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# ETIOLINE AS IMPORTANT PRECURSOR IN SOLANIDINE BIOSYNTHESIS IN VERATRUM GRANDIFLORUM ; (25S)-22,26-IMINOCHOLESTA-5,22(N)-DIENE-3β,16α-DIOL

Ko Kaneko, Mikako Watanabe, Yuhsuke Kawakoshi and Hiroshi Mitsuhashi

Department of Pharmacognosy and Plant Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan

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In a previous work[1] it was found that the etiolated Veratrum grandiflorum Loesen.fil. accumulated solanidine glycoside in the leaf, and subsequently, when the etiolated plant was illuminated, the accumulated solanidine glycoside was converted gradually to jerveratrum alkaloids. It seems reasonable to accept that, at the budding period and in the early stage of etiolation, the veratrum plant probably accumulates important precursors which are converted rapidly to solanidine (IV) under etiolated condition.

The glycosidic alkaloid in the part of a leaf during budding consists mainly of IV and two new alkaloids. These two new alkaloids decreased gradually during etiolation, while the content of IV increased and reached a maximum level by prolonged etiolation. Therefore, these two new alkaloids, which were accumulated in the budding plant and at the early stage of etiolation, appear to participate as an important precursor in the biosynthesis of IV.

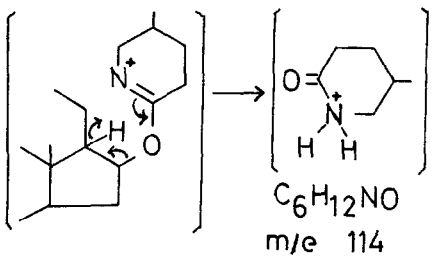
A total of 250 g of dried leaves of budding Veratrum grandiflorum gave 33.3 g of a glycoside mixture and 6.2 g of alkaloids after its acid hydrolysis. This 6.2 g of alkaloid mixture was purified through 200 g of alumina in a chromatographic column, which was eluted consecutively with benzene, 10% ether in benzene, 20% ether in benzene, 30% ether in benzene, and methylene chloride, as shown Table I. Fraction 3 was crystallized from acetone to 450 mg of fine needle crystals. After repeated recrystallization 3 or 4 times from acetone, the final crystals were dried overnight at 90° over $P_2O_5$ in vacuo. This was named etioline (I).

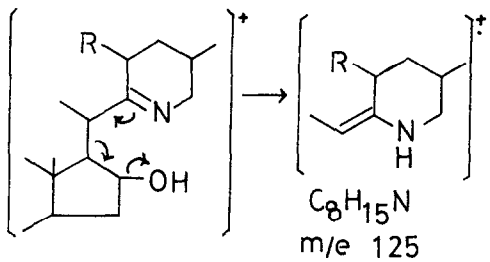
From the consideration of its empirical formula, $C_{27}H_{43}O_2N$, determined by elementary analysis and mass spectrography, I was assumed to be a glycosidic steroidal alkaloid. Presence of a double bond was revealed by nmr spectrum of I (olefinic proton, 5.36 ppm) and $SbCl_3$ colora-

tion on thin layer chromatogram, and I was converted to an α,ß-unsaturated oxo-derivatives (II), $\lambda_{max}^{EtOH}$ 240 nm (log ε 4.20) by Oppenauer oxidation. In the ir spectrum of I, absorption bands at 3450 to 3150 $cm^{-1}$ (broad) showed the presence of a hydroxyl group and that at 1660 $cm^{-1}$ is characteristic to C=N group, uv absorption maximum of I is at 238 nm (ε 183). From the ir and nmr spectra, it was proved that the C=N chromophore remained intact in I. Additional information on the nature of C=N chromophore was indicated by mass spectrum, $[M^+]$ 413 and m/e 125, 124 and 98. Although this molecular ion suggests the spirosolane formula[2] such as tomatid-5-ene-3ß-ol(25S) (VII) from Solanaceae by Schreiber[3] and solasodine(25R) (VIII) from Solanaceae, I did not agree well with VII and VIII on tlc and I did not show m/e 114 which is a base peak of spirosolane. The base peak of m/e 125 takes place as expected in McLafferty rearrangement of etioline, as same as verazine[4] (IX) and solacongestidine[5] (X).

Table I

| fraction No. | Solvents | Volume (l) | Constituent | Weight (mg) |
|---|---|---|---|---|
| 1 | benzene | 2 | solanidine | 510 |
| 2 | 10% ether in benzene | 3 | veratramine | trace |
| 3 | 20% ether in benzene | 4 | etioline | 520 |
| 4 | 30% ether in benzene | 4 | etioline and another new alkaloid | 350 |
| 5 | methylene chloride | 2 | etioline and another new alkaloid | 1,050 |
| 6 | methylene chloride | 2 | isojervine | trace |




Two oxygen functions in I were found to be alcoholic from the following evidences, I formed O,O,N-triacetate (III) by the usual method of acetylation, and this III, $C_{33}H_{49}O_5N$, mp 194-196° (needles), had signals at 1.96, 2.00 (OAc) and 2.10 (N-Ac) ppm in nmr spectrum. III displayed an enamine acetate functionality in the nmr (two olefinic protons at 5.4 and 5.2 ppm), uv, $\lambda_{max}^{EtOH}$ 234 nm (ε 7200), and ir, 1735 (OAc), 1665, 1642 (C=C-N-Ac) $cm.^{-1}$

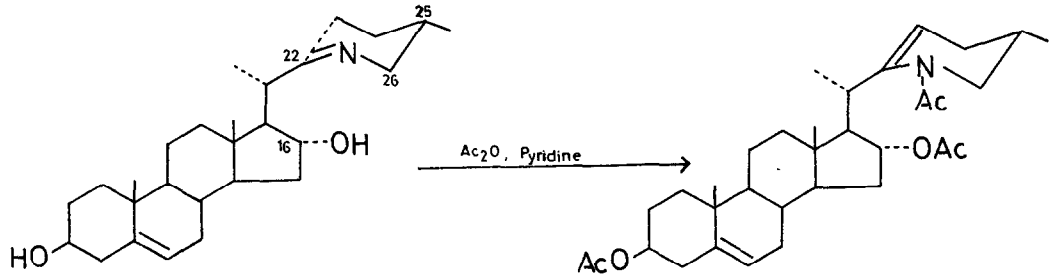
Hydrolysis of III with methanolic KOH gave a product which possessed the same Rf value as I, not that of spirosolane alkaloid (VII or VIII).

The location of the hydroxyl function of the steroidal ring in I appears to be at C-16 from chromic acid oxidation, the product of which showed absorption at 1745 $cm^{-1}$ (five-membered ring ketone) and 1715 $cm^{-1}$ (six-membered ring ketone) in its ir spectrum, and by reference to solanidine biosynthesis. The α orientation is assigned to the hydroxyl function at C-16 in I, I failed to cyclize to the spirosolane when refluxed in alcoholic KOH.[6]

In spite of a large number of steroidal alkaloids and sapogenins contained in Veratrum species, they possess only 25S-configuration and 25R-series of derivatives have not been found in veratrum plant up to present. I also appears to possess 25S-configuration.

From these evidences, I is the main glycosidic alkaloid in the budding Veratrum and its structure is determined (25S)-22,26-iminocholest-5,22(N)-diene-3ß,16α-diol.

During the past 10 years, some 22,26-iminocholestane derivatives were found in Solanum and Veratrum species and such specific steroidal structure was established, example, verazine,[4] solacongestidine,[5] solafloridine,[5] 22-oxo- and 24-oxo-solacongestidine[5] and tomatillidine.[6] These alkaloids, however, were only present as a novel and minor constituent. On the other hand, the budding Veratrum containes with etioline as major glycosidic alkaloid in the leaf as same as solanidine.




Etioline (I) Etioline-O,O,N-triacetate (III)

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