonate (5.42 g., 20%), $[\alpha]^{20}D$ +154° (*c* 2, H_2O), were collected. Recrystallization by the same procedure gave material with $[\alpha]^{20}D$ +160°, m.p. 240–242° dec.

Anal. Calcd. for $C_{15}H_{23}BrN_2OS$: C, 42.56; H, 5.47; Br, 18.89; N, 6.62. Found: C, 42.73; H, 5.57; Br, 18.80; N, 6.71.

The optical rotation could not be increased by further crystallization. No change was observed in the optical activity of aqueous solutions of the salt during 1 week at 20°.

The dextrorotatory salt (4.23 g., 10 mmole) from the first crop was dissolved in water (20 ml.) and percolated through Dowex 1 in the hydroxide ion form (50 ml.). The amide passed through immediately and the column was washed with water for complete recovery. The aqueous solution was evaporated as quickly as possible *in vacuo* leaving white, crystalline 3,4-dehydroprolinamide (1.067 g., 95%), $[\alpha]^{20}D$ +300° (*c* 2, H_2O). Recrystallization from benzene caused the specific rotation to drop to +260°. The optical activity of the amide in water (solution *p*H 9.5) decreased at 20° with a half-life time of 48 hr. The aqueous solution was racemized after 30 min. heating on the steam-bath. Removal of solvent left racemic 3,4-dehydroprolinamide, m.p. 93–95°, having infrared absorption in chloroform indistinguishable from that of authentic material.

3,4-Dehydroprolinamide (112 mg., $[\alpha]D$ +300°) in ethanol (5 ml.) and 1 *N* hydrochloric acid (2 ml.) was hydrogenated over Adams catalyst (50 mg.). After rapid, quantitative uptake, the catalyst was filtered off and the filtrate was evaporated leaving crystalline prolinamide hydrochloride having $[\alpha]^{20}D$ +59° (*c* 2, EtOH).