Synthesis of Four Diastereoisomers of Histopine

Yasuo KITAJIMA,* Michinori WAKI, and Nobuo IZUMIYA
Laboratory of Biochemistry, Faculty of Science, Kyushu University 33, Higashi-ku, Fukuoka 812
(Received June 15, 1982)

Four isomers of histopine, N^2-(1-carboxyethyl)histidine, were prepared to provide their physicochemical properties. A diastereomeric mixture, (*RS*),(*S*)-histopine (N^2-[(*RS*)-1-carboxyethyl]-(*S*)-histidine), was synthesized from 2-oxopropionic acid and (*S*)-histidine by reduction with sodium cyanoborohydride. The mixture was separated into its components, (*S*),(*S*)-histopine and (*R*),(*S*)-histopine, by an ion-exchange column chromatography. In a similar manner (*R*),(*R*)-histopine and (*S*),(*R*)-histopine were prepared. Configurations of the four isomers obtained were assumed from their ORD data by comparison with those of the isomers of octopine (N^2-(1-carboxyethyl)arginine) with a definite configuration.

Histopine is a natural compound isolated from crown gall tissue inoculated with *Agrobacterium tumefaciens* B_6.[1] A number of opines structurally related to histopine have recently been found in crown gall tumors of plants.[2] Octopine (N^2-[(*R*)-1-carboxyethyl]-(*S*)-arginine) first isolated from octopus muscle[3,4] is representative of such opines. Although little is known concerning the significance of the presence of these opines in plants and animals, the use of them as markers of a possible transfer of gene from the tumor-inducing bacterium into host plant cells[2,5] has roused further interest in these compounds.

The chemical structure of histopine was established by Kemp as N^2-(1-carboxyethyl)histidine.[1] The configuration of natural histopine, however, has not been determined. Kemp assumed that the configuration of the histidine (His) part was (*S*) only based on the following fact: natural histopine showed the same chromatographic behavior on thin-layer or ion-exchange column as that of a diastereomeric mixture of histopine which was synthesized from (*S*)-histidine and (*RS*)-2-bromopropionic acid.[1]

A synthetic attempt leading to a diastereomeric mixture of histopine has been reported.[1] However, none of the optically pure isomers of histopine has been isolated and characterized. Even for natural histopine, its physicochemical properties (elemental analysis, melting point and specific rotation) have not been given. Thus we were interested in preparing four isomers of histopine in pure state to provide their physicochemical properties.

This paper first describes the preparation of (*RS*),(*S*)-histopine (N^2-[(*RS*)-1-carboxyethyl]-(*S*)-histidine) by sodium cyanoborohydride reduction of a mixture of 2-oxopropionic acid and (*S*)-histidine, subsequent separation into its components by column chromatography, and similar experiments with (*R*)-histidine. Second, the configurations of the four isomers of histopine are assumed by comparison of their ORD patterns with those of octopine isomers having a definite configuration.

Results and Discussion

*Synthesis of (*RS*),(*S*)-Histopine (*N*²-[(*RS*)-1-Carboxyethyl]-(*S*)-histidine) from (*S*)-Histidine.* Kemp reported the preparation of (*RS*),(*S*)-histopine from (*RS*)-2-bromopropionic acid and (*S*)-histidine in the presence of $Ba(OH)_2$ at 55 °C without describing the yield.[1]

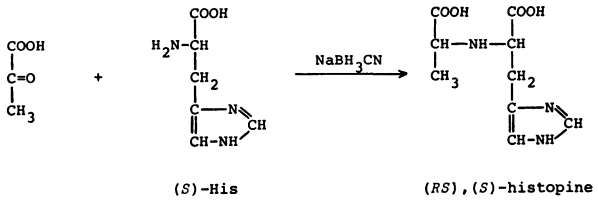

Fig. 1. Synthesis of a diastereomeric mixture of histopine from (*S*)-histidine.

Under these conditions, however, there is some risk of partial racemization of (*S*)-histidine part during the reaction. Recently Jensen *et al.* prepared a distereomeric mixture of nopaline (N^2-(1,3-dicarboxypropyl)arginine) in a high yield from 2-oxoglutaric acid and (*S*)-arginine by reduction with sodium cyanoborohydride ($NaBH_3CN$) under mild conditions at neutral pH and at room temperature.[6]

Taking advantages of this new reagent, $NaBH_3CN$, we intended to synthesize four isomers of histopine from 2-oxopropionic acid and (*S*)- or (*R*)-histidine by reductive amination (Fig. 1). We could obtain (*RS*),(*S*)-histopine in a high yield of 98% based on (*S*)-histidine after the reaction of 2-oxopropionic acid, (*S*)-histidine, and $NaBH_3CN$ in 5 : 1 : 3 molar ratio at pH 7.0 for 3 d. In a similar manner (*RS*),(*R*)-histopine was synthesized from (*R*)-histidine in a yield of 97%.

*Separation of (*RS*),(*S*)-Histopine into Components.* Separation of a diastereomeric mixture of octopinic acid (N^2-(1-carboxyethyl)ornithine) on an amino acid analyzer was already reported.[4] Then we tried to separate (*RS*),(*S*)-histopine into its components by an amino acid analyzer, and found the conditions to achieve the separation (see Experimental section). A faster eluting isomer was determined as (*S*),(*S*)-histopine, and a slower eluting isomer as (*R*),(*S*)-histopine by separate-runs using each pure isomer.

Preliminary experiments for separation of (*RS*),(*S*)-histopine were performed with a column of Dowex 50 at different concentration and pH in sodium citrate buffer system, in order to find optimal conditions for the preparative separation of histopine isomers. According to the above results (see Experimental section), we carried out preparative separation of (*RS*),(*S*)-histopine with a large column (1.8 cm × 190 cm) of Dowex 50. Thus (*S*),(*S*)-histopine with $[\alpha]_D$ +27.7° was isolated from the faster eluting fractions and (*R*),(*S*)-histopine

with $[\alpha]_D$ +30.2° from the slower eluting fractions respectively in a weight ratio of 1 : 1.7. In a similar manner, (*R*),(*R*)-histopine and (*S*),(*R*)-histopine were isolated by the column chromatography of (*RS*),(*R*)-histopine respectively in a weight ratio of 1 : 1.6. Interestingly each isomer in synthetic diastereomeric mixture was not in an equal ratio, which shows occurrence of diastereoselective reduction during the formation of diastereomeric mixture with $NaBH_3CN$.

Attempts to Prepare Histopine Isomers from (S)-Alanine. Chemical synthesis of histopine isomers was attempted by reactions starting from (*S*)-alanine to determine the configuration of the Ala part in histopine isomers. Biellmann *et al.* reported that octopine isomers were obtained from (*S*)-alanine and 2-oxo-5-guanidinopentanoic acid by reduction with $NaBH_3CN$.[7] In the present study, however, the reaction of (*S*)-alanine and 2-oxo-3-(5-imidazolyl)propionic acid with $NaBH_3CN$ under the same conditions gave no expected product. Changes of reaction conditions (*e.g.*, molar ratios of the reactants and pH) also resulted in no formation of histopine isomers. Instead, 2-hydroxy-3-(5-imidazolyl)-propionic acid was detected as a main product by ^1H NMR analysis of the reaction mixture. Previously, Izumiya *et al.* observed a similar result that palladium-catalyzed hydrogenation of (*S*)-alanine and 2-oxo-5-guanidinopentanoic acid did not produce octopine isomers.[8] We assume that easy formation of 2-hydroxy-3-(5-imidazolyl)propionic acid instead of the desired product is due to the instability of a Schiff's base formed from (*S*)-alanine and 2-oxo-3-(5-imidazolyl)-propionic acid or difficult formation of the Schiff's base because of the presence of basic imidazolyl group in the α-keto acid. Condensation of (*S*)-alanine and (*S*)-2-bromo-3-(5-imidazolyl)propionic acid derived from (*S*)-histidine was also unsuccessful.

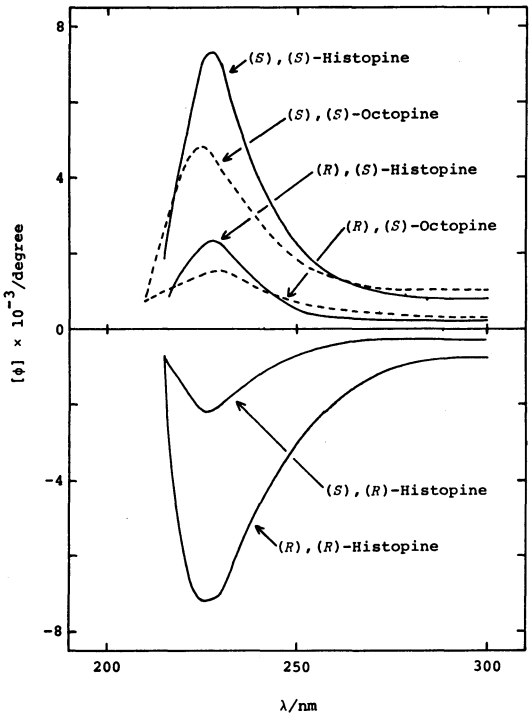

Fig. 2. ORD Curves of isomers of histopine and octopine in 5 M HCl.

Table 1. Configurations of diastereoisomers of histopine

Nomenclature	Configuration of Ala part	Configuration of His part	$[\alpha]_D$/° (5 M HCl)
(*S*),(*S*)-Histopine	(*S*)	(*S*)	+27.7
(*R*),(*S*)-Histopine	(*R*)	(*S*)	+30.2
(*R*),(*R*)-Histopine	(*R*)	(*R*)	−27.2
(*S*),(*R*)-Histopine	(*S*)	(*R*)	−29.8

Configurations of Four Diastereoisomers of Histopine. The configuration of the His part in histopine isomers obtained from 2-oxopropionic acid and (*S*)- or (*R*)-histidine is unequivocally defined, because the configuration of the His part is retained during the reduction with $NaBH_3CN$.

The configuration of the Ala part of histopine isomers was deduced from the results of ORD measurements (see Fig. 2) of four histopine isomers and two octopine isomers with a definite configuration as follows. ORD Pattern of (*S*),(*S*)-octopine (N^2-[(*S*)-1-carboxyethyl]-(*S*)-arginine)[4] is similar to that of a histopine isomer, which has an $[\alpha]_D$ value of +27.7°, prepared from (*S*)-histidine. Thus the configuration at the Ala part in this isomer of histopine is assumed to be (*S*), and the isomer is designated as (*S*),(*S*)-histopine as shown in Table 1. Similar ORD curves are obtained for (*R*),(*S*)-octopine[4] and for a histopine isomer, which has an $[\alpha]_D$ value of +30.2°, prepared from (*S*)-histidine. Thus the configuration of the Ala part in this isomer of histopine is assumed to be (*R*), and the isomer is designated as (*R*),(*S*)-histopine. It is apparent from Fig. 2 that histopine isomers, with $[\alpha]_D$ −27.2° and −29.8°, both prepared from (*R*)-histidine, are antipodes of (*S*),(*S*)- and (*R*),(*S*)-histopine, respectively.

Experimental

TLC was carried out on silica gel G (Merck) with the following solvent systems: R_f^1, *n*-BuOH–AcOH–pyridine–H_2O (4 : 1 : 1 : 2, v/v); R_f^2, MeOH–H_2O–pyridine (20 : 5 : 1, v/v). Paper chromatography shown as R_f^1 (PPC) was carried out on Toyo Roshi No. 52 paper with the same solvent system as for R_f^1. Material possessing an imidazolyl group was detected by spraying with the Pauly's reagent.[9] Other compounds on TLC were detected by spraying with 10% H_2SO_4, followed by heating on a hot plate. Optical rotations were measured on a Union high sensitivity polarimeter PM-71. Amino acid analyses were performed with a Hitachi amino acid analyzer KLA-5.

(RS),(S)-Histopine. A solution of (*S*)-histidine monohydrochloride (960 mg, 5 mmol) and freshly distilled 2-oxopropionic acid (2.20 g, 25 mmol) in water (7.5 ml) was adjusted to pH 7.0 with 2 M NaOH at 0 °C, and $NaBH_3CN$ (950 mg, 15 mmol) was added. After stirring for 3 d at room temperature, 12 M HCl (5 ml) was added to the reaction mixture. The solution was diluted with water and then put on a column (2.8 cm × 16.5 cm) of Dowex 50X8 (H^+ form), and the column was washed with water and eluted with 1 M aq pyridine (300 ml). The eluate was evaporated to give the desired product: yield, 1.11 g (98%); R_f^1 0.21, R_f^2 0.77, R_f^1 (PPC) 0.18. A part of the product was recrystallized from

water–EtOH for elemental analysis.

Found: C, 47.45; H, 5.76; N, 18.34%. Calcd for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.77; N, 18.49%.

(RS),(R)-Histopine. This was prepared from (*R*) histidine monohydrochloride and 2-oxopropionic acid as described above: yield, 97%; R_f^1 0.21, R_f^2 0.77.

Separation of (RS),(S)-Histopine into Components. By an Amino Acid Analyzer: A solution of (*RS*),(*S*)-histopine (3 mg) in 0.2 M sodium citrate buffer (0.5 ml) of pH 2.2 was subjected to the analyzer. The following conditions were sufficient to permit the separation of two isomers; a column with spherical fresin; 0.6 cm × 55 cm; buffer, 0.2 M sodium citrate buffer of pH 3.25; flow rate, 30 ml/h; jacket temperature, 55 °C. The eluate from the column was taken in a fraction collector (each fraction, 1-ml), the fractions being monitored as follows. An aliquot (0.1 ml) of each fraction was diluted with water (2.4 ml). To the solution, 10% Na_2CO_3 (1 ml) and a solution of the Pauly's reagent (0.5 ml)[9] were added. The absorbance of the mixture at 500 nm was measured on a Hitachi spectrophotometer 124. A peak for (*S*),(*S*)-histopine was at 32, ml and that for (*R*), (*S*)-histopine at 45 ml.

By Dowex 50 Column Chromatography: A column (0.9 cm × 100 cm) was packed with Dowex 50X8 (200—400 mesh; Na^+ form) equilibrated with a specified eluting solvent. The eluting solvents used were sodium citrate buffers at different concentrations and pHs as follows; 0.2 M at pH 2.51, 0.1 M at pH 3.25 or 0.2 M at pH 3.25. (*RS*),(*S*)-Histopine (6 mg) was applied to a column and eluted with one of these eluting solvents at room temperature. An aliquot (0.02 ml) of each fraction (2 ml) was spotted on a paper strip, and the spots containing histopine were visualized by spraying with the Pauly's reagent. Partial separations were observed with each buffer, however, the buffer of 0.2 M at pH 3.25 gave the most favorable pattern. (*RS*),(*S*)-Histopine was separated completely on a longer column (1.8 cm × 190 cm) with the buffer of 0.2 M at pH 3.25.

(S),(S)-Histopine. A solution of (*RS*),(*S*)-histopine (30 mg) in 0.2 M sodium citrate buffer (1 ml) of pH 3.25 was put on a column (1.8 cm × 190 cm) of Dowex 50X8 (Na^+ form). Elution was performed with the same buffer (each fraction, 8-ml) at a flow rate of 37 ml/h. The same chromatography was further repeated twice; thus total amount of 90 mg of (*RS*),(*S*)-histopine was separated into two components. The 3 faster eluting fractions 115—138 were combined and applied to a column (3.2 cm × 25 cm) of Dowex 50X8 (H^+ form). The column wa swashed with water and eluted with 2 M NH_3 aq (450 ml). The eluate was evaporated to afford a residue (33 mg), and the crude product was crystallized from water-EtOH to give pure (*S*),(*S*)-histopine: yield, 18 mg (20% from (*S*)-His); mp 252—253 °C (decomp); $[\alpha]_D^{20}$ +27.7° (*c* 1, 5 M HCl); R_f^1 0.21, R_f^2 0.53.

Found: C, 47.44; H, 5.80; N, 18.25%. Calcd for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.77; N, 18.49%'.

(R),(S)-Histopine. The 3 slower eluting fractions 143—165 obtained above were treated with Dowex 50X8 (H^+ form) as described for (*S*),(*S*)-histopine. Crude product (57 mg) was crystallized from water–EtOH: yield, 20 mg (22% from (*S*)-His); mp 240—241 °C (decomp); $[\alpha]_D^{20}$ +30.2° (*c* 1, 5 M HCl); R_f^1 0.21, R_f^2 0.53.

Found: C, 47.22; H, 5.83; N, 18.27%. Calcd for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.77; N, 18.49%.

(R),(R)-Histopine. This was prepared from (*RS*),(*R*)-histopine (90 mg) as described for the preparation of (*S*),(*S*)-histopine. The crude product (35 mg) obtained was crystallized from water–EtOH: yield, 25 mg (27% from (*R*)-His); mp 253—254 °C (decomp.); $[\alpha]_D^{20}$ −27.2° (*c* 1, 5 M HCl); R_f^1 0.21, R_f^2 0.53.

Found: C, 47.34; H, 5.90; N, 18.12%. Calcd for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.77; N, 18.49%.

(S),(R)-Histopine. This was prepared from the slower eluting fractions obtained above. The crude product (55 mg) was crystallized from water–EtOH: yield, 26 mg (28% from (*R*)-His); mp 241—242 °C (decomp); $[\alpha]_D^{20}$ −29.8° (*c* 1, 5 M HCl); R_f^1 0.21, R_f^2 0.53.

Found: C, 47.52; H, 5.76; N, 18.04%. Calcd for $C_9H_{13}O_4N_3$: C, 47.57; H, 5.77; N, 18.49%.

ORD Measurements. These were performed with a JASCO spectropolarimeter Model ORD/UV-5. A cell of the path length 0.1 cm was used and the runs were at ambient temperature. ORD Patterns of solutions of four histopine isomers, (*S*),(*S*)- and (*R*),(*S*)-octopine[4] in 5 M HCl (each, 1 mg/ml) are shown in Fig. 2.

We thank Dr. T. Kato and Dr. H. Aoyagi in this Laboratory for their helpful discussions.

References

1) J. D. Kemp, *Biochem. Biophys. Res. Commun.*, **74**, 862 (1977).
2) M. Drummond, *Nature*, **281**, 343 (1979).
3) K. Morizawa, *Acta Schol. Med. Univ. Imp. Kioto*, **9**, 285 (1927).
4) K. Goto, M. Waki, N. Mitsuyasu, Y. Kitajima, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, **55**, 261 (1982).
5) N. Murai and J. D. Kemp, *Proc. Natl. Acad. Sci. U. S. A.*, **79**, 86 (1982).
6) R. E. Jensen, W. T. Zdybak, K. Yasuda, and W. S. Chilton, *Biochem. Biophys. Res. Commun.*, **75**, 1066 (1977).
7) J. F. Biellmann, G. Branlant, and L. Wallén, *Bioorg. Chem.*, **6**, 89 (1977).
8) N. Izumiya, R. Wade, M. Winitz, M. C. Otey, S. M. Birnbaum, R. J. Koegel, and J. P. Greenstein, *J. Am. Chem. Soc.*, **79**, 652 (1957).
9) H. Pauly, *Hoppe-Seyler's Z. Physiol. Chem.*, **94**, 284 (1915).