Stereoselective effects in the solid-phase hydrogenation of unsaturated L-hydroxyproline derivatives

Yu. A. Zolotarev,[a] E. V. Laskatelev,[b] V. S. Kozik,[a] E. M. Dorokhova,[a] S. G. Rozenberg,[a] Yu. A. Borisov,[b] and N. F. Myasoedov[a]*

[a]Institute of Molecular Genetics, Russian Academy of Sciences, pl. Kurchatova, 123182 Moscow, Russian Federation. Fax: 007 (095) 196 0221. E-mail: zolotyua@img.ras.ru

[b]A. N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 28 ul. Vavilova, 117813 Moscow, Russian Federation. Fax: 007 (095) 135 5085. E-mail: compch@ineos.ac.ru

The solid-phase catalytic hydrogenation of (*R*)-4-*tert*-butoxy-Δ^1-pyrroline-2-carboxylic acid under the action of hydrogen spillover was studied. The reaction proceeds stereoselectively with the predominant formation of the L-amino acid. The configuration of the asymmetric center formed is determined by that of the asymmetric C(4) atom. The major portion of the isotope label is incorporated into the allylic C(3) and C(5) positions, and the β-H atoms are more mobile. Using quantum-chemical calculations, the geometric structure of the L-hydroxyproline molecule was calculated, and the spin-spin coupling constants for this tritium-labeled amino acid were determined.

Key words: solid-phase hydrogenation; quantum-chemical calculation; stereochemistry of addition; ^3H NMR; hydrogen spillover.

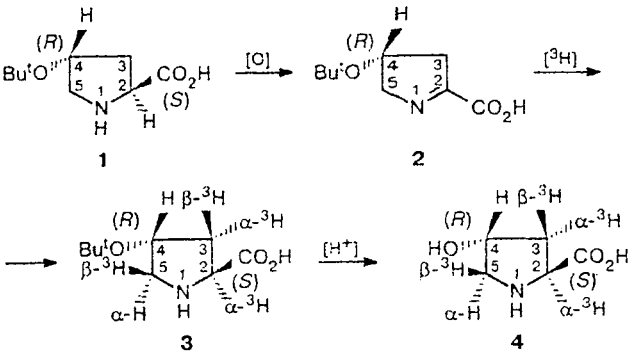
Tritium labeling of amino acids is widely used in medical biological studies. A multiple tritium label, which decreases the limits of determination of labeled compounds, is especially valuable. Liquid-phase catalytic reduction of unsaturated compounds-precursors is the main method for the incorporation of tritium into aliphatic amino acids.[1] If this reaction is carried out in a solid mixture of a finely dispersed platinum group metal, an inorganic support, and an unsaturated amino acid derivative, the degree of incorporation of tritium into the amino acid increases.[2,3] Solid-phase catalytic hydrogenation (SCH) can be performed in the absence of a direct contact between the catalyst and the substrate. The transfer of hydrogen activated on the catalyst to the reaction zone is called hydrogen spillover.[4] Asymmetric cyclohexanols were obtained by the reaction of hydrogen spillover (HS) with asymmetric crystals of substituted phenol.[5] In this work, the stereochemistry of the solid-phase hydrogenation of the diastereotropic multiple bond in (*R*)-4-*tert*-butoxy-Δ^1-pyrroline-2-carboxylic acid under the action of HS was studied.

Experimental

The studied transformations of L-hydroxyproline derivatives are presented in Scheme 1.

Synthesis of (*R*)-4-*tert*-butoxy-Δ^1-pyrrolin-2-carboxylic acid (2). (*R*)-4-*tert*-Butoxy-L-proline **1** (0.56 g, Bachem) was added to a 0.3 *M* solution of $CuSO_4$ (5 mL). Then 5 *M* KOH

Scheme 1



(6 mL) was added dropwise with stirring at $T \leq 20$ °C followed by 33% H_2O_2 (1 mL) (over 15 min). After 15 min, MnO_2 (30 mg) was added to decompose the excess of H_2O_2, and the mixture was stirred for 15 min. The solution was filtered, and 25% H_2SO_4 (4 mL) was added dropwise to the filtrate ($T \leq 20$ °C) to pH 7. An equal volume of EtOH was added to the solution, the precipitate of K_2SO_4 that formed was filtered off, and the filtrate was concentrated *in vacuo* to give the Cu salt of acid **2** (0.38 g). To isolate acid **2**, the salt was dissolved in H_2O (5 mL), and the solution was passed through a column (8×50 mm) with Dowex A1 (Serva). The solution was concentrated *in vacuo*. Acid **2** (0.29 g, overall yield 52%) was obtained as a yellow oil. ^1H NMR (D_2O), δ: 1.25 (s, 9 H, Bu^t); 2.45 (m, 2 H, ABX, $H_2C(3)$); 3.98 (dd, 1 H, α-HC(5), ^3J = 9.1 Hz, ^3J = 2.46 Hz); 4.37 (t, 1 H, β-HC(5), $^2J_1$ = 9.1 Hz, $^3J_2$ = 9.1 Hz); 4.42—4.56 (m, 1 H, HC-4).

Translated from *Izvestiya Akademii Nauk. Seriya Khimicheskaya*, No. 10, pp. 1820—1823, October, 1997.

1066-5285/97/4610-1726 $18.00

Synthesis of 4-(2)-hydroxy-L-[G-^{3}H]proline (4). Following the procedure for the synthesis of tritium-labeled amino acids,[2] acid **2** (2 mg) was supported onto Al_2O_3 (40 mg) from an aqueous solution and mixed with the catalyst, 5% Pd on $BaSO_2$ (20 mg). The solid mixture was placed in a 10-mL tube, which was evacuated, filled with gaseous tritium to a pressure of 20 kPa, and heated at 100 °C for 1 h. Then the tube was cooled, evacuated, and flushed with hydrogen. Amino acid **3** was desorbed by 0.1 *N* aqueous NH_3 containing 20% EtOH. The solution was evaporated to dryness, then 20% aqueous EtOH was twice added, the solvent was evaporated to remove labile tritium, and [G-^{3}H](O-Bu^t)Hyp (**3**) was obtained. Chromatographic purification and determination of the tritium distribution were performed after removal of the *tert*-butyl ether group. This was carried out by treatment with 1 *M* hydrochloric acid at 20 °C for 1 h. Amino acid **4** was chromatographed on an Amberlite CG carboxyl cation-exchange resin (Serva) in the Cu^{2+}-form[3] using 0.1 *M* aqueous ammonia as the eluent. The solution of amino acid **4** was concentrated to obtain [G-^{3}H](O-Bu^t)Hyp (**3**) (148 mCi). The amino acid was separated into optical isomers by ligand-exchange chromatography on chiral sorbents.[6] L-[G-^{3}H]Hyp (**4**) (122 mCi) with a molar radioactivity of 50 Ci mol^{-1} was obtained in a chemical yield of 23%. D-[G-^{3}H]Hyp (20 mCi) was also isolated. The tritium distribution in the labeled amino acids was studied by ^{3}H NMR spectroscopy. The incorporation of tritium into amino acids was determined by liquid scintillation counting.

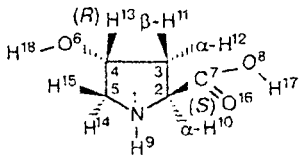
^{3}H NMR spectroscopy. ^{3}H and ^{1}H NMR spectra of solutions of amino acids in D_2O were recorded on a Bruker AC 250 spectrometer (266.8 and 250 MHz, respectively). The tritium-labeled amino acid (50 mCi) dissolved in D_2O (500 mL) was used for measurements.[7]

The refined data on stereochemistry of the isotope label distribution were obtained by a procedure described previously.[8]

Calculation methods. Geometric parameters of hydroxyproline were calculated by the semiempirical AM1 method realized in the AMPAC program.[9] More exact calculations were performed by the GAUSSIAN-94 program[10] in the Hartree—Fock approximation using the 3-21G basis. All calculations of molecular systems in the ground electronic state were performed with full optimization of geometry. The electron density distribution in a neutral amino acid molecule was analyzed according to Mulliken. A DEC AXP 3000-400 working station was used for calculations.

Results and Discussion

It is known that L,D-proline can be obtained by the catalytic hydrogenation of an aqueous solution of Δ^1-pyrroline-2-carboxylic acid.[11] The solid-phase catalytic hydrogenation of this compound using gaseous tritium resulted in the formation of L,D-[G-^{3}H]Pro with the incorporation of tritium exceeding the stoichiometric addition to the C=N bond.[2] The effect of the asymmetric C(4) atom on the stereochemistry of tritium incorporation was studied in the SCH of compound **2**. The results of quantum-chemical (*ab initio*) calculations of 4-hydroxy-L-proline were used for analysis of the ^{1}H and ^{3}H NMR spectra. The geometric parameters of this amino acid are presented in Table 1. The numeration of atoms is shown in the structure **5**.



5

The spin-spin coupling (SSC) constants of protons depend on the dihedral angles between C—H bonds.[12] The analysis of the ^{1}H NMR spectra with account of the geometry of the molecule allowed one to determine the SSC constants ($^3J_{10-11}$ = 10.1, $^3J_{10-12}$ = 7.9,

Table 1. Interatomic distances (r), bond angles (ω), and dihedral angles (φ) in the 4-hydroxy-L-proline molecule obtained by the HF 3-21G method

Distance	r/Å	Angle	ω/deg
N(1)—C(2)	1.49	N(1)—C(2)—C(3)	107.4
C(2)—C(3)	1.55	C(2)—C(3)—C(4)	103.9
C(3)—C(4)	1.54	C(3)—C(4)—C(5)	103.6
C(4)—C(5)	1.53	C(4)—C(5)—N(1)	103.2
C(5)—N(1)	1.49	C(5)—N(1)—C(2)	103.6
C(2)—C(7)	1.53	Angle	φ/deg
C(7)—O(8)	1.34	N(1)—C(2)—C(3)—C(4)	3.7
O(8)—H(17)	0.98	C(2)—C(3)—C(4)—C(5)	21.2
C(7)—O(16)	1.20	C(3)—C(4)—C(5)—N(1)	−39.9
C(4)—O(6)	1.45	C(4)—C(5)—N(1)—C(2)	42.3
O(6)—H(18)	0.97	α-H(10)—C(2)—C(3)—β-H(11)	−114.0
N(1)—H(9)	1.01	α-H(10)—C(2)—C(3)—α-H(12)	8.6
C(2)—α-H(10)	1.08	H(13)—C(4)—C(3)—β-H(11)	26.6
C(3)—β-H(11)	1.08	H(13)—C(4)—C(3)—α-H(12)	−95.0
C(3)—α-H(12)	1.08	H(13)—C(4)—C(5)—H(14)	76.4
C(4)—H(13)	1.08	H(13)—C(4)—C(5)—H(15)	−47.0
C(5)—H(14)	1.08	β-H(11)—C(3)—C(5)—H(14)	79.7
C(5)—H(15)	1.08	α-H(12)—C(3)—C(5)—H(14)	−163.7

Table 2. Tritium distribution in 4-hydroxy-L-[G-^{3}H]proline (**4**) obtained by the solid-phase hydrogenation of (*R*)-4-*tert*-butoxy-Δ^1-pyrroline-2-carboxylic acid (I) and solid-phase isotope exchange at 150 °C (II)

δ	Assignment	Distribution	
		I	II
2.10	3β	29	1
2.40	3α	20	6
3.30	5α	0	59
3.45	5β	35	21
4.25	2	16	9
4.65	4	0	4

Table 3. Tritium distribution in L,D-[G-^{3}H]proline with a molar radioactivity of 56 Ci mmol^{-1} obtained by the solid-phase hydrogenation of Δ^1-pyrroline-2-carboxylic acid at 100 °C

δ	Assignment	Tritium distribution (%)
1.95	4	0
2.05	3β	34
2.35	3α	34
3.35	5	6
4.25	2	26

$^2J_{11-12}$ = 13.3, $^3J_{11-13}$ = 4.0, $^3J_{12-13}$ = 1.6, $^4J_{12-14}$ = 1.6, $^3J_{13-15}$ = 3.2, $^2J_{14-15}$ = 9.8 Hz) and assign the peaks in the spectrum of 4-hydroxy-L-proline.

The reaction between compound **2** and activated tritium was performed with the spatial separation of the unsaturated compound and the heterogeneous catalyst. Thus, the hydrogenation involved HS. The addition at the diastereotropic multiple bond occurs stereoselectively. The content of the L-form exceeded that of the D-amino acid more than sixfold. The tritium distribution for compound **4** is presented in Table 2. The degree of substitution of the H atom by tritium at the C(2) atom was only 27%. It can be assumed that the addition of hydrogen at C(2) was due to the intramolecular transfer from the allylic positions of the molecule. The configuration of the asymmetric center appearing at the C(2) atom is determined by the asymmetric C(4) atom. The substitution of the hydrogen atoms at C(3) and C(5) by tritium is also stereoselective. The allylic β-H atoms that are located at the same side as the carboxyl group are predominantly substituted. It has been shown previously that the carboxyl group participates in the stabilization of the transition state in the isotope exchange of hydrogen in alanine involving HS.[13] It can be assumed that this group also participates in the stabilization of the transition state, which is formed when HS is added to the unsaturated compound.

The incorporation of tritium due to the isotope exchange of hydrogen accompanying the solid-phase hydrogenation can be compared to the tritium distribution obtained by the solid-phase catalytic isotope exchange. The refined data on the stereochemistry of the isotope label distribution are presented in Table 2. The substitution of hydrogen in the diastereotropic CH_2 groups of 4-hydroxy-L-proline is also stereoselective. However, the C—α-H bonds with the same arrangement relative to the plane of the ring as the C(4)OH group are more reactive. The mechanism of the solid-phase catalytic isotope exchange can be presented as the reversible monomolecular electrophilic interaction of HS in the form of a proton with the saturated C atom of the solid organic compound.[13] Positively charged complexes with the five-coordinate C atom can be formed in this interaction. The stability of the complexes with the proton at the C(3) and C(5) atoms of 4-hydroxy-L-proline was calculated by the quantum-chemical semiempirical AM1 method. The predominant substitution of the α-H atoms is related to the participation of the C(4)OH group in the stabilization of the complexes formed with the protons at C(3) and C(5).

The solid-phase catalytic hydrogenation of the Δ^1-pyrroline-2-carboxylic acid was carried out to elucidate the effect of the asymmetric C(4)OH group on the reactivity (Table 3). The ^{3}H NMR spectroscopic data were assigned as described previously.[14] The isotope label is incorporated predominantly into the allyl C(3) atom and is uniformly distributed between α-H(3) and β-H(3). The hydrogenation reaction results in the formation of racemic proline.

Thus, the solid-phase catalytic hydrogenation of (*R*)-4-*tert*-butoxy-Δ^1-pyrroline-2-carboxylic acid under the action of HS occurs stereoselectively with the predominant formation of the L-amino acid. The configuration of the asymmetric center appearing at the C(2) atom is determined by the asymmetric C(4) atom. The major portion of the isotope label is predominantly incorporated at the allylic C(3) and C(5) atoms, and the β-H atoms are more mobile. The observed stereoselectivity of hydrogen exchange at these C atoms can be explained by an additional stabilization of the intermediate product of the addition of one HS particle with the participation of the carboxyl group.

This work was carried out on a setup for work with multicurie quantities of gaseous tritium.

This work was financially supported by the Ministry of Science of the Russian Federation (Project No. 96-03-04), the International Science Foundation (Grant N6A 000), and the Russian Foundation for Basic Research (Project Nos. 94-03-09015 and 96-03-34443).

References

1. E. A. Evans, in *Tritium and Its Compounds*, Butterworth, London, 1974, 314.
2. Yu. A. Zolotarev, V. S. Kozik, E. M. Dorokhova, and N. F. Myasoedov, in *Proceedings of the Fourth International Symposium "Synthesis and Application of Isotopically Labelled Compounds,"* Eds. T. A. Baillie and J. R. Jones, Elsevier, Amsterdam, 1992, 498.
3. Yu. A. Zolotarev, V. S. Kozic, E. M. Dorochova, and N. F. Myasoedov, *J. Labelled Comp.*, 1991, **29**, 997.
4. P. A. Sermon, *Catalysis Reviews*, 1973, **8**, 211.
5. R. Lamatrine and R. Perrin, *Stud. Surf. Sci. Catal.*, 1983, **17**, 251.
6. Yu. A. Zolotarev, N. F. Myasoedov, V. I. Penkina, O. V. Petrenik, and V. A. Davankov, *J. Chromatography*, 1981, **207**, I, 231.
7. S. G. Rosenberg, Yu. A. Zolotarev, and N. F. Myasoedov, *Amino Acids*, 1992, **3**, 95.
8. Yu. A. Zolotarev, E. M. Dorokhova, V. N. Nezavibatko, Yu. A. Borisov, S. G. Rosenberg, and N. F. Myasoedov, *Amino Acids*, 1995, **8**, 353.
9. M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
10. C. C. J. Roothaan, *Rev. Mod. Phys.*, 1951, **23**, 69.
11. L. Macholan and J. Vencalkova, *Chem. Ber.*, 1963, **96**, 237.
12. *Modern NMR Techniques for Chemistry Research*, Ed. E. Derome, Pergamon Press, Oxford—New York, 1987.
13. Yu. A. Zolotarev, E. V. Laskatelev, V. S. Kozik, E. M. Dorokhova, S. G. Rozenberg, Yu. A. Borisov, and N. F. Myasoedov, *Izv. Akad. Nauk, Ser. Khim.*, 1997, 757 [*Russ. Chem. Bull.*, 1997, **46**, 726 (Engl. Transl.)].
14. E. A. Evans, D. C. Warreii, J. A. Elvidge, and J. R. Jones, *Handbook of Tritium NMR Spectroscopy and Applications*, J. Wiley and Sons, Chichester, 1985, 50.

Received December 27, 1996