

mixed melting point and comparisons of thin-layer chromatography and IR spectra. This result established the 14-position of the tertiary hydroxyl group in digiprogenin.

The formation of  $\beta$ -digiprogenin (VIII) from  $\alpha$ -digiprogenin (II) with acid may be explained\*<sup>1</sup> by 1,4-elimination of water in the sequence indicated in Chart 1 from IX to XII. An analogous elimination of water was recently reported with erythrophleguine by Norin, *et al.*<sup>9)</sup>

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\*<sup>1</sup> Prof. C. W. Shoppee informed us in private communication that he developed independently the same explanation of this dehydration.

9) O. Lindwall, F. Sandberg, R. Thorsén, T. Norin: *Tetrahedron Letters*, No. 47, 4203 (1965).

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UDC 581.19 : 582.38 : 547.94

### Structure of Serratine

In previous publication,<sup>1,9)</sup> we have described the isolation and characterization of four new alkaloids, serratinine, serratinidine, serratine and serratanine from *Lycopodium serratum* THUNB. var. *Thunbergii* MAKINO (ホソバトウゲンバ) and the structures of serratinine (I)<sup>2)</sup> and serratinidine (II)<sup>3)</sup> which are unique among the lycopodium alkaloids, have been established.

Serratine (III), m.p. 253°,\*<sup>1</sup> C<sub>18</sub>H<sub>25</sub>O<sub>3</sub>N,\*<sup>2</sup>  $[\alpha]_D^{25} -15.0^\circ$  (c=1.02 in EtOH), IR,\*<sup>3</sup>  $\nu_{\max}$  cm<sup>-1</sup>: 3185 (OH), 1730 (C=O), NMR\*<sup>3</sup>: in pyridine, 8.69  $\tau$  (3H, s.,  $\text{>C-CH}_3$ ).

At the beginning of this study, it was anticipated that serratine would possess the serratinine skeleton because the mass spectrum of this alkaloid showed the prominent peaks at M<sup>+</sup>-28 (in this case, m/e 251), m/e 152 and m/e 150 which seem to be diagnostically important fragments for the mass spectra of serratinine type alkaloids.\*<sup>4</sup>

Acetylation of serratine (III) with Ac<sub>2</sub>O-pyridine at room temperature for six days afforded monoacetylserratine (IV), m.p. 264~265.5°, C<sub>18</sub>H<sub>27</sub>O<sub>4</sub>N, IR,  $\nu_{\max}$  cm<sup>-1</sup>: 3550 (OH), 1718 (ester and ketone carbonyl groups), NMR: 8.79 (3H, s.,  $\text{>C-CH}_3$ ), 8.05 (3H, s., -CO-CH<sub>3</sub>), 5.21 (1H, m., >CH-OAc). Further treatment of (IV) with Ac<sub>2</sub>O-pyridine at

\*<sup>1</sup> All melting points were observed on a microscopic hotstage and are uncorrected.

\*<sup>2</sup> The molecular weight establishment by mass spectrometry made revision of the earlier proposed molecular formula, C<sub>17</sub>H<sub>27</sub>O<sub>3</sub>N,<sup>1)</sup> of serratine to the present one. All compounds given by molecular formulae gave satisfactory elementary analyses.

\*<sup>3</sup> IR spectra were measured on Nujol mulls and unless otherwise noted, NMR spectra were taken in CDCl<sub>3</sub> on a Varian A-60 at 60 Mc. Chemical shifts are reported in  $\tau$  values, using tetramethylsilane as an internal reference.

\*<sup>4</sup> The mass spectrometric analyses of this series of alkaloids will be presented in elsewhere.

1) Y. Inubushi, Y. Tsuda, H. Ishii, T. Sano, M. Hosokawa, T. Harayama: *Yakugaku Zasshi*, 84, 1108 (1964).

2) Y. Inubushi, H. Ishii, B. Yasui, M. Hashimoto, T. Harayama: *Tetrahedron Letters*, No. 14, 1537 (1966); Y. Inubushi, H. Ishii, B. Yasui, T. Harayama: *Ibid.*, No. 14, 1551 (1966).

3) B. Yasui, H. Ishii, T. Harayama, R. Nishino, Y. Inubushi: *Tetrahedron Letters*, No. 33, 3967 (1966).

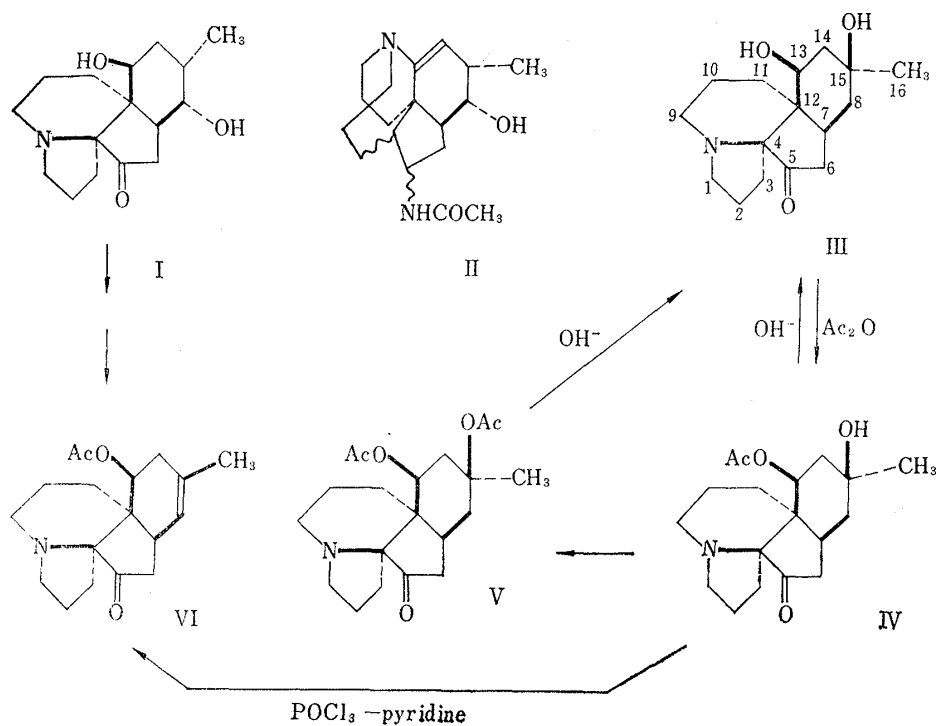


Chart 1.

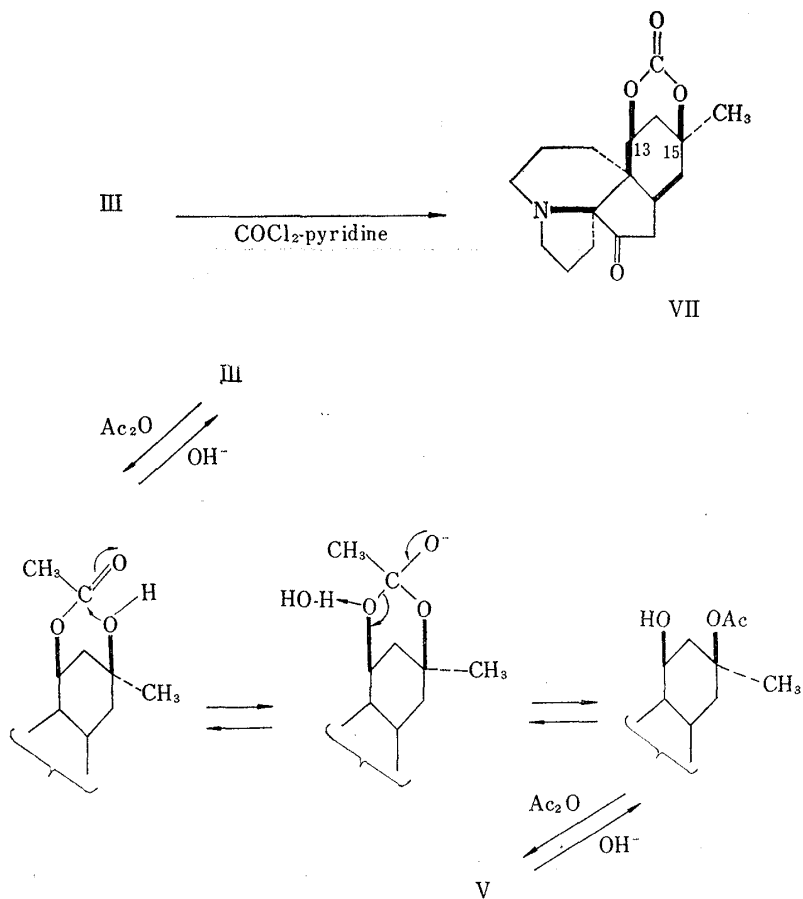


Chart 2.

100° for 4 hr. gave diacetylserratine (V), m.p. 212~214°,  $C_{20}H_{29}O_5N$ , IR,  $\nu_{max}$   $cm^{-1}$ : 1740 (C=O), no OH band, NMR: 8.51 (3H, s.,  $\rightarrow C-CH_3$ ), 8.10 (3H, s.,  $-CO-CH_3$ ), 8.04 (3H, s.,  $-CO-CH_3$ ), 5.23 (1H, t.,  $J=2.5$  c.p.s.,  $\rightarrow CH-OAc$ ). Hydrolysis of both (IV) and (V) regenerated serratine. In the NMR spectra below 6.5  $\tau$ , these two compounds revealed only one signal corresponding to one proton. This observation together with the  $\tau$  values of the tertiary methyl group suggested the presence of  $\left\langle \begin{smallmatrix} C \\ C \end{smallmatrix} \right\rangle C \begin{smallmatrix} OH \\ CH_3 \end{smallmatrix}$  system in the serratine molecule.

Dehydration of (IV) with  $POCl_3$ -pyridine at room temperature provided anhydromonoacetylserratine (VI), which was identified with an authentic sample of anhydromonoacetylserratinine II<sup>2)</sup> derived from serratinine (I), by comparison of IR spectra, specific rotations and mixed melting point determination. From this result it is certain that serratine should be represented by the formula (III) with only the configuration at C-15 to be settled, since the structure and the full absolute stereochemistry of serratinine (I)<sup>2)</sup> have been established.

Finally, the *cis* relationship between the C-13 and C-15 hydroxyl groups was shown by formation of serratine carbonate (VII), m.p. 295~297°,  $C_{17}H_{23}O_4N$ , IR,  $\nu_{max}$   $cm^{-1}$ : 1738 (C=O), no OH band, which was obtained by treatment of serratine with phosgene-pyridine at room temperature for one day.

From this steric relationship, the facile acetylation and hydrolysis of the tertiary hydroxyl group of serratine can be well explained by intramolecular transesterification through the cyclic intermediate as shown in the chart.

Consequently, serratine is represented by the absolute stereostructure of III.

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### Recovery of Biologically Active Peptides from Their 1-Dimethylaminonaphthalene-5-sulfonyl Derivatives

1-Dimethylaminonaphthalene-5-sulfonyl chloride (DNS-Cl), a reagent of fluorometry for amino compounds,<sup>1)</sup> has been utilized for identification of minute quantities of amines and amino acids.<sup>2)</sup> Recently, one of the authors applied this reagent to microanalysis of peptides whose identification was successfully achieved on thin-layer chromatogram.<sup>3)</sup> We will report a new method to recover biologically active peptides from purified DNS-peptides by reductive elimination of DNS groups with metal sodium in anhydrous liquid ammonia at  $-70^\circ$ .

Commercial colistin of 70% purity was treated with 50 folds of DNS-Cl in 50% acetone at pH 8.2. After standing for several hours at room temperature, the reaction

1) G. Weber: Biochem. J., **51**, 155 (1952).

2) W. R. Cray, B. S. Hartley: *Ibid.*, **89**, 59 (1963); Biochim. et Biophys. Acta, **21**, 58 (1956); N. Seiler, J. Wiechmann: *Experientia*, **20**, 559 (1964); *Ibid.*, **21**, 203 (1965).

3) Z. Tamura, J. J. Pisano, S. Udenfriend: Unpublished work.