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Serinol (2-Amino-1,3-propanediol) and 3-Amino-1,2-propanediol in Soybean Nodules

Kiwamu Minamisawa and Hiroyuki Watanabe

Department of Agricultural Chemistry, Ibaraki University, Ami, Ibaraki 300-03, Japan

The compound X, which had previously been found to be accumulated in the soybean nodules formed by infection with wild-type H_2-uptake negative *Bradyrhizobium japonicum* strains, was identified as serinol (2-amino-1,3-propanediol) by means of elementary analysis, infrared spectrometry, ^{1}H-nuclear magnetic resonance, ^{13}C-nuclear magnetic resonance, high-performance liquid chromatography and gas chromatography/mass spectrometry. During the process of purification of compound X, it was also elucidated that 3-amino-1,2-propanediol was present in the soybean nodules as a minor component.

Key words: Aminoalcohol — 3-Amino-1,2-propanediol — *Bradyrhizobium japonicum* — *Glycine max* — Serinol (2-amino-1,3-propanediol) — Soybean nodules.

In a previous paper (Minamisawa et al. 1984), it was found that a large quantity of an unidentified compound (compound X) was present in the soybean nodules formed by infection with wild-type Hup$^-$ *Bradyrhizobium japonicum* strains. It was suggested that compound X might be involved in the process of hydrogen metabolism in soybean nodules, since the content of compound X in the nodules formed by infection with Hup$^+$ strains was remarkably lower than that in the nodules formed by infection with Hup$^-$ strains. It was also elucidated that, among the tissues of soybean plants, compound X is presnt only in the nodules and that free-living Hup$^-$ strains synthesize the compound X in a yeast extract mannitol medium.

This report describes the purification and identification of compound X.

Materials and Methods

Plant materials—Soybean nodules evolving H_2 in air (Arima et al. 1981) were detached from soybean plants (*Glycine max* cv. Enrei) at the full-bloom stage for the purification of compound X.

Preparation of amine fraction from soybean nodules—One kilogram of the soybean nodules was homogenized in 7.2 liters of 80% hot ethanol and extracted at 80°C for 1 h. The extract separated by centrifugation (6,000 × *g*, 30 min) was evaporated to dryness in vacuo, redissolved in 1.5 liters of distilled water, and centrifuged at 12,000 × *g* for 30 min. Amberlite IR-120B (H$^+$type; resin volume, 3 liters) was added to the supernatant solution, stirred and washed with distilled water. The amino compound fraction eluted from the resin with 30 liters of 2 M

Abbreviations: 3APD, 3-amino-1,2-propanediol; IR, infrared; ^{1}H-NMR, ^{1}H-nuclear magnetic resonance; ^{13}C-NMR, ^{13}C-nuclear magnetic resonance; TFAA, trifluoroacetic anhydride; TFA, trifluoroacetyl; HPLC, high-performance liquid chromatography; GC, gas chromatography; MS, mass spectrometry; SIM, selected ion monitoring; TIM, Total ion monitoring; Hup$^-$ strain, H_2-uptake negative strain; Hup$^+$ strain, H_2-uptake positive strain.

NH_4OH was evaporated to dryness in vacuo and dissolved in distilled water. The amino compound fraction was then passed through a Dowex 1 column (OH^- type, 50–100 mesh, 4.7×35 cm) to remove amino acids from the fraction. The effluent from the Dowex 1 column was loaded on a Dowex 50 column (H^+ type, 50–100 mesh, 4.7×17.3 cm), which was then washed with 3 liters of distilled water. The amine fraction eluted from the Dowex 50 column with 700 ml of 2 M NH_4OH was evaporated in vacuo, dissolved in methanol and filtered through quartz wool.

Ion exchange column chromatography—A one-third portion of the amine fraction was subjected to chromatography on a Dowex 50 column (pyridine type, 200–400 mesh, 1.8×110 cm). The column was eluted with a linear gradient of 0.2 M–1.0 M pyridine buffer (pH 4.4–5.1). The pH of the buffer was adjusted with acetic acid. Flow rate and fraction size were 1.6 ml/min and 11.5 ml, respectively. Two hundred microliters of each fraction was mixed with 10 ml of OPA solution, consisting of *o*-phthalaldehyde (0.8 g/liter), ethanol (10 ml/liter) and 2-mercaptoethanol (2.0 ml/liter) in 0.4 M borate buffer, pH 10.5. The fluorescence intensity of the reaction mixture was then determined after 4 min using a fluorescence spectrophotometer (Hitachi, 204-S).

Chemicals—Serinol and 3APD were purchased from Sigma Chemical Company and Aldrich Chemical Company, respectively. Other reagents were special-grade products from Wako Pure Chemical Industries, Ltd.

Elementary analysis—The elementary analysis was requested of the Institute of Physical and Chemical Research (Japan).

IR spectrometry—The IR spectrum was obtained using a JIR-40 FT-IR spectrophotometer (JEOL) with a KBr disk.

^{1}H-NMR and ^{13}C-NMR—^{1}H-NMR spectra were obtained using a JEOL GX-400 spectrometer at 400 MHz in D_2O. Values of chemical shifts were obtained when the signal of the internal HDO was fitted to 4.80 ppm. ^{13}C-NMR spectra were obtained using a JEOL FX-100 spectrometer at 25 MHz in CD_3OD. Values of chemical shifts were obtained using tetramethylsilane as an internal standard.

HPLC analysis—HPLC analysis was performed on a column (4×150 mm) packed with Hitachi Costom Ion-Exchange Resin (#2619F). The buffer solutions employed as eluant, shown in Table 1, were pumped through the column at a rate of 0.3 ml/min. The reagent, which consisted of a solution of *o*-phthalaldehyde (0.40 g/liter), ethanol (5.0 ml/liter) and 2-mercaptoethanol (1.0 ml/liter) in 0.4 M borate buffer, pH 10.5, was mixed with the effluent from the column at a rate of 1.0 ml/min. The reaction mixture was introduced into the flow cell of a fluorescence spectrophotometer (Hitachi, 204-S) the excitation and emission wavelengths of which were set at 340 and 450 nm, respectively.

Preparation of TFA derivative for GC/MS—A sample in hydrochloride form was dissolved in TFAA, and the mixture kept at 70°C for 30 min in a sealed glass tube. Thereafter, excess TFAA

Table 1 Buffer solution employed as eluant for HPLC

	NA buffer	4A buffer	10A buffer	NB buffer
Trisodium citrate dihydrate (g/liter)	15.55	15.55	15.55	19.26
Sodium chloride (g/liter)	25.98	25.98	25.98	35.44
Citric acid (g/liter)	15.04	15.04	15.04	12.06
Ethanol (ml/liter)	0	40	100	0
n-Caprylic acid (ml/liter)	0.10	0.10	0.10	0.10
25% Brij-35 (ml/liter)	4.0	4.0	4.0	4.0

was evaporated under a N_2 stream at room temperature and residual TFA derivative was dissolved in dichloromethane.

GC/MS—A Hitachi M-80A gas chromatograph/mass spectrometer was used. The reactant gas was isobutane (5×10^{-5} Torr) and GC conditions were as follows: column, OV-1 capillary column (0.25 mm × 25 m); carrier gas, He; flow rate of carrier gas, 2.8 ml/min; column temperature 80–130°C (5°/min).

Results and Discussion

Fig. 1 shows the ion exchange column chromatogram of the amine fraction from the soybean nodule extract. Three peaks appeared when assayed with the OPA reagent. The second large peak and the third peak were ascertained to be those of compound X and ethanolamine according to the previously reported HPLC analysis (Minamisawa et al. 1984). The first peak, which appeared to overlap the second peak of compound X, was considered to indicate the presence of another compound (unknown Y). Fraction A and B were collected as illustrated in Fig. 1.

After the fraction A, containing compound X, had been evaporated in vacuo to remove pyridine and acetic acid, a yellowish viscous liquid remained. This viscous liquid was dissolved in 0.1 M HCl and evaporated in vacuo in order to transform compound X into its hydrochloride form. This compound X hydrochloride was crystallized in ethanol containing a little HCl and recrystallized in the same solvent. After the crystals had been dried in vacuo, 73 mg of crystals was obtained. These crystals were considered to be the pure substance, as they appeared as thin, clear plates.

Elementary analysis of the compound X hydrochloride yielded the following values: C, 28.15%; H, 7.78%; N, 10.89%; O, 25.09%; Cl, 27.53%, corresponding to an empirical formula of $C_3H_{10}NO_2Cl$. The values calculated for $C_3H_{10}NO_2Cl$ are as follows: C, 28.25%; H, 7.90%; N, 10.98%; O, 25.08%; Cl, 27.79%; the values for all elements are thus in accord with those obtained from elementary analysis.

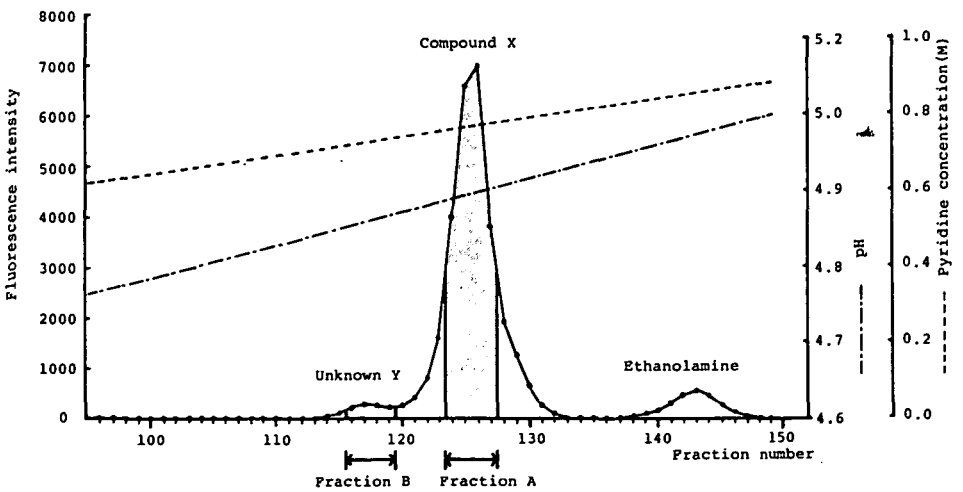

Fig. 1 Ion exchange column chromatogram of amine fraction from soybean nodule extract. An aliquot portion of the amine fraction (see text) was put on a Dowex 50 column (pyridine type, 200–400 mesh, 1.8 × 110 cm). The column was eluted with a linear gradient of 0.2 M pyridine buffer (pH 4.4) to 1.0 M pyridine buffer (pH 5.1). The pH of each buffer was adjusted with acetic acid. Flow rate, 1.6 ml/min; fraction size, 11.5 ml.

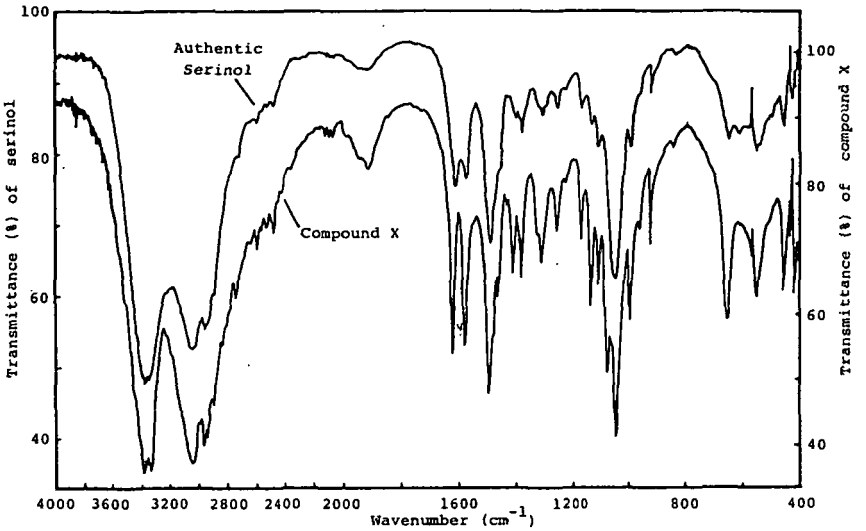

Fig. 2 IR spectra of authentic serinol and purified compound X.

Since $C_3H_{10}NO_2Cl$ was the empirical formula of compound X hydrochloride, the molecular formula of compound X was considered to be $(C_3H_9NO_2)_n$ (n=1, 2, 3, ...). The reaction mode between *o*-phthalaldehyde and compound X (data not shown) suggested that compound X contained one amino group per molecule. Thus, the possible molecular formula of the compound X was considered to be $C_3H_9NO_2$. The ^{13}C-NMR spectrum of the purified compound X exhibited only two signals for a methyne group (δ, 55.32 ppm) and a methylene group (δ, 63.94 ppm), which suggested that compound X has two kinds of carbon in terms of molecular structure.

From the above results, it appeared that compound X is serinol (2-amino-1,3-propanediol). Therefore, the IR spectra, ^1H-NMR, HPLC chromatograms and GC/MS of authentic serinol were compared with those of purified compound X.

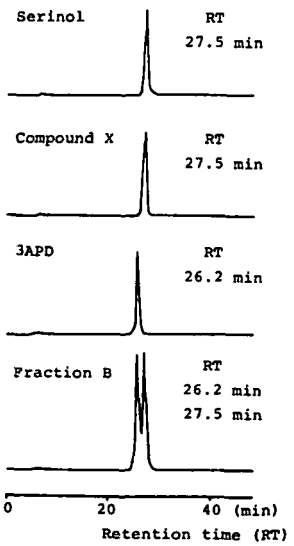

Fig. 3 HPLC chromatograms of authentic serinol, purified compound X, authentic 3APD and fraction B with 4A buffer solution as eluant.

Table 2 Retention time (min) of authentic serinol, purified compound X, authentic 3APD and fraction B in HPLC analysis with various buffer solutions

Buffer solution	Serinol	Compound X	3APD	Fraction B	
				First peak	Second peak
NA	30.0	30.0	28.1	28.1	30.0
4A	27.5	27.5	26.2	26.2	27.5
10A	25.2	25.2	24.4	24.4	25.2
NB	23.1	23.1	21.7	21.7	23.1

Fig. 2 shows the IR spectra of authentic serinol and purified compound X. The IR spectrum of authentic serinol agreed with that of compound X within all ranges including a fingerprint region.

The ^{1}H-NMR spectrum of authentic serinol showed 3.45 ppm (1H, m), 3.74 ppm (2H, dd), and 3.85 ppm (2H, dd). The ^{1}H-NMR spectrum of compound X was exactly the same as that of authentic serinol.

Fig. 3 shows the HPLC chromatograms of authentic serinol, purified compound X, authentic 3APD and fraction B using the 4A buffer solution (Table 1) as eluant. The HPLC chromatogram of the compound X showed only one peak, the retention time of which was exactly the same as that of authentic serinol. The HPLC chromatogram of fraction B gave two peaks, both of which showed the same retention time as the peaks of authentic 3APD and serinol, respectively. Even when the other buffer solutions were employed as eluant, the retention times of authentic serinol and 3APD also agreed with that of the peak for compound X and the first peak of fraction B, respectively (Table 2).

Fig. 4 shows the GC/SIM·TIM chromatograms of authentic serinol, purified compound X, authentic 3APD and fraction B TFA derivatives. Authentic serinol TFA derivative showed the same retention time as the purified compound X TFA derivative in the TIM and SIM

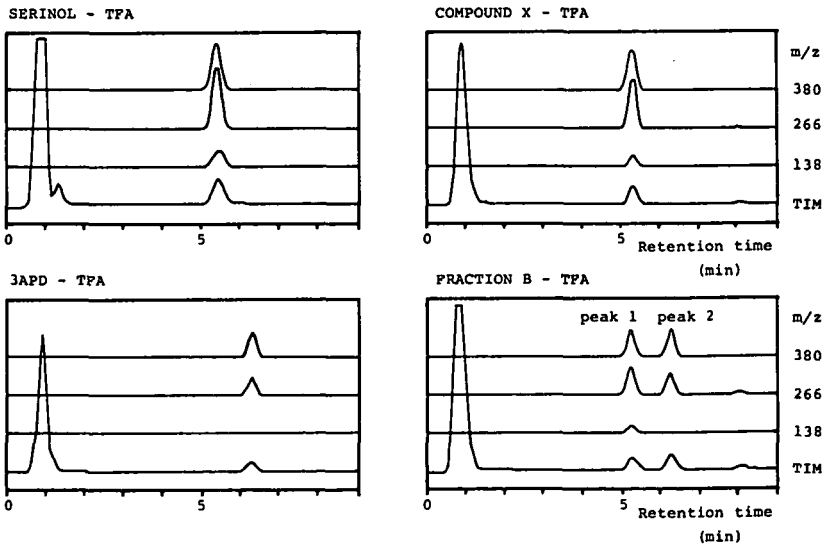

Fig. 4 GC/SIM·TIM chromatograms of authentic serinol, purified compound X, authentic 3APD and fraction B TFA derivatives.

(m/z, 380, 266, 138) chromatograms. Moreover, authentic 3APD TFA derivative showed the same retention time as the peak 2 of fraction B TFA derivative in the TIM and SIM (m/z, 380, 266) chromatograms.

The mass spectra corresponding to each peak are shown in Fig. 5 and Fig. 6. In the mass spectrum of the authentic serinol TFA derivative, a quasi-molecular ion (MH^+) was observed at m/z=380 as an N,O-trifluoroacetyl derivative (Fig. 5). The quasi-molecular ion of the compound X TFA derivative was also detected at m/z=380, the fragmentation pattern in the spectrum of the compound X derivative being consistent with that in the spectrum of the authentic serinol derivative.

The quasi-molecular ion of the peak 2 of fraction B TFA derivative was detected at m/z=380, the fragmentation pattern in the spectrum of the peak 2 of the fraction B derivative being consistent with that in the spectrum of the authentic 3APD derivative (Fig. 5) and inconsistent with that in the spectrum of the authentic serinol derivative (Fig. 6).

From the results of the elementary analysis, ^{13}C-NMR, IR spectra, ^1H-NMR, HPLC and GC/MS, it was demonstrated that compound X is identical with serinol (2-amino-1,3-propanediol). It was also elucidated that the unknown Y contained in fraction B is identical with 3APD (3-amino-1,2-propanediol), from the results of HPLC analysis and GC/MS. It remains unclear whether the absolute configuration of the 3APD in the soybean nodules is in the S or the R form.

Serinol has numerous citations in the chemical literature (Karrer et al. 1948, Zimmerman et al. 1962, Fink et al. 1963, Neuzil et al. 1965, Szammer 1969, Pitre et al. 1979, Wong et al. 1985) and in the literature on enzymatic studies (Dunkerton et al. 1975, Gillard et al. 1977,

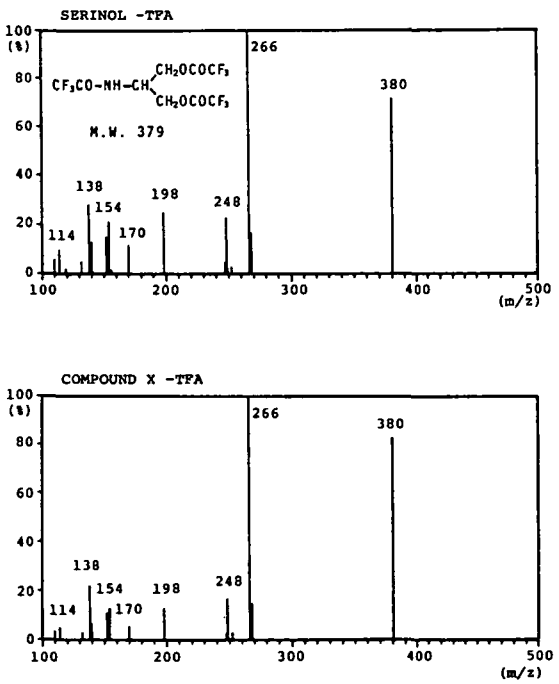

Fig. 5

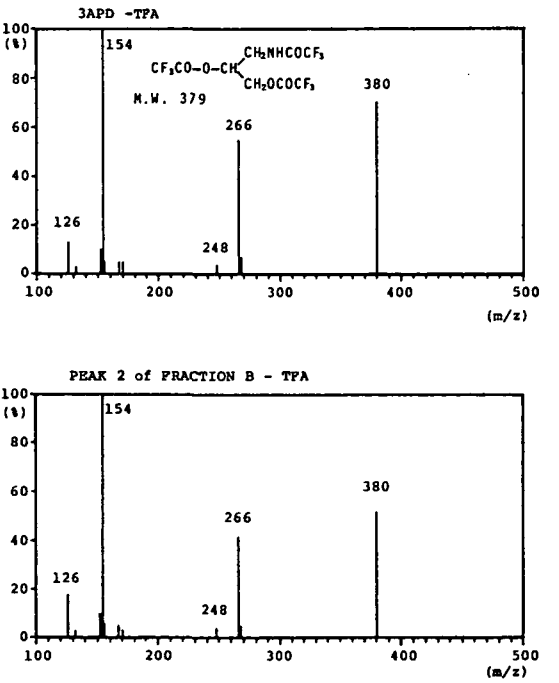

Fig. 6

Fig. 5 Mass spectra of authentic serinol-TFA and purified compound X-TFA.

Fig. 6 Mass spectra of authentic 3APD-TFA and peak 2 of fraction B-TFA.

Huber et al. 1982). However, we could find only one reference to its isolation from biological material (Pinkerton et al. 1976). Pinkerton et al. reported that serinol was present on the leaf surface of sugarcane susceptible to *Helminthosporium sacchari* and suggested that serinol acted as one of the activators of toxin (helminthosporoside) production in attenuated cultures of *Helminthosporium sacchari*. Hence, our present report is the first describing the existence of serinol in the legume nodules and appears to be the second describing the presence of serinol in living organisms.

The concentration of serinol in the soybean nodules formed by infection with wild-type Hup$^-$ strains was found to approach almost the level of free alanine or glutamate on a molar basis, ranging from 0.8 to 2.0 mg/g nodule dry weight, when recalculation was carried out from the previously reported results (Minamisawa et al. 1984). Thus, serinol is considered to form a comparatively large pool among the free nitrogenous compounds in the soybean nodules formed by infection with the wild-type Hup$^-$ strain, although it is still unclear why such an accumulation of serinol occurs.

We have not been able to find any reference to the isolation of free 3APD from biological material, though 3APD is known to be a moiety of bacterial sphingophospholipid (Kemp et al. 1976) or mephenoxalone in human urine (Eckhardt et al. 1977). Although the amount of 3APD was around one thirtieth that of serinol in the soybean nodule extract prepared for this work, 3APD may be related to serinol with regard to metabolism, since 3APD and serinol differ from each other only in the position of their functional groups.

Further investigation is needed to clarify the metabolism of serinol and 3APD in legume nodules and in free-living *Bradyrhizobium* and *Rhizobium*. To our knowledge, the metabolism of low-molecular-weight aminoalcohols has not yet been investigated in detail except for ethanolamine. Therefore, further studies on serinol and 3APD will be significant for elucidating aminoalcohol metabolism in living organisms.

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