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Identification and Determination of *sym*-Homospermidine in Roots of Water Hyacinth, *Eichhornia crassipes* SOLMS

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An unusual polyamine, *sym*-homospermidine, was identified in water hyacinth, *Eichhornia crassipes* SOLMS, by gas chromatography-mass spectrometry as its *N*-ethoxycarbonyl derivative. This polyamine predominated in the root and its contents determined by gas chromatography were in the range of 9.9—46.4 nmol/g fresh weight.

Keywords——*Eichhornia crassipes*; Pontederiaceae; homospermidine; polyamine; GC-MS; GC; *N*-ethoxycarbonyl derivative

The water hyacinth, *Eichhornia crassipes* SOLMS (Japanese name: Hoteiaoi; Pontederiaceae), is an aquatic plant having vegetative character. Recently, much attention has been focused on the use of this plant as a possible nutrient removal agent for waste-water treatment.[1]

While examining the polyamine content in this plant, we detected an unknown peak which appeared slightly later than spermidine under our gas chromatographic (GC) conditions. This paper describes the identification of the unknown component as *sym*-homospermidine [$NH_2(CH_2)_4NH(CH_2)_4NH_2$], and shows that this polyamine is a major constituent in the root. Among higher plants, *sym*-homospermidine has only been found so far in the leaf of the sandal wood tree (*Santalum album*)[2] and in the sword bean (*Canavalia gladiata*),[3] although it has also been found sporadically in vertebrates,[4] algae[5] and bacteria.[6]

Experimental

Reagents——The hydrochloride salts of 1,3-diaminopropane, putrescine, spermidine and spermine were purchased from Nakarai Chemicals Ltd., and the hydrochloride salt of *sym*-homospermidine was kindly supplied by Dr. T. Oshima of Mitsubishi-Kasei Institute of Life Sciences. 1,8-Diaminooctane, used as an internal standard, was obtained from Nakarai Chemicals Ltd.; the hydrochloride salt was prepared by reaction with concentrated HCl and recrystallized from EtOH. Prior to use, all standard amines were dried overnight in a vacuum desiccator over P_2O_5, and a polyamine standard solution (each 125 nmol/ml) and an internal standard solution (125 nmol/ml) were each prepared in water. Ethyl chloroformate (bp 95 °C) was obtained from Tokyo Kasei Kogyo Co. and stored at 4 °C after distillation. Diethyl ether was purified by distillation. All other chemicals were reagent grade products available from usual commercial sources.

Instruments——A Shimadzu GC 4CM gas chromatograph equipped with a flame ionization detector and a linear temperature programmer was used. The column packing, 0.5% SP-1000 on Uniport HP (100—120 mesh), was prepared by using 1-BuOH–$CHCl_3$ (1 : 1) as a coating solvent according to the filtration method.[7] SP-1000 (Supelco) and Uniport HP were purchased from Gasukuro Kogyo. The packed glass column (0.5 mm × 3 mm i.d.) was preconditioned at 280 °C for 20 h at an N_2 flow rate of 30 ml/min. GC conditions were as follows: oven temperature, programmed at 10 °C/min from 120 to 280 °C; N_2 flow rate, 80 ml/min; injection and detector temperature, 285 °C. For gas chromatographic-mass spectrometric (GC-MS) analysis, a Shimadzu-LKB 9000 gas chromatograph-mass spectrometer with the same type of column as used for GC analysis was employed under the following conditions: GC oven temperature, programmed at 5 °C/min from 90 to 240 °C; He flow rate, 40 ml/min; trap current, 60 μA; ionizing voltage, 70 eV; accelerating voltage, 3.5 kV; ion source temperature, 270 °C; separator temperature, 260 °C.

Extraction and Isolation of Polyamines——Samples of *E. crassipes* were collected during Nov. 1982. Each fresh sample was divided into root (including root hair), petiole and leaf after being repeatedly washed with running water. A known weight (20—30 g) of each portion was homogenized for 15 min with 150 ml of 4% $HClO_4$ and left to stand overnight at room temperature. The suspension was centrifuged and the precipitate was washed twice with 20 ml of 4% $HClO_4$. The supernatant and washings were put in a 200-ml measuring flask and made up to 200 ml with 4% $HClO_4$. An aliquot of this solution (10—20 ml) was applied to a column of Amberlite CG-120 (100—200 mesh, H^+ form, 3 ml bed volume), and the polyamine fraction was collected according to the procedure of Yamamoto *et al.*[8] After addition of the internal standard solution (0.5 ml), the eluate containing polyamines was evaporated to dryness at 60 °C in a rotary evaporator under reduced pressure. The residue was transferred with 2 ml of water to a 10-ml glass tube (Corning No. 9826) having a screw top with a PTFE-lined cap.

Identification and Determination of Polyamines——Polyamines thus obtained were converted to the *N*-ethoxycarbonyl (EOC) derivatives,[8] which were analyzed by GC and GC-MS. Calibration curves for the polyamines in the range of 10—125 nmol were constructed by using the peak height ratios relative to the internal standard. Each calibration plot was found to be linear and reproducible. Therefore, the amounts of polyamines in plant samples were practically determined by comparing the peak height ratios obtained from samples with those from one standard mixture, usually containing 75 nmol of each polyamine. On the other hand, according to the procedure of Smith,[6a] the polyamine fraction obtained after the ion-exchange column chromatography was dansylated and thin layer chromatography (TLC) of the resulting products was carried out in $CHCl_3$–Et_3N (5 : 1) on a precoated Silica gel 60 plate (E. Merck, 0.25 mm thickness).

Results and Discussion

The gas chromatogram of the *N*-EOC derivatives of polyamines obtained from the root of *E. crassipes* revealed an unknown peak (X) besides the peaks of the usual polyamines, as shown in Fig. 1. The peak X exhibited the same retention time as that of the *N*-EOC derivative of authentic *sym*-homospermidine. TLC analysis of the dansylated polyamine fraction also demonstrated the presence of an unknown component (*Rf* 0.67) as well as

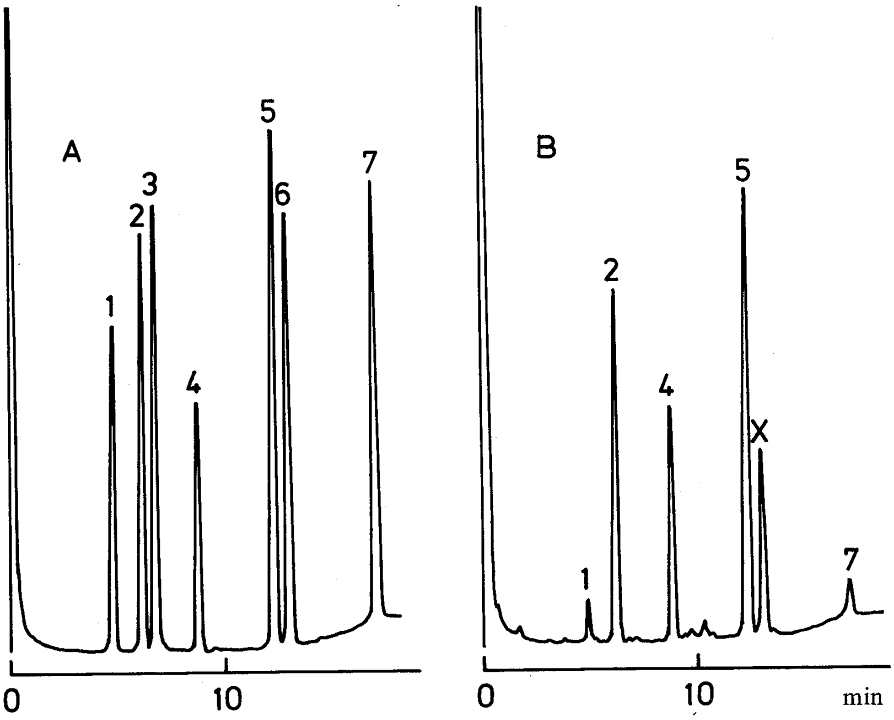

Fig. 1. Gas Chromatograms of the *N*-EOC Derivatives of Polyamines Obtained from (A) a Standard Mixture and (B) Root of *E. crassipes*

GC conditions are described in "Experimental." Peaks: 1, 1,3-diaminopropane; 2, putrescine; 3, cadaverine; 4, internal standard (1,8-diaminooctane); 5, spermidine; 6, *sym*-homospermidine; 7, spermine.

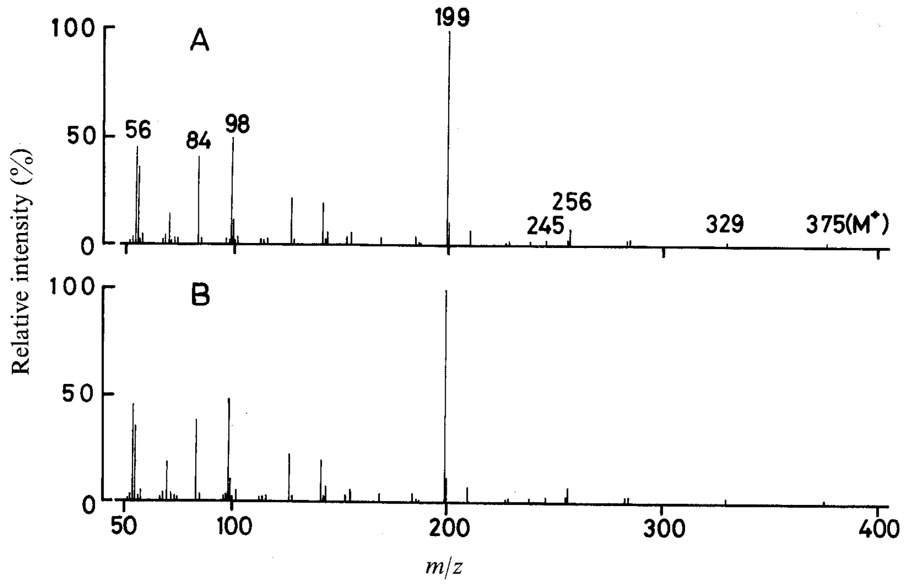

Fig. 2. Mass Spectra of (A) the *N*-EOC Derivative of Authentic *sym*-Homospermidine and (B) Peak X

putrescine (*Rf* 0.35), spermidine (*Rf* 0.62) and spermine (*Rf* 0.79), and the unknown was identical with the authentic *sym*-homospermidine on co-chromatography. For an unambiguous identification of the peak X, the sample for GC was subjected to GC-MS. A molecular ion peak (M^+) with the expected *m*/*z* 375 was observed for the peak X, and finally the identity of the unknown compound was definitely shown to be *sym*-homospermidine by comparing the spectrum with that obtained from the authentic *N*-EOC derivative (Fig. 2). The base peak at *m*/*z* 199 is probably produced by the loss of $C_2H_5OCONH(CH_2)_3$ from the peak at *m*/*z* 329 ($M^+ - C_2H_5OH$), which also gave the peak at *m*/*z* 256 with the loss of C_2H_5OCO. The loss of $C_2H_5OCONH(CH_2)_3$ from the molecular ion peak provides the peak at *m*/*z* 245. Three prominent peaks at *m*/*z* 56, 84 and 98 are presumably assigned to CH_2NCO, $(CH_2)_3NCO$ and $(CH_2)_4NCO$, respectively. The identification of 1,3-diaminopropane together with the other polyamines was also achieved by GC-MS.

In order to determine whether *sym*-homospermidine is generally present in this plant, five samples harvested from different ponds were analyzed by both GC and GC-MS. In each instance, the peak corresponding to *sym*-homospermidine was observed and the GC-MS spectrum obtained was indistinguishable from that of the authentic derivative of *sym*-homospermidine.

Recovery experiments were carried out to elucidate the quantitative accuracy of this method. The 4% $HClO_4$ extract from each portion was fortified with the standard solution at the level of 50 nmol/10 ml extract, and the recovery rates were determined from triplicate analyses of these samples and of the corresponding plant specimens. The results showed that the recoveries of polyamines throughout the ion-exchange column chromatography followed by derivatization and GC were almost quantitative. The mean recovery rates with standard deviations were as follows: putrescine 94.9 ± 3.9%, spermidine 99.4 ± 5.7%, *sym*-homospermidine 97.9 ± 5.2% and spermine 102.1 ± 7.3%.

The polyamine contents in roots as determined by GC are shown in Table I. *sym*-Homospermidine was one of the major constituents of polyamines in the root, but was present only in small amounts in the other portions; its contents in petioles and leaves were found to be in the range of 1.19—2.36 and 1.43—3.93 nmol/g fresh weight, respectively. Similarly, water (500 ml) from each pond was filtered and evaporated to dryness after addition of 1 ml of concentrated HCl, and the residue was analyzed as described above. No *sym*-homospermidine

Table I. Polyamine Contents in the Roots of *E. crassipes*

Sample	Polyamine (nmol/g fresh weight)			
	Putrescine	Spermidine	*sym*-Homospermidine	Spermine
A	28.2	29.0	10.7	3.0
B	83.9	19.7	15.9	2.8
C	206.7	124.1	41.8	13.0
D	29.2	25.7	9.9	2.9
E	70.9	54.9	46.4	13.7

was detectable. Therefore, it appears that *sym*-homospermidine in this plant is endogenous.

In view of the report indicating that polyamines (spermidine and spermine) have a stabilizing effect on the beet root membrane,[9] it is possible that *sym*-homospermidine along with the other polyamines plays a similar regulatory role in regard to the root membrane permeability of this plant in its highly hypotonic environment. The distribution of *sym*-homospermidine in allied plant as well as its physiological significance in this plant seem worthy of further study.

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