of DOPA, *p*-tyrosine, *m*-tyrosine, *o*-tyrosine and phenylalanine were 3.2, 4.3, 5.7, 9.2, and 11.5 min, respectively.

Standard curves obtained by plotting the peak area against the amount of substance injected were linear in the range of 100—500 ng/50 μl. The smallest amount that could be determined was 10 ng of hydroxyphenylalanine per injection.

In conclusion, the separation of the isomers of hydroxyphenylalanine by HPLC has advantages over conventional methods in terms of high sensitivity, simplicity and rapidity. The method may be adaptable to studies on phenylalanine metabolites in biological samples.

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Synthesis of Some Cyclic Derivatives of Spermine and Spermidine

EIICHI KIMURA and TAKASHI YATSUNAMI

Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine[1]

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Some cyclic derivatives of spermine and spermidine, 1,5,8,12-tetraazacyclohexadecane **7**, 1,5,9,13-tetraazacycloheptadecane **9**, and 1,5,9-triazacyclotridecane **11** were synthesized as part of our studies on the functions of natural polyamines and the effects of structural modification of the biological activities.

Keywords——synthesis; cyclic spermine; cyclic spermidine; protonation constants; polyamines

The possible biological roles of biogenic polyamines such as spermine **1** and spermidine **2** in numerous growth processes have been scrutinized recently.[2] Promoting effects in DNA replication,[3] stimulatory effects in the synthesis of nucleic acids[4] and proteins,[5] and inhibitory effects in lipid peroxidation[6] have been extensively studied. However, the mechanisms of these functions remain unclear, except for an ealier suggestion[7] that the polyamines interact with nucleic acids, ribosomes, enzymes or lipids as organic polycations, thereby stabilizing higher structures of the macromolecules.

We have now prepared some cyclic derivatives of spermine (trivially called "cyclic spermine"), 1,5,8,12-tetraazacyclohexadecane **7** and 1,5,9,13-tetraazacycloheptadecane **9**, and spermidine, 1,5,9-triazacyclotridecane **11**[8] ("cyclic spermidine"). Our previous studies[9] show-

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8) This compound has already been prepared as a ligand for metal complexation, but was not recognized as a spermidine derivative: M. Nonoyama, *Transition Met. Chem.*, **1**, 70 (1976); F.L. Weitl and K.N. Laymond, *J. Am. Chem. Soc.*, **101**, 2728 (1979).
9) E. Kimura and M. Kodama, *Yuki Gosei Kagaku Kyokai Shi*, **35**, 632 (1977); I. Tabushi, T. Nishiya, E. Kimura, K. Hattori, K. Odashima, K. Koga, and F. Toda, "Hosuto-Gesuto no Kagaku," Kyoritsu Shuppan, Tokyo, 1979, pp. 84—94; M. Kodama and E. Kimura, *J. Chem. Soc. Dalton*, **1978**, 1081.

ed that properties such as the conformation, amine basicities, metal affinities and lipophilicities of the linear polyamines are dramatically altered by cyclization. Such structural modification of the natural linear polyamines should thus cause modified binding to biologically important macromolecules, possibly triggering modified biological activities.

The protonation constants presented herein for the cyclic spermine and spermidine clearly indicate alteration in their chemical properties.

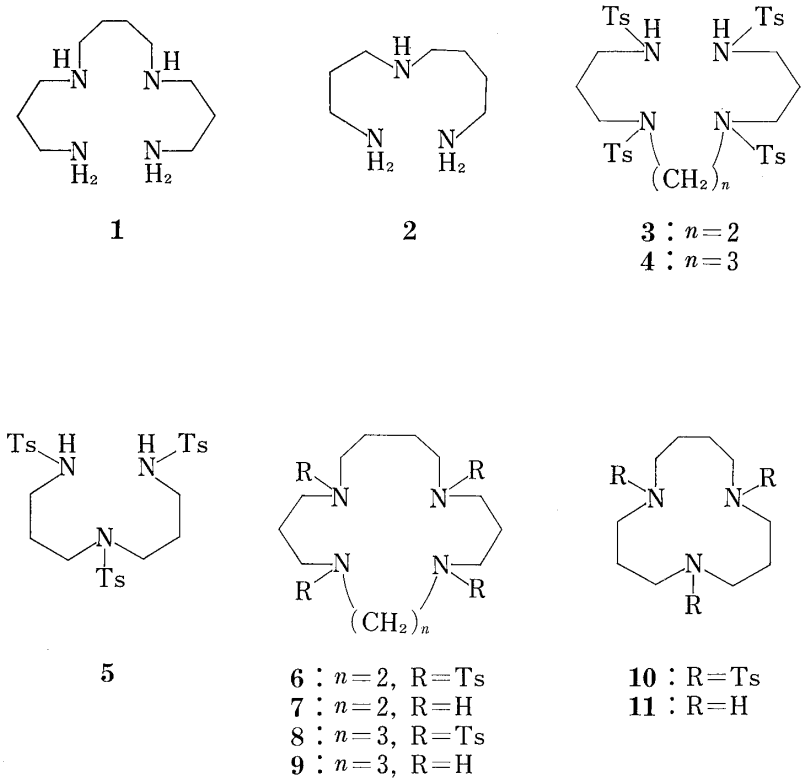

Fig. 1

All the cyclic polyamines were synthesized by a slight modification of the procedure of Koyama and Yoshino.[10] 1,4-Dibromobutane was treated with the tetratosylated tetraamine **3** or **4**, or the tritosylated triamine **5**, in DMF at high dilution to produce the cyclized product, 1,5,8,12-N,N′,N″,N‴-tetrakis(*p*-toluenesulfonyl)tetraazacyclohexadecane **6**, 1,5,9,13-N,N′,-N″,N‴-tetrakis(*p*-toluenesulfonyl)tetraazacycloheptadecane **8** or 1,5,9-N,N′,N″-tris(*p*-toluenesulfonyl)triazacyclotridecane **10**, respectively, in fairly good yield. The cyclic structures of **6**, **8**, and **10** were supported by their proton NMR spectra ($CDCl_3$), which showed no signal assignable to terminal NH groups. The 1:1 stoichiometry of the products was confirmed by their molecular ion peaks and fragmentation patterns in the mass spectra and by elemental analyses (see "Experimental"). Hydrolytic removal of the tosyl groups of **6**, **8**, and **10** with 48% hydrobromic acid in acetic acid afforded the desired compounds, 1,5,8,12-tetraazacyclohexadecane **7**, 1,5,9,13-tetraazacycloheptadecane **9** and 1,5,9-triazacyclotridecane **11**, respectively, as the hydrobromide salts. Their elemental analyses were consistent with the structures depicted.

The mixed-mode protonation constants of **7**, **9**, and **11** were determined by potentiometric acid-base titration with carbonate-free sodium hydroxide solution. The results for the macro-

10) H. Koyama and T. Yoshino, *Bull. Chem. Soc. Jpn.*, **45**, 481 (1972).

Table I. Comparison of the Protonation Constants ($K_i=[LH^{i+}]/[LH^{(i-1)+}][H^+]$) of the Cyclic Compounds **7**, **9** and **11** with those of Linear Spermine **1** and Spermidine **2** at 25° and $I=0.20$ mol dm^{-3} ($NaClO_4$)

Compound	log K_1	log K_2	log K_3	log K_4
7	10.04	9.69	6.80	3.54
9	10.23	9.66	7.40	5.31
11	9.79	8.13	4.18	
1[a]	10.80	10.02	8.85	7.96
2[a]	10.89	9.81	8.34	

a) B.N. Palmer and H.K.J. Powel, *J. Chem. Soc. Dalton*, **1974**, 2089.

cyclic polyamines are summarized for comparison with those of the linear homologs spermine and spermidine.

The effects of cyclization on the amine basicities can be seen in the third and fourth protonation constants of **7** and **9** and the third protonation constant of **11**. These lower basicities for the cyclized polyamines can be ascribed to the close proximity of the $^+$NH groups initially formed, resulting in the repulsion of protons approaching the third (and/or fourth) nitrogen atoms. Such trends are more marked with smaller-sized macrocyclic tetra- and triamines.[9] It should be noted that at physiological pH the cyclized compounds **7** and **9** are present as di- or trications in contrast to spermine (tetracation), and that compound **11** is present as a dication in contrast to tricationic spermidine.[11]

Experimental

All the melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were measured on a JNM-PS-100 (100 MHz) spectrometer using tetramethylsilane as an internal standard, and chemical shifts are given in δ values. Mass spectra were recorded on a JEOL-01SG spectrometer. Protonation constants were determined potentiometrically using the procedure described in ref. 9.

Reactions of the Tetratosylated Tetraamines 3 and 4, and the Tritosylated Triamine 5 with 1,4-Dibromobutane——General Procedure: A mixture of **3**, **4** or **5** (9 mmol) and 1,4-dibromobutane (9 mmol) in DMF (100 ml) was added dropwise over 48—72 hr to a suspension of anhydrous potassium carbonate (12 g) in DMF (600 ml) maintained at 115—120° with vigorous stirring. After stirring for an additional 24 hr at the same temperature, the mixture was cooled and filtered. The filtrate was concentrated to about one-tenth of the original volume and was partitioned between benzene (500 ml) and saturated sodium chloride solution (100 ml). The benzene layer was washed with water twice, dried over sodium sulfate and evaporated to dryness. The products were purified by direct recrystallization or chromatography of the residue. 1,5,8,12-N,N′,N″,N‴-Tetrakis(*p*-toluenesulfonyl)tetraazacyclohexadecane **6** (34%) was obtained by direct recrystallization. mp 218—220°. *Anal.* Calcd for $C_{40}H_{52}N_4O_8S_4$: C, 56.85; H, 6.20; N, 6.63. Found: C, 57.38; H, 6.29; N, 6.58. NMR ($CDCl_3$): 1.6—2.0 (8H, broad m, $NCH_2C\underline{H}_2$), 2.44 (12H, s, $C_6H_4C\underline{H}_3$), 2.8—3.4 (16H, broad m, $NC\underline{H}_2$), 7.2—7.8 (16H, m, $C_6\underline{H}_4$). MS *m/e*: 844 (M^+), 689, 534. 1,5,9,13-N,N′,N″,N‴-Tetrakis(*p*-toluenesulfonyl)tetraazacycloheptadecane **8** (56%) was obtained as an amorphous solid by chromatography on silica gel. *Anal.* Calcd for: $C_{41}H_{54}N_4O_8S_4$: C, 57.32; H, 6.34; N, 6.52. Found: C, 57.15; H, 6.36; N, 6.66. NMR ($CDCl_3$): 1.4—2.1 (10H, broad m, $NCH_2C\underline{H}_2$), 2.40 (12H, s, $C_6H_4C\underline{H}_3$), 2.8—3.4 (16H, broad m, $NC\underline{H}_2$), 7.2—7.8 (16H, m, $C_6\underline{H}_4$). MS *m/e*: 858 (M^+), 703, 548, 393, 238. 1,5,9-N,N′,N″-Tris(*p*-toluenesulfonyl)triazacyclotridecane **10** (33%) was obtained by direct recrystallization. mp 232—235°. *Anal.* Calcd for $C_{31}H_{41}N_3O_6S_3$: C, 57.47; H, 6.38; N, 6.49. Found: C, 57.75; H, 5.94; N, 6.50. NMR ($CDCl_3$): 1.6—2.0 (8H, broad m, $NCH_2C\underline{H}_2$), 2.43 (9H, s, $C_6H_4C\underline{H}_3$), 2.8—3.6 (12H, broad m, $NC\underline{H}_2$), 7.3—7.8 (12H, m, $C_6\underline{H}_4$). MS *m/e*: 647 (M^+), 492, 337, 182.

Hydrolysis of 6, 8 or 10——General Procedure: Compound **6**, **8** or **10** was dissolved in 15 ml of a 1: 1 mixture of 48% hydrobromic acid and acetic acid per gram of the substrate and refluxed for 72 hr. After removing the solvent *in vacuo*, the residue was dissolved in water, filtered to remove water-insoluble materials, and the filtrate was evaporated to dryness. The residue was recrystallized from acetic acid–48%

11) Preliminary experiments with the cyclized polyamines indicated significantly different biological activities from those of the linear counterparts. A detailed investigation is in progress.

hydrobromic acid. The tetrahydrobromide of 1,5,8,12-tetraazacyclohexadecane **7** (80%) was obtained as needles, mp 256.5—257° (dec.). *Anal.* Calcd for $C_{12}H_{32}Br_4N_4$: C, 26.11; H, 5.84; N, 10.15. Found: C, 25.94; H,5.74; N, 10.18. The tetrahydrobromide of 1,5,9,13-tetraazacyxloheptadecane **9** (66%) was obtained as pillars, mp 278—279° (dec.). *Anal.* Calcd for $C_{13}H_{34}Br_4N_4$: C, 27.58; H, 6.05; N, 9.90. Found; C, 27.61; H, 5.75; N, 9.76. The trihydrobromide of 1,5,9-triazacyclotridecane **11** (88%) was obtained as prisms, mp 269.5—272° (dec.). *Anal.* Calcd for $C_{19}H_{26}Br_3N_3$: C, 28.06; H, 6.12; N, 9.82. Found; C, 27.90; H, 5.95; N, 9.79.

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Co-occurrence of (—) and (+)-Germacrene-D in *Solidago altissima* L.: Determination of the Optical Rotation of optically Pure Germacrene-D[1]

MASATAKE NIWA, MASANOBU IGUCHI, and SHOSUKE YAMAMURA

Faculty of Pharmacy, Meijo University[2]

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Germacrene-D, which has sexual attractant properties similar to those of periplanone-B, has been isolated in an optically impure state from many plants, *i.e.*, (—) and (+)-germacrene-D co-occur in different ratios in various plants. (—)-Germacrene-D isolated from *Solidago altissima* L. in this work was determined to have an optical purity of 18.0%. In addition, the optical rotation of optically pure germacrene-D was estimated to be 305°.

Keywords——sesquiterpene; germacrene; optical rotation; chemical transformation; transannular reaction; biogenesis

Introduction

Germacrene-D, a typical ten-membered ring sesquiterpene, has been found in many plants. (—)-Germacrene-D (**1**) was first isolated by Yoshihara *et al.* from *Pseudotsuga japonica* S. and its structure, including the absolute configuration ($[\alpha]_D^{23}$ —240°), was also elucidated.[3] The same authors later obtained (+)-germacrene-D (**2**) ($[\alpha]_D^{23}$ +305°) from *Dendropanax trifidus* M.[4] Recently, (—)-germacrene-D ($[\alpha]_D^{20}$ —145° in EtOH) was found in the steam distillates of the plant *Solidago altissima* L. collected in Osaka early in September.[5]

In view of the finding that germacrene-D has a sexual stimulant activity similar to that of periplanone-B,[6] which is a sexual stimulant for the American cockroach,[7] we were interested in attempting to isolate germacrene-D from *Solidago altissima* L. on a preparative scale.

Results and Discussion

The fresh leaves of the plant were collected in Nagoya early in August and extracted with benzene at room temperature. The benzene extract was roughly separated by column chro-

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