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ORIGIN OF NITROGEN IN THE BIOSYNTHESIS OF SOLANIDINE BY *VERATRUM GRANDIFLORUM*

KO KANEKO, MIKAKO W. TANAKA and HIROSHI MITSUHASHI
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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Key Word Index—*Veratrum grandiflorum*; Liliaceae; veratrum alkaloid biosynthesis; origin of nitrogen; solanidine.

Abstract—*Veratrum* plants accumulate verazine as the major alkaloid in the rhizome during the dormant stage 4 months after cold treatment. The quantitative changes in the L-arginine content during dormancy appear to be related to the accumulation of verazine. Moreover, the results of feeding experiments with budding *Veratrum* suggested that L-arginine is the most likely nitrogen source for solanidine biosynthesis, as L-arginine-[^{15}N] was incorporated into solanidine twenty times more efficiently than $^{15}NH_4Cl$.

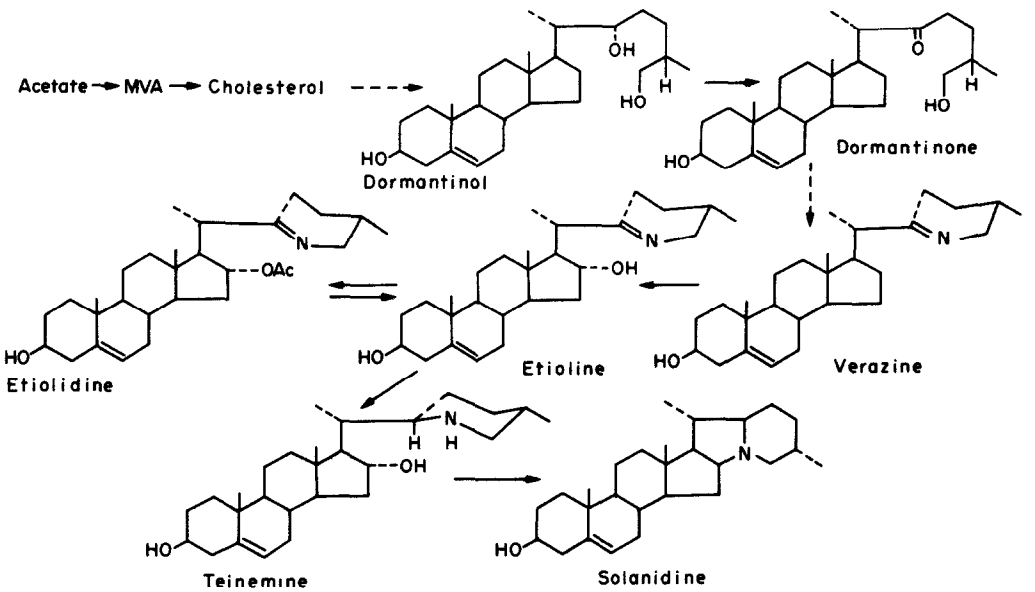
INTRODUCTION

In our previous studies on the biosynthesis of *Veratrum* alkaloids [1–3], etioline and teinemine [4], 22,26-epiminocholestene derivatives, and dormantinone [5] and dormantinol [6] oxygenated cholestene derivatives, were isolated from budding *Veratrum grandiflorum*. Their structures were determined as follows: etioline: (25*S*)-22,26-epiminocholesta-5,22(N)-diene-3β,16α-diol; teinemine: (25*S*)-22,26-epiminocholest-5-en-3β,16α-diol; dormantinone: (25*S*)-3β,26-dihydroxycholest-5-en-22-one; dormantinol: (25*S*)-cholest-5-en-3β,22α,26-triol. The presence of these steroidal compounds, in addition to verazine [7], in budding *Veratrum*, which most actively synthesizes solanidine, suggests a new hypothetical pathway of solanidine biosynthesis [8] as shown in Scheme 1. An important problem is to determine the origin of the nitrogen required in the biosynthesis of solanidine from cholesterol.

RESULTS AND DISCUSSION

A careful analysis of the alkaloids extracted from *Veratrum* during the dormant to mature period showed that verazine accumulated in the rhizome, while solanidine and etioline accumulated in the bud of dormant *Veratrum*. This result suggests that the production of solanidine in *Veratrum* begins before the budding period, and verazine is synthesized at an early step of the biosynthesis of solanidine in the dormant *Veratrum* rhizome.

Based on this assumption, we attempted to find a reasonable origin of the nitrogen required for the biosynthesis of solanidine in *Veratrum* during the dormant



Scheme 1. Hypothetical biogenetic pathway of solanidine.

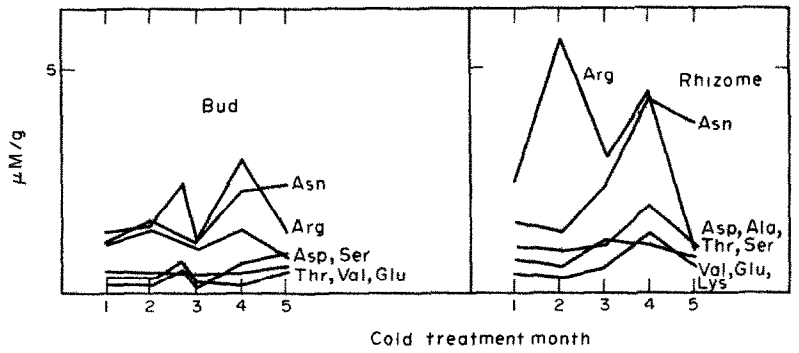


Fig. 1.

period to the budding stage. When *Veratrum* was harvested and kept at 5° in a cold room under moist conditions, budding began 5 months later. This period of cold treatment before budding was designated as the dormant period.

Figures 1 and 2 respectively show the quantitative changes of free amino acids and ammonia in *Veratrum* during the dormant period, and Fig. 3 shows the accumulation of verazine in the rhizome during the same period. During this period there was a large increase followed by a decrease in the concentration of L-arginine in the rhizome. The amount of L-arginine in the rhizome was about four times higher than that of other amino acids and ammonia, and it reached a maximum value 2 months after the beginning of the cold treatment. On the other hand, the content of other amino acids did not change during the dormant period, as shown in Figs. 1 and 2. This fact indicates the possibility that L-arginine is the origin of the nitrogen required for the biosynthesis of solanidine in *Veratrum*.

The dormant plant does not absorb an external substrate, because the plant only has a low transpiration rate. Therefore, the budding *Veratrum* were fed with L-arginine-[^{15}N], $^{15}NH_4Cl$ and cholesterol-[4-^{14}C] by the cotton wick method.

The results of mass spectroscopic analysis of ^{15}N content and the radioactivity of solanidine are shown in Table 1, in which an atom excess of ^{15}N in solanidine was calculated per mM of each compound fed, because the purchased L-arginine-[^{15}N] and $^{15}NH_4Cl$ possessed different atom excesses of ^{15}N and they were applied to *Veratrum* in different amounts. On the basis of this calculation, the incorporation of ^{15}N from L-arginine was 20 times higher than that from NH_4Cl, and the ratio of $^{15}N/^{14}C$ from L-arginine feeding was 10 times higher than that from NH_4Cl. It is concluded from these results, that L-arginine is the most likely origin of nitrogen in the biosynthesis of solanidine in *Veratrum*.

EXPERIMENTAL

Plant material. Resting plants of *Veratrum grandiflorum* (Max.) Loesen were harvested at Teine, Hokkaido, Japan, at the end of August, and washed rhizomes stored in a cold room as described previously [1].

Extraction and isolation of Veratrum *alkaloid.* Plants were dried at 60° and extracted with a mixture of ammoniacal $CHCl_3$ and MeOH. The extract was hydrolyzed with 1 M HCl in MeOH, and resulting crude alkaloid applied to a chromatographic column of alumina, as described previously [1].

Determination of amino acids in the plant. Veratrum plants at each stage were separated into 2 parts, bud and rhizome, and each part was homogenized with H_2O. The free amino acids in the homogenate, after removal of protein and peptide by addition of EtOH, were applied to a chromatographic column of Dowex 50 W × 8 (H^+ form), eluted with 1 M NH_4OH, and analyzed by a Hitachi KLA-3B amino acid analyzer.

Determination of ammonia in the plant. Veratrum plants at each stage were separated into 2 parts, bud and rhizome, and each part was homogenized with 5% KH_2PO_4. The ammonia was collected in 0.05 M H_2SO_4 soln by bubbling of air after addition of conc NaOH. The amount of ammonia in the soln was determined at 500 nm after addition of Nessler reagent.

Feeding experiment. Four plants of just-budding *Veratrum* were cultivated under dark conditions for 14 days, as described previously [1]. Two plants were fed by the cotton wick method with 55 mg of 51.3 atom excess % of L-arginine-[^{15}N] and the remaining 2 plants with 80 mg of 91.3 atom excess % of $^{15}NH_4Cl$ per plant, each together with 31.25 μCi of cholesterol-[4-^{14}C] as a marker of incorporation. The cholesterol-[4-^{14}C] was dissolved in a few drops of EtOH, 5% Tween

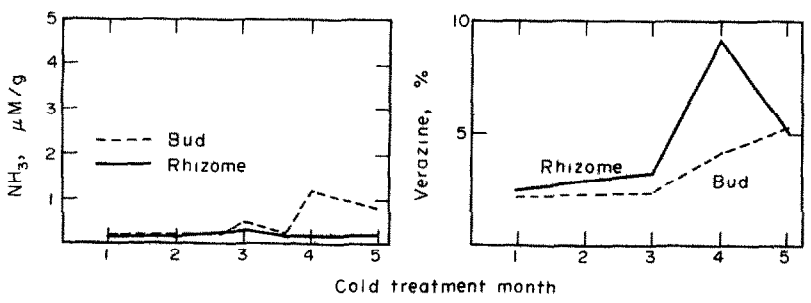


Fig. 2.

Table 1. Incorporation of ^{15}N from L-arginine-[^{15}N] and $^{15}NH_4Cl$ into solanidine, when fed with 31.25 μCi of cholesterol-[4-^{14}C] in *Veratrum grandiflorum*

Solanidine	Feeding substance: L-arginine-[^{15}N] (51.3 atom %) Fed (110 mg)	Calcd (per mM)	$^{15}NH_4Cl$ (96.3 atom %) Fed (160 mg)	Calcd (per mM)
^{15}N-Atom excess %	0.11	0.18	0.05	0.017
^{15}N-Incorporation %	0.21	0.36	0.054	0.018
^{14}C-Incorporation %	0.03		0.016	
$^{15}N/^{14}C$	7.1	11.9	3.39	1.13

80 soln added, the EtOH evaporated and the emulsion diluted with H_2O.

Measurement of radioactivity. Radioactivity of solanidine was determined by a liquid scintillation counter as described previously [1].

MS analysis of ^{15}N. Doubly labeled solanidine was decomposed by the micro-Kjeldahl method, and generated ammonia was absorbed in 0.05 M H_2SO_4. The soln contained about 1 mg of N/ml. The MS analysis was performed at Japan Isotopic Center, the Institute of Physical and Chemical Research, Komagome, Bunkyoku, Tokyo.

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