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CONVERSION OF SOLANIDINE TO JERVERATRUM ALKALOIDS IN *VERATRUM GRANDIFLORUM*

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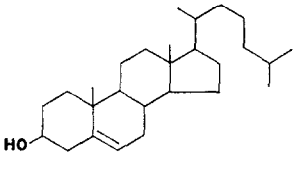
Key Word Index—*Veratrum grandiflorum*; Liliaceae; biosynthesis; jerveratrum alkaloid; solanidyl glycoside precursors.

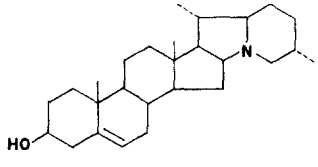
Abstract—Etiolated *Veratrum grandiflorum* Loesen. fil. accumulates solanidyl glycoside in the leaf, and this glycoside is converted into jerveratrum alkaloids in the rhizome during subsequent illumination.

INTRODUCTION

PAST evidence[1,2] has led to the view that the biogenetic pathway of jerveratrum alkaloids and ceveratrum alkaloids in *Veratrum grandiflorum* is essentially the same as that of normal steroidal sapogenins[3,4] and steroidal alkaloids,[5–7] and that the 27 carbon skeleton of cholestane is converted directly into *C*-nor-D-homo-steroidal alkaloids.



(I)



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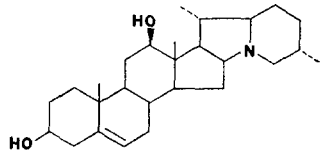

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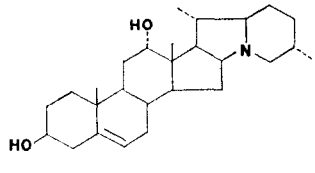

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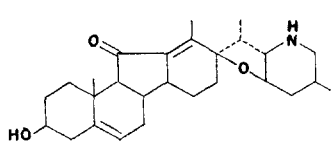

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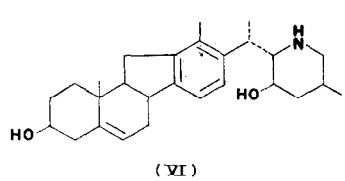

(VI)

Narayanan suggested[8] that an equatorial hydroxyl group at C-12 in solanidine would induce the *C*-nor-D-homo-rearrangement and that the jerveratrum and ceveratrum alkaloids would readly be synthesized from this hydroxylated solanidine in *Veratrum*. A possible mechanism for this rearrangement has been proposed earlier,[1,2] using *Veratrum grandiflorum*. Epirubijervine (III), which is hydroxylated equatorially at C-12, has not been

[1] K. KANEKO, H. MITSUHASHI, K. HIRAYAMA and N. YOSHIDA, *Phytochem.* **9**, 2489 (1970).
[2] K. KANEKO, H. MITSUHASHI, K. HIRAYAMA and S. OHMORI, *Phytochem.* **9**, 2497 (1970).
[3] E. HEFTMANN, E. R. LIEBER and R. D. BENNETT, *Phytochem.* **6**, 225 (1967).
[4] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 577 (1965).
[5] R. TSCHESCHE and H. HULPKE, *Z. Naturforsch.* **21b**, 494 (1966).
[6] R. TSCHESDHE and H. HULPKE, *Z. Naturforsch.* **22b**, 791 (1967).
[7] R. TSCHESCHE and H. HULPKE, *Z. Naturforsch.* **21b**, 893 (1966).
[8] C. R. NARAYANAN, *Fortschr. Chem. Org. Naturstoffe* (edited by L. ZECHMEISTER), Vol. XX, p. 298, Springer, Geneva (1962).

isolated from the natural source up to the present but both solanidine (II) and rubijervine (IV) were isolated as minor components from resting *Veratrum* rhizome.[9]

On the other hand, although solanidine (II) in *Solanum tuberosum* at the budding period, it is hardly detectable in the growing plant. There is thus the possibility that precursors formed from cholesterol (I) accumulate at the budding period and then give rise to jerveratrum or ceveratrum alkaloids during the early growing period. This is supported by the fact that incorporation of acetate-1-^{14}C into *Veratrum* alkaloids is highest during the early growing period as described previously.[1]

When *Veratrum* plants were fed with acetate-1-^{14}C in total darkness, a large amount of radioactive solanidyl glycoside (II′) accumulated in the etiolated leaf but not in the rhizome. When the etiolated plant was subsequently illuminated, the radioactivity disappeared rapidly from the accumulated solanidyl glycoside (II′) and was incorporated into jerveratrum alkaloids.[10] The present report describes the relationship between solanidine and jerveratrum alkaloids under etiolated and illuminated growth conditions, and the accumulation of a monohydroxylated solanidine in the rhizome following a relatively short illumination period.

RESULTS

The amount of solanidine (II) as glycoside (see Experimental) in etiolated *Veratrum* leaf reached 50–60 mg per plant after 10 days in the dark. On the other hand, jervine (V) and veratramine (VI) were each present as about 100 mg per individual plant, and consisted of 10% of free alkaloid and 90% of glycoside. Almost twice the amount of solanidine (II) was present in dark-grown plants after cultivation for 7 days. Both free solanidine and its glycoside (II′) were present in small amounts in the rhizome during either dark conditions or in the naturally growing plant.

From these facts, it appears that solanidine (II) accumulates in the leaf as a glycoside in the dark and that the solanidyl glycoside (II′) is then incorporated into *Veratrum* alkaloids on illumination. Similar changes probably take place under natural conditions.

To examine this phenomenon, *Veratrum* plants were fed with 50 or 100 μCi of acetate-1-^{14}C in the dark. Radioactivity increased gradually in the leaf. These etiolated plants were then illuminated for 12 hr per day, and allowed to grow for several more days.

In the dark, *Veratrum* plants grew very rapidly, (height after 10 days was about 40–50 cm), but did not show the usual hook-shaped form. Growth continued but at a reduced rate on illumination, and after 2 days the chlorophyll content was almost equal to that in fully illuminated plants. The incorporation of radioactivity from solanidyl glycoside (II′) to jerveratrum alkaloids in *Veratrum* during the dark period and successive illumination is shown in Table 1.

The radioactivity of solanidine (II) increased continuously in the dark (Table 1). The radioactivity of jerveratrum alkaloids, jervine (V) and veratramine (VI), only increased slightly in this period. It proved difficult to maintain completely dark conditions because H_2O and $FeCl_3$ were supplied every day. Thus even the plants grown in the dark for 20 days were slightly green and contained a small amount of chlorophyll. It is possible that the relative high radioactivity of jervine (Table 1), may be due to this trace of illumination. When the etiolated plant was illuminated, the radioactivity of jervine (V) and veratramine

[9] T. MASAMUNE, Y. MORI, M. TAKASUGI, A. MORI, S. OHUCHI, N. SATO and N. KATSUI, *Bull. Chem. Soc. Japan* **38**, 1374 (1965).

[10] K. KANEKO, M. WATANABE, S. TAIRA, K. HIRAYAMA and H. MITSUHASHI, Abstr. papers, *Ann. Meeting of Japan. Pharma. Assoc.* **II**, 143 (1970).

(VI) increased rapidly as the radioactivity in solanidine (II) decreased. After 10 days, solanidine (II) could not be isolated, and was only identified in trace amounts by TLC. The radioactivity of jervine (V) and veratramine (VI) reached a maximum 2 days after illumination. These results seem to suggest that solanidine (II) plays a part in the biogenesis of the jerveratrum alkaloids in *Veratrum*, and supports Narayanan's hypothesis[8] on the formation of *C*-nor-D-homosteroidal skeleton by rearrangement, perhaps due to increases in NADPH on illumination.[11,12]

TABLE 1. RADIOACTIVITY IN STEROIDAL ALKALOIDS OF MATURE *Veratrum* PLANT GROWN IN THE LIGHT AND IN THE DARK

Cultivation (days)		Radioactivity dpm per mmole $\times 10^{-4}$		
Dark	Light	Solanidine	Veratramine	Jervine
10	0	47·5	9·0	6·8
10	2	30·4	20·3	46·2
10	6	11·0	5·0	7·1
10	10	—	14·7	27·0
20	0	146·0	11·1	28·9

The growth of etiolated plants at the budding stage was significant, but the incorporation of radioactivity from solanidine (II) to the jerveratrum alkaloids was very much slower than in the mature plant (Table 2). These plants did not fully recover their green color when placed in the light, suggesting that they had lost some ability to synthesize chlorophyll. From these facts, it seems likely that the transition of solanidine (II) to jerveratrum alkaloids is correlated with the synthetic activity of the chloroplast.

TABLE 2. CHANGES IN THE RADIOACTIVITY IN STEROIDAL ALKALOIDS OF ETIOLATED *Veratrum* PLANTS AT THE BUDDING STAGE AFTER ILLUMINATION

Cultivation (days) with illumination*	Radioactivity dpm per mmol $\times 10^{-4}$			
	Solanidine	Veratramine	Jervine	Hydroxylated solanidine
0	68·8			
2	26·4	0·8	1·6	
4	34·4	0·4	0·6	
6	10·8	0·6	0·6	
10	2·9	3·1	0·9	8·2

* After 5 days in the dark.

The alkaloidal fraction, isolated after illumination for 10 days, showed a new main unknown spot on TLC between solanidine (II) and veratramine (VI). The compound was separated on an alumina column and by TLC; it had a relatively high radioactivity (Table 2) and was also isolated from plants cultivated without acetate-1-^{14}C. It had m.p. 181–187°, [M^+] 413 (by MS) and, from this evidence, it corresponds to a monohydroxy-derivative of solanidine (II), but not rubijervine (IV). Further work is in progress on the structure of this alkaloid and its role in the biogenesis of the jerveratrum alkaloids will be reported at a later date.

[11] T. OH-HAMA and S. MIYACHI, *Biochim. Biophys. Acta* **34**, 202 (1959).
[12] W. L. OGREN and D. W. KROGMANN, *J. Biol. Chem.* **240**, 4603 (1965).

EXPERIMENTAL

Plant material. The rhizomes of *Veratrum grandiflorum* Loesen. fil. were harvested at Teine, Hokkaido, Japan, in the end of summer, and the washed rhizomes were stored in a cold room for 5 months. Such plants were cultivated in quarter strength Hoagland solution.[1] The growth of the plant and its ability to synthesize *Veratrum* alkaloids were most active at the time of budding.

Etiolated and illuminated cultures. A plant fed with acetate-1-^{14}C,[1] was cultivated in darkness. When fully etiolated, the plant was irradiated 12 hr per day with an artificial light placed 20 cm from each plant.

Comparison of solanidine content in etiolated and illuminated plants. The plants were cultivated for 7 days both in the dark and in the light. Leaves (10 g) from each were dried and extracted with ammoniacal MeOH. The MeOH extract did not contain free solanidine, but on hydrolysis with 1 N-HCl in MeOH, solanidine was obtained as the main alkaloid (TLC). *Veratrum* leaves in both light and dark contained traces of jervine and veratramine.

Extraction and isolation of radioactive alkaloids. Plants were dired at 60°, mixed with an equal vol. of sand powdered, and extracted with ammoniacal mixture of $CHCl_3$ and MeOH (1:1). The extract was concentrated to dryness and the solid re-extracted with Et_2O (Soxhlet). The residue was hydrolyzed with 1N HCl in MeOH for 6 hr. The hydrolyzate was diluted with H_2O (1:1), made alkaline with 50% NaOH, and extracted with Et_2O. The Et_2O was extracted with 5% tartaric acid, giving jervine (m.p. 240°), veratramine (m.p. 204°), and solanidine (m.p. 217°), which were isolated and purified as described previously.[1] A small part of jervine was converted into isojervine,[13] and isojervine was separated from jervine by TLC. Free jervine and vertramine were also obtained by column chromatography and TLC from the Et_2O phase of the Soxhlet extraction. Free and hydrolyzed alkaloids were individually combined and recrystalized to constant its radioactivity.

Separation of hydroxylated solanidine. The *Veratrum* rhizome from a plant illuminated for 2 days, after 12 days in the dark, was dried and powdered. 79 g of this powder was extracted and worked up as before. The hydrolyzed alkaloid fraction (2·2 g) was separated on alumina as described previously.[1] After the elution of solanidine, the following fraction, with 10% Et_2O in benzene, was collected. This fraction was further purified by repeated TLC, and gave 34 mg of alkaloid, m.p. 181–187°.

Measurement of radioactivity. The radioactivity of all the samples was measured by a liquid scintillation, as described previously.[1]

[13] W. A. JACOBS and Y. SATO, *J. Biol. Chem.* **181**, 55 (1949).