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NATURAL OCCURRENCE OF GUANIDINOOXYPROPYLAMINE IN *Wistaria floribunda* AND THE SWORD BEAN *Canavalia gladiata*

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SUMMARY: We found a new guanidinooxyamine in *Wistaria floribunda* seeds and seedlings of the sword bean, *Canavalia gladiata*. This amine was not only ninhydrin-positive but also gave a positive alkaline nitroprusside-ferricyanide reaction. It was characterized as γ-guanidinooxypropylamine [$H_2N(NH=)CNHO(CH_2)_3NH_2$] by comparison with the authentic compound on column and thin-layer chromatograms visualized with specific reagents and by reductive cleavage. Evidence for the occurrence of another unusual guanidino amine, homoagmatine in *W. floribunda* seeds was also presented.

Polyamines have been shown to be implicated in the control of macromolecular biosynthesis and growth (1). Both common and "unusual" polyamines have been found in higher plants (2). Putrescine, spermidine and spermine have been detected in many different plants, while 1,3-diaminopropane, cadaverine and agmatine are found in a limited number of them. Several "unusual" polyamines have also been found in particular plants, for example, homospermidine in sandalwood (3), canavalmine in sword bean, *Canavalia gladiata* (4) and homoagmatine in *Lathyrus sativus* seedlings (5).

In the course of investigations on polyamines in leguminous plants, we have observed several unknown peaks on our column chromatograms. One of them gave a positive nitroprusside-ferricyanide reaction, though it did not corresponded to either L-canavanine, agmatine or homoagmatine. Since leguminous plants contain several guanidino amino acids including L-canavanine (6-8), we speculated it would be one of the decarboxylation products of these amino acids. This unknown compound was indeed a decarboxylation product of L-canavanine. The present communication deals with the

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isolation and characterization of a hitherto unrecognized guanidinooxyamine in *Wistaria floribunda* and *C. gladiata*.

MATERIALS AND METHODS

Chemicals — L-Arginine·HCl, L-homoarginine·HCl, L-canavanine·H_2SO_4, putrescine·2HCl, cadaverine·2HCl, spermidine·3HCl, spermine·4HCl, arcaine·H_2SO_4 hirudonine·H_2SO_4, agmatine·H_2SO_4, octopine, *o*-methylisourea sulfate and palladium-barium sulfate were purchased from Sigma (St. Louis, MO). 3-Hydroxypropylamine was obtained from Wako Pure Chemicals (Osaka). Ten aliphatic pentaamines which contain aminopropyl and/or aminobutyl moieties were kindly supplied by Dr. K. Samejima of Josai University. Homospermidine and aminobutylhomospermidine (homospermine) were synthesized according to Okada *et al* (9). The synthesis of homoagmatine (monoamidinocadaverine) and audouine (diamidinocadaverine) was achieved by guanidation of cadaverine with *o*-methylisourea (5). Briefly, cadaverine·2HCl was treated with *o*-methylisourea-HCl in 2M NaOH at pH 10.5 for 5 days at 25°C. Homoagmatine and audouine were separated from unreacted cadaverine with a column (9 x 120 mm) of ion-exchange resin (Hitachi Custom 2612). The conversion of sulfate forms of L-canavanine, agmatine and *o*-methylisourea to hydrochloride forms was achieved by passing through a column of Dowex 1-X8 before use.

Guanidinooxypropylamine was enzymatically synthesized from L-canavanine. L-Canavanine·HCl was incubated with acetone-dried *Escherichia coli* 7020 (IFO 3544) in 0.2M acetate buffer (pH 5.25) at 30°C for 1 hr (10). After the termination of the reaction with cold 0.5N $HClO_4$, the decarboxylation product of L-canavanine was purified by column chromatography as described above.

Materials — *Canavalia gladiata* seeds were purchased from a local seed dealer. *Wistaria floribunda* seeds were collected in botanical gardens in Maebashi. After water imbibition for 12 hr, the seeds were germinated and allowed to grow in the dark on moistened filter paper discs.

Polyamine analysis — Seeds and seedlings were homogenized in 3 volumes of 0.5N $HClO_4$. After centrifugation of the homogenates, the supernatants were passed through a column (2.5 x 2 cm) of Dowex-50 W to concentrate polyamines and to eliminate amino acids (11). Polyamines were analyzed by high-performance ion-exchange column chromatography as described previously (12) with slight modifications. The identity of polyamines was also established by thin-layer chromatography on cellulose and silica gel (13). Amines on chromatograms were visualized by spraying either ninhydrin, Sakaguchi or alkaline nitroprusside-ferricyanide (pentacyanoferrate) reagent.

Large scale isolation of guanidinooxypropylamine — Approximately 1 kg of *Wistaria* seeds and *Canavalia* seedings were homogenized in 10 l of 0.5N $HClO_4$. Polyamines and unknown compounds in $HClO_4$ extracts were concentrated by the use of a Dowex-50 W column, and then separated by a column of Hitachi Custom 2612 (13).

RESULTS

When analyzed with the use of our highly sensitive high-performance liquid chromatography, putrescine, spermidine, homospermidine, spermine and agmatine were detected in the seed of *Wistaria floribunda* (Fig. 1A). Cadaverine appeared in seedlings of *W. floribunda* (1B) and *Canavalia gladiata* (1D).

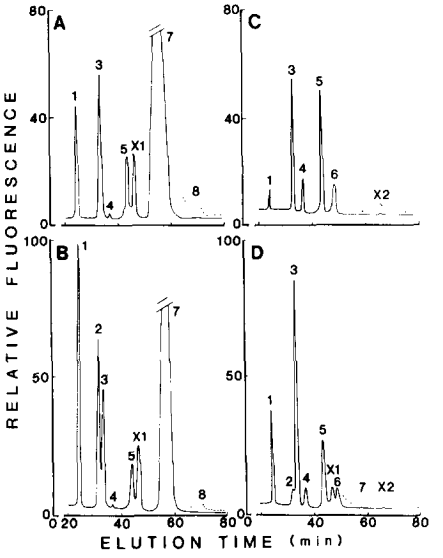

Fig. 1. Elution profiles of polyamines found in *Wistaria floribunda* and *Canavalia gladiata*. Polyamines were separated by high-performance ion-exchange chromatography from the seeds (A and C) and seedlings (B and D) of *W. floribunda* and *C. gladiata*, respectively. Elution patterns of the polyamines were followed by *o*-pthalaldehyde. The numbers above the peaks correspond to: (1) putrescine; (2) cadaverine; (3) spermidine; (4) homospermidine; (5) spermine; (6) canavalmine; (7) agmatine and (8) homoagmatine. X1 and X2, unknown compounds. Dotted lines indicate the curves at 25 times the standard sensitivity.

Besides these polyamines a prominent peak X1 was found between spermine and agmatine. This peak eluted a little prior to canavalmine. It was not identical to such tetraamines as norspermine, thermospermine and homospermine. The retention time of X1 did not correspond to that of any of the 10 pentaamines including caldopentamine and homocaldopentamine. Other compounds tested such as aminopropylcadaverine, arcaine (diamidinoputrescine), audouine (diamidinocadaverine), hirudonine (diamidinospermidine), octopine [N^2-(1-carboxyethyl)-L-arginine], norepinephrine, normetanephrine, octopamine, dopamine, serotonin, tryptamine, tyramine, cystamine, histamine, 1-methylhistamine, and N-carbamylputrescine were also excluded. The same peak as X1 appeared in *C. gladiata* seedlings (Fig. 1D), though not in the seeds. X1 from both *Wistaria* seeds and *Canavalia* seedlings behaved chromatographically like authentic guanidinooxypropylamine whenever different columns and solvent systems were used (data not shown).

When pooled fractions A-I and B-I both of which contained X1 (Fig. 2) were analyzed by thin-layer chromatography, X1 behaved just like guanidinooxypropylamine (Fig. 3). It gave a positive nitroprusside-ferricyanide reaction, which is specific for guanidinooxy compounds. Unlike agmatine and homoagmatine, X1 was not Sakaguchi-positive. When reduced in the presence of palladium-barium sulfate according to Natelson (14), X1 disappeared and new compounds which corresponded to guanidine and 3-hydroxypropylamine were produced. All these findings show that X1 is indeed identical to guanidinooxypropylamine.

A-II was identified as agmatine by column and thin-layer chromatographic techniques (Fig. 3). Agmatine is the most abundant polyamine in both seeds and seedlings of *W. floribunda* (0.6-0.8 μmoles/g weight), while only a small amount of this guanidino amine was detected in *Canavalia* seedlings (Fig. 1D). A minor peak which corresponded to homoagmatine was observed in both seeds and seedlings of *Wistaria*, but not in *Canavalia*. Several other minor peaks were

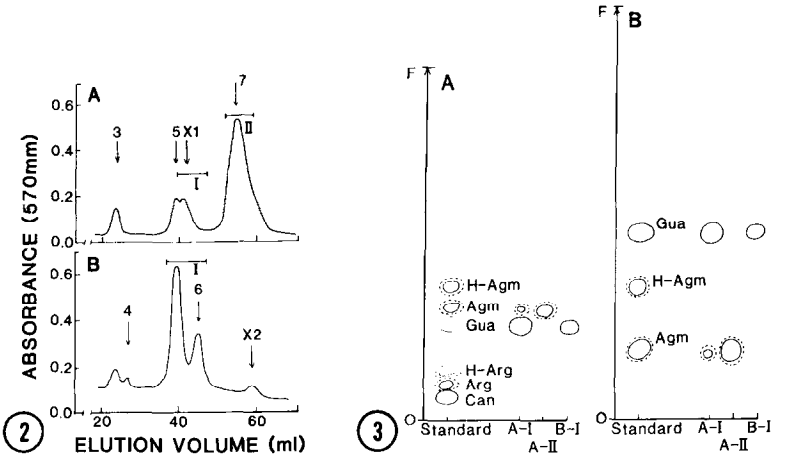

Fig. 2. Large-scale separation of polyamines and X1 from *W. floribunda* (A) and *C. gladiata* (B). Polyamines were detected by ninhydrin-reagent at 570 nm. Pooled fractions A-I, A-II and B-I were used for further analysis.

Fig. 3. Thin-layer chromatographic separation of A-I, A-II and B-I on cellulose (A) and silica gel G (B). Arginine (Arg), homoarginine (H-Arg), canavanine (Can), agmatine (Agm), homoagmatine (H-Amg) and guanidinooxypropylamine (Gua) were run stimulataneously. Spots on chromatograms were visualized by spraying alkaline nitroprusside-ferricyanide (open circle) or Sakaguchi (dotted circle) reagent. Solvent systems used were isopropanol-ammonia (7:3, V/V) (A) and chloroform-methanol-ammonia (2:2:1, V.V) (B). O, origin; F, solvent front.

found in *Canavalia* seeds and seedlings. The unknown peak X2 was tentatively identified from the retention times as pentaamine of the constitutional formula, $H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$ among the 10 pentaamines examined.

DISCUSSION

L-Canavanine (α-amino-γ-guanidinooxybutyric acid) is a nonprotein amino acid first found in the jack bean, *Canavalia ensiformis* (7) and has been shown to occur in a number of species of Leguminosae (6, 8). This amino acid is especially abundant in the seeds of various species of *Canavalia* and *Wistaria* (8). It can be converted to guanidinooxypropylamine by decarboxylation in these plants. In fact, this amine has been shown to be formed from L-canavanine by *E. coli* 7020 (10), though this bacterium normally contains no guanidinooxypropylamine. The present study has shown that this amine occurs naturally in *Wistaria floribunda* and *Canavalia gladiata*. Possibly L-canavanine is metabolized by decarboxylation as well as by arginase in these plants (15). Our preliminary experiments revealed that other species of Leguminosae which contain L-canavanine also contain guanidinooxypropylamine as a normal constituent. It is not known, however, whether it is merely an inert or detoxicated metabolite of L-canavanine or a biologically active amine. It is of interest to note that this amine content increases in *Canavalia* seedlings. Probably a decarboxylase for L-canavanine is induced during germination like other amino acid decarboxylases (16).

Since *W. floribunda* seeds contain enormous amounts of agmatine and large amounts of guanidinooxypropylamine, it is possible that a common enzyme is responsible for the formation of these amines. Although L-canavanine is a substrate for arginine decarboxylase of *E. coli* (17), it does not serve as a substrate but an inhibitor for argine decarboxylase purified from oat seedlings (18). Thus, it remains unknown whether or not guanidinooxypropylamine is formed by arginine decarboxylase or by a specific decarboxylase in leguminous plants.

Our present study has provided evidence for the occurrence of homoagmatine in *Wistaria* seedlings. This amine was first reported in *Lathyrus sativus*

seedlings (5). We also found other minor peaks which corresponded to none of the hitherto recognized amines. Our preliminary experiments showed that they corresponded to some of the 10 aliphatic pentaamines. Attempts to characterize these compounds are under way in our laboratories.

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