# Configuration of Alanine Part in Natural Octopine[1)]

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Octopine has a structure of $N^2$-(1-carboxyethyl)arginine or *N*-(1-carboxy-4-guanidinobutyl)alanine. Configuration of the arginine part in natural octopine is determined as (*S*), whereas that of the alanine part has been assumed as (*R*). The configuration of the alanine part was concluded to be (*R*) by the present synthesis of (+)-octopine, which was identical with the natural one, by a sequence of reactions starting from (*R*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid. The reaction of (*R*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid in alkaline conditions gave $N^2$-((*R*)-1-carboxyethyl)-$N^5$-acetyl-(*S*)-ornithine which was converted to $N^2$-((*R*)-1-carboxyethyl)-(*S*)-ornithine ((*R*),(*S*)-octopinic acid) by hydrolysis with hydrochloric acid. (*R*),(*S*)-Octopinic acid gave (+)-octopine ((*R*),(*S*)-octopine) by guanidination. The reaction of (*S*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid led to give (*S*),(*S*)-octopinic acid and subsequently (+)-isooctopine ((*S*),(*S*)-octopine).

Octopine is an unusual amino acid first isolated from octopus muscle in 1927[2)] and from crown-gall tissue.[3)] A number of imino acids structurally related to octopine have recently been found in crown-gall tumors of plants, and the use of these imino acids as markers of a possible transfer of gene from the tumor-inducing bacterium into host plant cells has sparked further interest in these compounds.[4)]

The chemical structure of octopine was established as $N^2$-(1-carboxyethyl)arginine or *N*-(1-carboxy-4-guanidinobutyl)alanine.[5,6)] The configuration of the arginine (Arg) part in natural octopine has been determined to be (*S*) by either enzymic method or synthetic approach as follows. Akasi demonstrated that natural octopine is susceptible to arginase which decomposes (*S*)-arginine, but not (*R*)-arginine.[7)] Akasi also prepared identical compound with natural octopine from (*S*)-arginine and (*S*)-2-bromopropionic acid; in this reaction the configuration of the (*S*)-Arg part is retained.[5)]

However, no definite conclusion in respect to the configuration of the alanine (Ala) part has been reached. (*R*)-Configuration of the Ala part in natural octopine has been assumed by two groups. Izumiya *et al.* assumed the (*R*)-configuration by data of optical rotatory dispersions of natural octopine and its diastereomer.[8)] They also assumed the (*R*)-configuration by occurrence of Walden inversion of (*S*)-2-bromopropionic acid from kinetic data on the reaction of (*S*)-arginine and (*S*)-2-bromopropionic acid in alkaline conditions.[8)] Biellmann *et al.* demonstrated the (*R*)-configuration by an elegant method; they prepared octopine mixture from (*R*)-alanine and 2-oxo-5-guanidinopentanoic acid by reduction with cyanoborohydride, and observed the presence of natural octopine in the octopine mixture by enzymic method.[9)] They, however, failed to separate the octopine diastereomers mentioned above, despite that they could isolate crystalline (+)-octopine corresponding to the natural one and its diastereomer in good yields after reduction of (*S*)-arginine and 2-oxopropionic acid by cyanoborohydride.[9)] Previously, Izumiya *et al.* observed a similar result that hydrogenation of a mixture of (*S*)-arginine and 2-oxopropionic acid in the presence of palladium black produced octopine, but hydrogenation of a mixture of (*R*)-alanine and 2-oxo-5-guanidinopentanoic acid did not.[8)]

This paper presents a definite conclusion for (*R*)-configuration of the Ala part by the synthesis of (+)-octopine corresponding to natural octopine starting from (*R*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid.

## Results and Discussion

*Approach in Determination of Configuration of Ala Part in Natural Octopine.* Two groups reported that the reaction of (*S*)-arginine and (*S*)-2-bromopropionic acid produces (+)-octopine which is confirmed to be identical with natural octopine.[5,8)] Since the configuration at an asymmetric carbon of the Arg part is in full retention, the configuration of Ala part of natural octopine should be either (*R*) or (*S*) as shown in Scheme 1. Supposed natural octopine containing (*R*)-alanine residue is designated as (+)-**1a**, and the one containing (*S*)-alanine residue as (+)-**1b**. We designed to synthesize an octopine starting from (*R*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid ((*R*)-2-Br-5-Acpa) as shown in Scheme 2. We postulated that the configuration of the Ala part in natural octopine should be (*R*) if we would obtain (+)-octopine ((+)-**1a**) which is identical with natural one, whereas the configuration should be (*S*) if we obtain (−)-octopine ((−)-**1b**) which is an antipode of supposed natural octopine ((+)-**1b**).

*Conclusion for Configuration of Ala Part in Natural Octopine.* We could isolate (+)-octopine ((+)-**1a**) after a sequence of the reactions shown in Scheme 2, therefore we conclude that the configuration of the Ala part in natural octopine is (*R*). Then, we propose new nomenclature for four isomers of octopine as given in Table 1. It is also concluded that the asymmetric carbon with (*R*)-configuration in (*R*)-2-Br-5-Acpa is converted to (*S*) by Walden inversion during the formation of (*R*),(*S*)-Ac-octopinic acid.

*Reaction of (*R*)-Alanine and (*R*)-2-Br-5-Acpa.* The route to synthesize (*R*),(*S*)-octopine is shown in Fig. 1. Izumiya *et al.* reported that (+)-octopine and (+)-isooctopine were obtained in good yields

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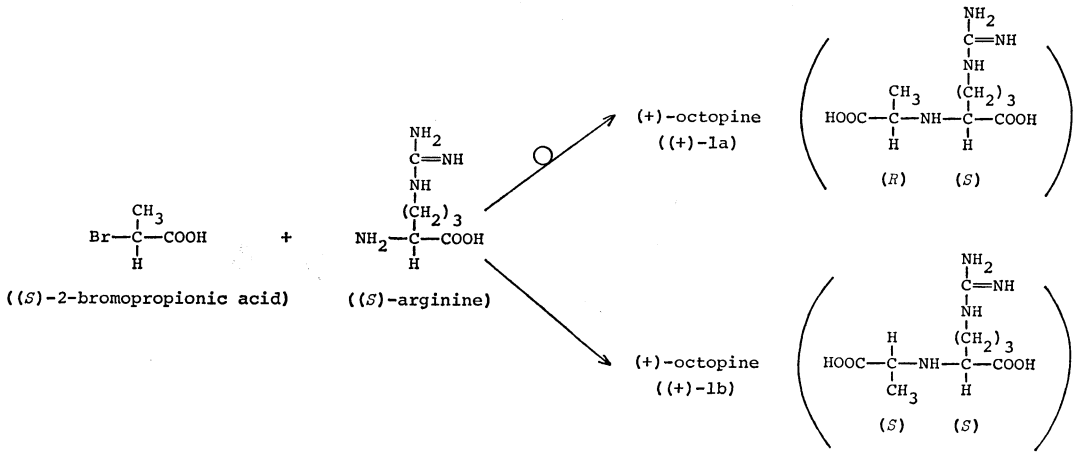
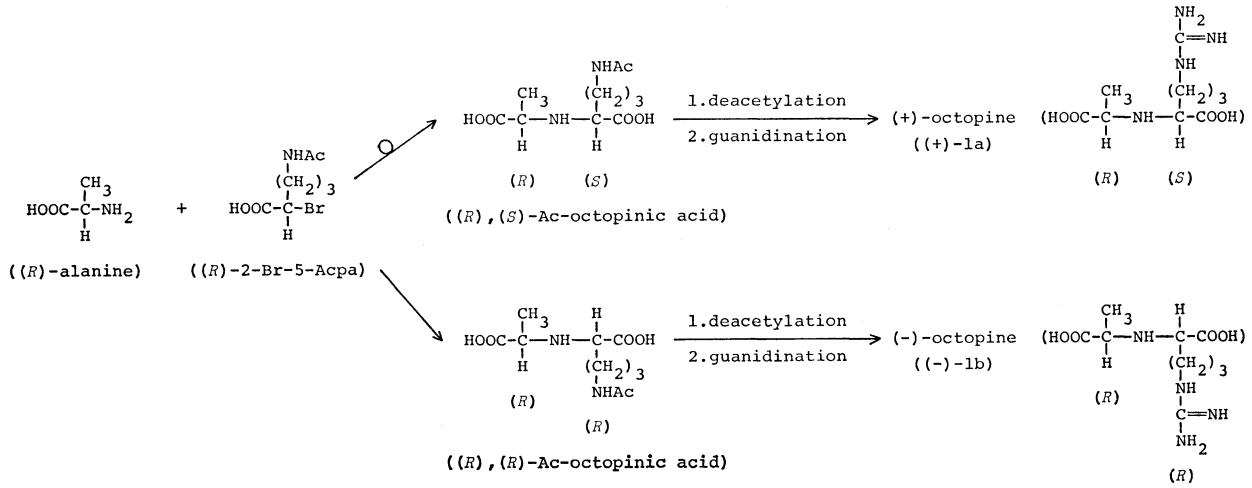



Scheme 1. Formation of (+)-octopine ((+)-**1a** or (+)-**1b**) from (*S*)-arginine and its supposed configuration.




Scheme 2. Supposed formation of (+)-octopine ((+)-**1a**) or (−)-octopine ((−)-**1b**) from (*R*)-alanine.

Table 1. Configurations of isomers of octopine

| Nomenclature | | Configuration of | | Source | $[\alpha]_D(H_2O)$ |
|---|---|---|---|---|---|
| By Akasi[a] and Izumiya *et al.*[b] | By present authors | Ala part | Arg part | | ° |
| (+)-Octopine | (*R*),(*S*)-Octopine | (*R*) | (*S*) | Natural or Synthetic | +20.8 |
| (−)-Octopine | (*S*),(*R*)-Octopine | (*S*) | (*R*) | Synthetic | −20.6[b] |
| (+)-Isooctopine | (*S*),(*S*)-Octopine | (*S*) | (*S*) | Synthetic | +26.7 |
| (−)-Isooctopine | (*R*),(*R*)-Octopine | (*R*) | (*R*) | Synthetic | −26.6[b] |

a) Cited from Ref. 10. b) Cited from Ref. 8.

(*ca.* 50%) from (*S*)-arginine, (*RS*)-2-bromopropionic acid, and $Ba(OH)_2$ in 1:2:2 molar ratio at 37 °C for 2 d.[8] However, the reaction of (*R*)-alanine, (*R*)-2-Br-5-Acpa, and $Ba(OH)_2$ under the same conditions gave the desired $N^2$-((*R*)-1-carboxyethyl)-$N^5$-acetyl-(*S*)-ornithine ((*R*),(*S*)-Ac-octopinic acid) in only trace amount. We assume that poor yield compared with the reaction of arginine and 2-bromopropionic acid is due to the presence of a bulky 3-acetamidopropyl group in 2-Br-5-Acpa and/or absence of basic guanidino group in alanine moiety. Then, we had to search for the conditions to give Ac-octopinic acid in fairly good yield.

In order to estimate a yield of Ac-octopinic acid and a possible occurrence of epimerization in $N^5$-acetylornithine part, we tried to separate (*R*),(*S*)- and (*S*),(*S*)-forms of $N^2$-(1-carboxyethyl)ornithine (octopinic acid) by an amino acid analyzer,[11] and found the conditions (see Experimental) to achieve the separation as shown in Fig. 2. A mixture of (*R*)-alanine, (*R*)-2-Br-5-Acpa, and $Ba(OH)_2$ was incubated for certain intervals, and a part of the mixture was refluxed with 2 M HCl, and the yield and the degree of epimerization of (*R*),(*S*)-octopinic acid in the acid hy-

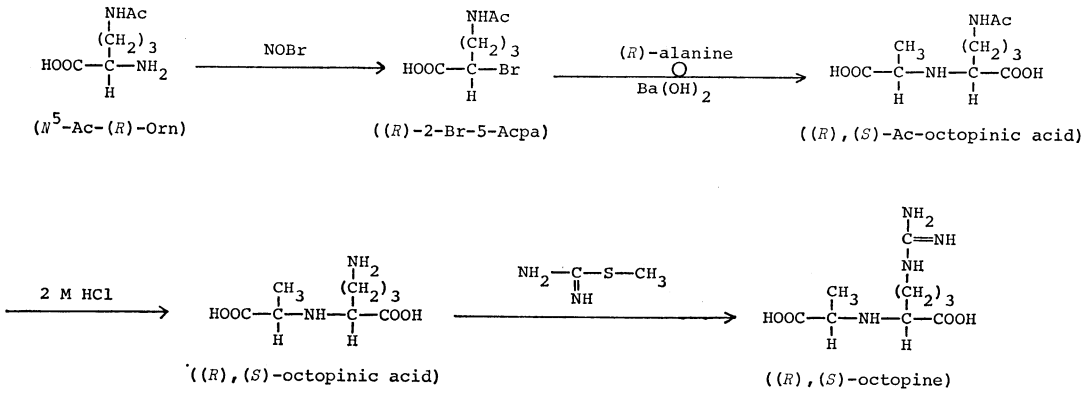



Fig. 1. Synthetic route for (*R*),(*S*)-octopine from (*R*)-alanine and (*R*)-2-Br-5-Acpa.

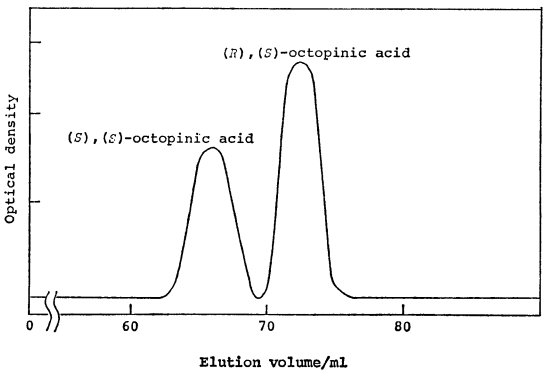



Fig. 2. Chromatogram of isomers of octopinic acid by an amino acid analyzer.

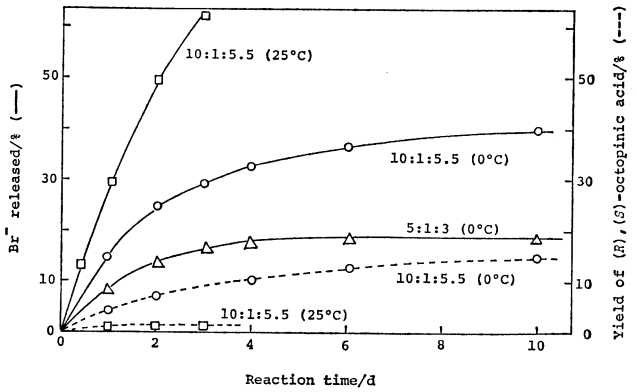



Fig. 3. Liberation of $Br^-$ and formation of (*R*),(*S*)-Ac-octopinic acid during the reaction of (*R*)-alanine and (*R*)-2-Br-5-Acpa. Ratio of *e.g.* 10:1:5.5 means that of (*R*)-Ala:(*R*)-2-Br-5-Acpa:$Ba(OH)_2$ in molar term.

drolysate were determined by an amino acid analyzer using (*R*),(*S*)- and (*S*),(*S*)(instead of (*R*),(*R*))-forms of octopinic acid as reference substances under the conditions described above. At the same time, $Br^-$ released during the reaction was analyzed by the Volhard titration method.

In Fig. 3, time-courses of $Br^-$ release and (*R*),(*S*)-octopinic acid formation are given. The increase of the reaction temperature to 25 °C or 37 °C resulted in rapid liberation of $Br^-$, but the formation of Ac-octopinic acid was very poor. Molar ratios of the

Table 2. Effect of ratio of reactants on formation of acetyl octopinic acid[a]

| Reactant in molar ratio | | | | Yield/% of (*R*),(*S*)-Ac-octopinic acid[b] |
|---|---|---|---|---|
| (*R*)-Alanine | | (*R*)-2-Br-5-Acpa | $Ba(OH)_2$ | |
| 1 | : | 2 | : 2 | 1 |
| 2 | : | 1 | : 1.5 | 5 |
| 5 | : | 1 | : 3 | 12 |
| 10 | : | 1 | : 5.5 | 15 |

a) The reaction was carried out at 0 °C for 10 d. b) Yields are calculated from (*R*)-2-Br-5-Acpa and estimated from (*R*),(*S*)-octopinic acid derived from (*R*),(*S*)-Ac-octopinic acid by an amino acid analyzer.

reactants gave also some influences on a yield; some of the results of the experiments are summarized in Table 2. We observed that the formation of Ac-octopinic acid was favored when the ratios of (*R*)-alanine to (*R*)-2-Br-5-Acpa were increased. Based on the above results, the favorable conditions to yield Ac-octopinic acid were settled as follows: a mixture of (*R*)-alanine, (*R*)-2-Br-5-Acpa, and $Ba(OH)_2$ in molar ratio of 10:1:5.5 is allowed to react at 0 °C for 10 d. It was observed, however, that the occurrence of possible (*R*),(*R*)-Ac-octopinic acid was 5—6% of the desired (*R*),(*S*)-isomer. We assume that the reasons of epimerization will be that (*R*)-2-Br-5-Acpa contains already some (*S*)-form and/or racemization at $N^5$-acetyl-(*R*)-ornithine part occurs during the reaction. Nevertheless, an undesirable (*R*),(*R*)-isomer in a small amount could be removed by recrystallization as mentioned latter.

*Syntheses of* (R),(S)- *and* (S),(S)-*Forms of Ac-octopinic Acid, Octopinic Acid, and Octopine.* Employing the favorable conditions mentioned above, recrystallized (*R*),(*S*)-Ac-octopinic acid was obtained from (*R*)-alanine and (*R*)-2-Br-5-Acpa in a yield of 16%. This compound still contained 3% of its possible isomer, (*R*),(*R*)-Ac-octopinic acid. However, (*R*),(*S*)-octopinic acid, obtained from the (*R*),(*S*)-Ac-octopinic acid by hydrolysis and recrystallized from water–ethanol, was free from (*R*),(*R*)-octopinic acid; the (*R*),(*R*)-isomer might be removed during the recrystallization. Alternatively, octopinic acid was also obtained from (+)-octopine ("natural octopine"), derived from (*S*)-

arginine and (*S*)-2-bromopropionic acid, with the treatment of $Ba(OH)_2$ at 80 °C. The octopinic acid obtained above showed the same properties as those of the (*R*),(*S*)-octopinic acid derived from the (*R*),(*S*)-Ac-octopinic acid (*e.g.*, melting point, $R_f$ values on paper, and specific rotations). Finally, crystalline (*R*),(*S*)-octopine was prepared from (*R*),(*S*)-octopinic acid, derived from (*R*),(*S*)-Ac-octopinic acid, and *S*-methylisothiourea in a yield of 61%. Identity between this (*R*),(*S*)-octopine and (+)-octopine ("natural octopine") was established by comparison of chromatographies on paper and silica gel-thin layer, and specific rotations. In the same manner, (*S*),(*S*)-Ac-octopinic acid was synthesized from (*S*)-alanine and (*R*)-2-Br-5-Acpa. Subsequently, (*S*),(*S*)-forms of octopinic acid and octopine were prepared by the same procedure as for the (*R*),(*S*)-forms. Their physical properties were different from those of the corresponding octopinic acid and octopine derived from (+)-octopine ("natural octopine") or (+)-octopine itself.

## Experimental

All the melting points are uncorrected. Paper chromatography was carried out on Toyo Roshi No. 52 paper with the solvent system: $R_f$, *n*-BuOH–AcOH–pyridine–$H_2O$ (15:3:10:12, v/v). TLC was carried out on silica gel G (Merck) with the same solvent system as described above: $R_f$ (TLC). Material possessing an amino group was detected by spraying with 0.2% ninhydrin solution in acetone, followed by heating at 100 °C. Material having a guanidino group was detected by spraying with an alkaline pentacyanonitrosylferrate(III)–hexacyanoferrate(III) reagent.[12] Material having blocked amino group was detected by spraying with 10% $H_2SO_4$, followed by heating on a hot plate. Optical rotations were measured on a Yanagimoto polarimeter OR-20. Amino acid analyses were performed with a Hitachi amino acid analyzer KLA-3B.

*N⁵-Acetyl-(R)-ornithine (N⁵-Ac-(R)-Orn).* This was prepared from (*R*)-ornithine monohydrochloride (16.9 g, 100 mmol) in the same manner as the preparation of $N^5$-Ac-(*S*)-Orn,[13] and was recrystallized from water–EtOH; yield, 15.9 g (91%); mp 233—234 °C (dec); $[\alpha]_D^{20}$ −24.6° (*c* 1, 5 M HCl); $R_f$ 0.29. Reported value for $N^5$-Ac-(*S*)-Orn; $[\alpha]_D^{25}$ +24.0° (*c* 1, 5 M HCl).[13]

*(R)-2-Bromo-5-acetamidopentanoic Acid ((R)-2-Br-5-Acpa).* This was prepared from $N^5$-Ac-(*R*)-Orn (8.71 g, 50 mmol), KBr (20.9 g, 175 mmol) and sodium nitrite (5.58 g, 80 mmol) in 1.25 M $H_2SO_4$ (105 ml) according to the procedure of Izumiya[14]; yield of an oil, 6.43 g (54%); $R_f$ (TLC) 0.70.

*Chromatography of (R),(S)- or (S),(S)-Form of Octopinic Acid with Amino Acid Analyzer.* Certain amount (5 μmol) of either (*R*),(*S*)- or (*S*),(*S*)-form of octopinic acid was dissolved in 0.2 M citrate buffer of pH 2.2, and an aliquot (0.5 μmol) of the solution was subjected to the analyzer. The following conditions were sufficient to permit the separation of two isomers; column with spherical resin, 0.9 cm × 50 cm; buffer, the standard citrate at pH 3.25; flow rate, 60 ml/h; jacket temperature, 55 °C. A chromatogram with a mixture of both isomers is shown in Fig. 2. The ratio of color intensities in the chromatogram of equimolar amount of (*R*),(*S*)-form and (*S*),(*S*)-form of octopinic acid was found to be 140:100.

*Reaction of (R)- or (S)-Alanine and (R)-2-Br-5-Acpa. Determination of Bromide Ion*: (*R*)- or (*S*)-Alanine, (*R*)-2-Br-5-Acpa and $Ba(OH)_2 \cdot 8H_2O$ in each specified amount were dissolved in water (20 ml). An aliquot (1 ml) of the solution was withdrawn for certain intervals, and was subjected to the Volhard titration. An amount of $Br^-$ released was determined, the result being shown in Fig. 3.

*Determination of (R),(S)- or (S),(S)-Form of Ac-octopinic Acid*: An aliquot (1 ml) of the reaction mixture described above for certain intervals was put on a column (1.1 cm × 6 cm) of Dowex 50X8 ($H^+$ form), and the column was washed with water and eluted with 2 M $NH_3$ aq. The eluate was evaporated, and the residue was dissolved in 2 M HCl. After being refluxed for 2 h, the solution was evaporated, and the residue was dissolved in 0.2 M citrate buffer (pH 2.2). An aliquot was subjected to an amino acid analyzer as described previously. Yield of (*R*),(*S*)- or (*S*),(*S*)-form of Ac-octopinic acid was calculated from a peak of corresponding octopinic acid. Elution volume of unchanged alanine on the analyzer was 98 ml, and the ratio of color intensities in the chromatogram of equimolar amount of (*R*),(*S*)-octopinic acid and alanine was found to be 85:100.

*(R),(S)-Ac-octopinic Acid.* A solution of (*R*)-alanine (4.45 g, 50 mmol), (*R*)-2-Br-5-Acpa (1.19 g, 5 mmol), and $Ba(OH)_2 \cdot 8H_2O$ (8.68 g, 27.5 mmol) in water (50 ml) was stirred at 0 °C. After being stirred for 10 d at 0 °C, the solution was applied to a column (2.8 cm × 26 cm) of Dowex 50X8 ($H^+$ form), which was washed with water and eluted with 2 M $NH_3$ aq (300 ml). The eluate was evaporated and the residual solid was dissolved in a small amount of 0.1 M AcOH. The solution was put on a column (1.1 cm × 50 cm) of Amberlite IR-45 pre-equilibrated with 0.1 M AcOH. Elution was performed with 300 ml of 0.1 M AcOH and then 2 M AcOH (each fraction, 25-ml), fractions being monitored by TLC; the fractions 2—6 contained (*R*)-alanine. The fractions 17—21 were evaporated, and recrystallized from water–EtOH–acetone; yield, 197 mg (16% based on (*R*)-2-Br-5-Acpa); $R_f$ (TLC) 0.36. This compound contained small amount (*ca.* 3%) of possible (*R*),(*R*)-Ac-octopinic acid, which was determined by an amino acid analyzer on an acid hydrolysate of a part of the product (197 mg).

Found: C, 46.85; H, 7.41; N, 10.46%. Calcd for $C_{10}H_{18}O_5N_2 \cdot 2/3H_2O$: C, 46.51; H, 7.55; N, 10.85%.

*(S),(S)-Ac-octopinic Acid.* This was prepared from (*S*)-alanine (4.45 g, 50 mmol) and (*R*)-2-Br-5-Acpa (1.19 g, 5 mmol) as described above; yield, 172 mg (14%); $R_f$ (TLC) 0.36. This compound contained small amount (*ca.* 4%) of possible (*S*),(*R*)-Ac-octopinic acid.

Found: C, 47.92; H, 7.31; N, 11.19%. Calcd for $C_{10}H_{18}O_5N_2 \cdot 1/4H_2O$: C, 47.90; H, 7.44; N, 11.17%.

*(R),(S)-Octopinic Acid. From (R),(S)-Ac-octopinic Acid*: A solution of (*R*),(*S*)-Ac-octopinic acid (123 mg, 0.5 mmol) in 2 M HCl was refluxed for 2 h and evaporated. The residue dissolved in a small amount of water was put on a column (1.1 cm × 6 cm) of Dowex 50X8 ($H^+$ form), and the column was washed with water and eluted with 2 M $NH_3$ aq. The eluate was evaporated and recrystallized from water–EtOH; yield, 76 mg (74%); mp 275—277 °C (dec); $[\alpha]_D^{20}$ +19.2° (*c* 0.5, $H_2O$); $R_f$ 0.11. This compound was pure (*R*),(*S*)-octopinic acid showing no contamination of possible (*R*),(*R*)-isomer.

Found: C, 45.90; H, 7.84; N, 13.23%. Calcd for $C_8H_{16}O_4N_2 \cdot 1/4H_2O$: C, 46.04; H, 7.97; N, 13.42%.

*From (+)-Octopine*: (+)-Octopine (246 mg, 1 mmol)[8] derived from (*S*)-arginine and (*S*)-2-bromopropionic acid was dissolved in saturated aqueous $Ba(OH)_2$ solution (100 ml) at 0 °C, and the solution was left to stand at 75—80 °C for 6 h. After removal of $Ba^{++}$ by $H_2SO_4$, the filtrate was treated with a column of Dowex 50X8 as described above.

The eluate with 2 M $NH_3$ aq was evaporated and the residue dissolved in 0.1 M pyridine-formate buffer (pH 3.1) was put on a column (1.1 cm×40 cm) of Dowex 50X8 pre-equilibrated with the same buffer. Elution was performed with the same buffer (each fraction, 10-ml), and the fractions 10—15 were evaporated to afford crude (*R*),(*S*)-octopinic acid which contained *ca.* 1.5% possible (*R*),(*R*) (or (*S*),(*S*))-octopinic acid. Crude product was recrystallized from water–EtOH to give pure (*R*),(*S*)-octopinic acid; yield, 80 mg (39%); mp 276—278 °C (dec); $[\alpha]_D^{20}$ +19.1° (*c* 0.5, $H_2O$); $R_f$ 0.11. Reported values[15] for octopinic acid derived from natural octopine: mp 270—271 °C; $[\alpha]_D^{20}$ +18.48° (*c* 0.5, $H_2O$).

*(S),(S)-Octopinic Acid.* This was prepared from (*S*),(*S*)-Ac-octopinic acid (123 mg, 0.5 mmol) as described for the preparation of (*R*),(*S*)-octopinic acid from (*R*),(*S*)-Ac-octopinic acid; yield, 73 mg (72%); mp 237 °C (dec); $[\alpha]_D^{20}$ +24.6° (*c* 0.5, $H_2O$); $R_f$ 0.11.

Found: C, 45.80; H, 7.89; N, 13.28%. Calcd for $C_8H_{16}O_4N_2 \cdot 1/3H_2O$: C, 45.71; H, 7.99; N, 13.33%.

*(R),(S)-Octopine.* A solution of (*R*),(*S*)-octopinic acid (41 mg, 0.2 mmol), derived from (*R*),(*S*)-Ac-octopinic acid, and *S*-methylisothiourea sulfate (56 mg, 0.4 mmol) in 0.1 M NaOH (4 ml) was kept at 35 °C for 6 d, and the solution was put on a column (1.1 cm×6 cm) of Dowex 50X8 ($H^+$ form). The column was washed with water and eluted with 2 M $NH_3$ aq. The eluate was concentrated and applied to a column (1.4 cm×3 cm) of Dowex 1X2 ($OH^-$ form), and the column was washed with water and eluted with 1 M AcOH. The eluate was evaporated, and the residue dissolved in 0.1 M pyridine-formate buffer (pH 3.1) was put on a column (0.9 cm×33 cm) of Dowex 50X8 (pyridinium form). The column was washed with the same buffer (75 ml), and eluted with 1 M pyridine. The eluate containing octopine was evaporated and recrystallized from water–EtOH–acetone; yield, 30 mg (61%); mp 280—283 °C (dec); $[\alpha]_D^{20}$ +20.8° (*c* 1, $H_2O$); $R_f$ 0.15.

Found: C, 43.78; H, 7.32; N, 22.83%. Calcd for $C_9H_{18}O_4N_4$: C, 43.89; H, 7.37; N, 22.75%. Reported values[15] for natural octopine: mp 281—282 °C; $[\alpha]_D^{17}$ +20.94° (*c* 2.6, $H_2O$). Reported values[8] for (+)-octopine prepared from (*S*)-arginine and (*S*)-2-bromopropionic acid; mp 283—284 °C; $[\alpha]_D^{24}$ +20.8° (*c* 2, $H_2O$).

Identity of (*R*),(*S*)-octopine obtained from (*R*)-alanine and (*R*)-2-bromo-5-acetamidopentanoic acid, and (+)-octopine[8] prepared from (*S*)-arginine and (*S*)-2-bromopropionic acid was ascertained by co-chromatographies on paper and silica gel-thin layer with several solvent systems in addition to the same specific rotations described above.

*(S),(S)-Octopine.* (*S*),(*S*)-Octopinic acid (41 mg, 0.2 mmol) was treated with *S*-methylisothiourea sulfate followed by work-up as described above. The crude product was recrystallized from water–EtOH–acetone; yield 32 mg (65%); mp 263—264° (dec); $[\alpha]_D^{20}$ +26.7° (*c* 1, $H_2O$); $R_f$ 0.15.

Found: C, 43.67; H, 7.48; N, 22.65%. Calcd for $C_9H_{18}O_4N_4$: C, 43.89; H, 7.37; N, 22.75%. Reported values[8] for (+)-isooctopine prepared from (*S*)-arginine and (*R*)-2-bromopropionic acid; mp 264—265 °C; $[\alpha]_D^{24}$ +26.8° (*c* 2, $H_2O$).

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