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The Structures of Serratine and Serratanidine[1,2]

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The structures of two new lycopodium alkaloids, serratine and serratanidine, which have been isolated from *Lycopodium serratum* Thunb. var. *serratum* f. *serratum* (=*L. serratum* Thunb var. *Thunbergii* Makino)[4,5] were studied. From the chemical and spectroscopic evidence, the structures of these alkaloids were established as serratine (VI) and serratanidine (VII), respectively.

The alkaloid constituents of *Lycopodium serratum* Thunb. var. *serratum* f. *serratum* (=*L. serratum* Thunb. var. *Thunbergii* Makino) have been examined exhaustively and four known alkaloids, lycodoline (I), lycodine (II), clavolonine (III), lycopodine (trace) (IV) and seven new alkaloids, serratinine, serratine, serratanidine, serratinidine, serratidine, lycoserrine and serratanine have been isolated from this plant.[4,5] The structure of serratinine,[6] one of the major alkaloids, has been shown as an absolute stereostructure (V) which is a unique skeletal structure among those of lycopodium alkaloids.

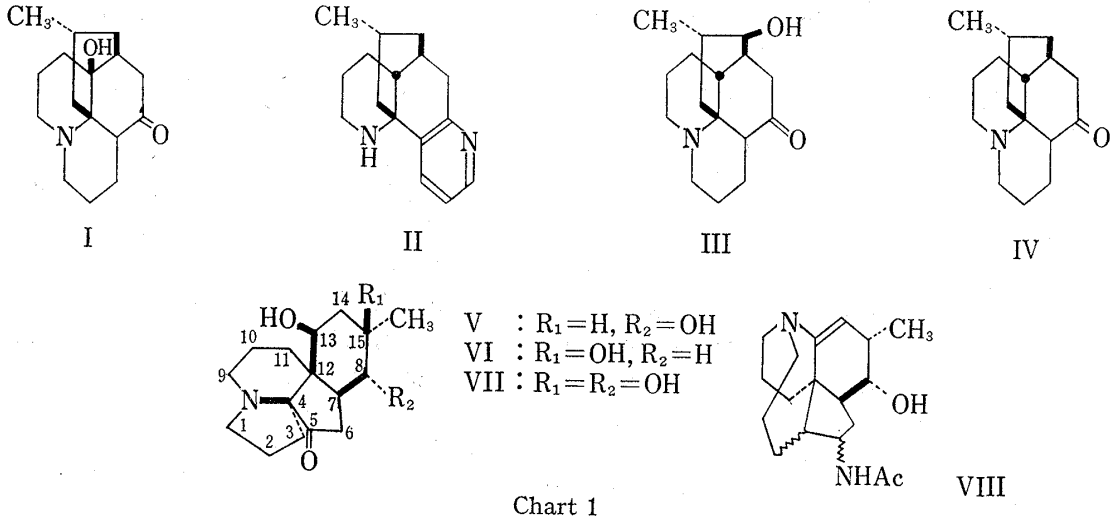


Chart 1

1) Studies on the Constituents of Domestic Lycopodium Genus Plants, Part IX: Part VIII; *Tetrahedron*, **24**, 3541(1968).
2) The preliminary reports of this work appeared in *Chem. Pharm. Bull.* (Tokyo), **15**, 250 (1967) and *ibid.*, **16**, 561(1968).
3) Location: *a) Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto; b) 6-5, Toneyama, Toyonaka, Osaka.*
4) Y. Inubushi, Y. Tsuda, H. Ishii, T. Sano, M. Hosokawa and T. Harayama, *Yakugaku Zasshi*, **84**, 1108 (1964).
5) Y. Inubushi, H. Ishii, B. Yasui, T. Harayama, M. Hosokawa, R. Nishino and Y. Nakahara, *Yakugaku Zasshi*, **87**, 1394 (1967).
6) Y. Inubushi, H. Ishii, B. Yasui, M. Hashimoto and T. Harayama, *Tetrahedron Letters*, **1966**, 1537, 1551; *Chem. Pharm. Bull.* (Tokyo), **16**, 82, 92, 101 (1968).

Successively, we have reported the structure establishments of serratine (VI),[7] serratanidine (VII)[8] and serratinidine (VIII)[9] by chemical correlation with serratinine in the preliminary communications.

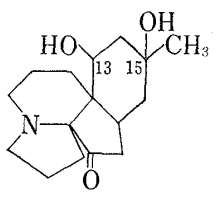
In the present paper we wish to report a full detail of structure establishments of serratine (VI) and serratanidine (VII) and a practical usefulness of mass spectrometry to the structure elucidation of serratinine type alkaloid.[10]

Serratine was obtained as colorless pillars, mp 253° and the molecular formula $C_{16}H_{25}O_3N$ was fixed on the basis of analytical and mass spectral data. The infrared (IR) spectrum showed the presence of one or more hydroxyl groups by absorption band at 3185 cm^{-1} and a ketone function at 1730 cm^{-1}. It was inferred from the nuclear magnetic resonance (NMR) spectrum of serratine in pyridine that a C-methyl group (8.69 τ, s.,) is tertiary. Acetylation of serratine with acetic anhydride in pyridine at room temperature for six days gave monoacetylserratine (IX), $C_{16}H_{24}O_2N(OCOCH_3)$, which still showed a hydroxyl band at 3550 cm^{-1} in the IR spectrum and a 3H singlet at 8.05τ and a 1H multiplet at 5.21τ attributable to an acetyl methyl and a proton geminal to an acetoxyl group, respectively, in the NMR spectrum.

On the other hand, acetylation of serratine with acetic anhydride in pyridine at 100° for 5 hours or with acetic anhydride and sodium acetate at 100° for 4 hours provided diacetylserratine (X), $C_{16}H_{23}ON(OCOCH_3)_2$, whose IR spectrum showed no hydroxyl band. The NMR spectrum of diacetyl serratine showed two singlets at 8.04 and 8.10 τ due to two acetyl methyls. However, there was found only a 1H triplet (J=2.5 cps,) at 5.23 τ which should be assigned to the proton geminal to an acetoxyl group. That no rearrangement had occurred during acetylation was certain because both monoacetylserratine (IX) and diacetylserratine (X) were hydrolyzed to afford serratine. In addition to these facts, lack of any signal due to an olefinic proton and N-methyl group in the NMR spectrum of diacetylserratine led us to a conclusion that serratine must be a tetracyclic alkaloid having the expanded formula, $C_{11}H_{19}$(C=O) (>CH–OH) (⩾C–CH_3) (⩾C–OH) (>N–).

Striking evidence of the skeleton of serratine came from the mass spectrum of serratine. Its mass spectrum showed three characteristic peaks at M-28, *m/e* 152 and *m/e* 150, which are diagnostically important for the serratinine skeleton carrying a hydroxyl group at C_{13}.[10] From these spectral data, we can presume that the plane structure of serratine is represented by the formula (A).

Dehydration of monoacetylserratine (IX) with phosphorus oxychloride in pyridine afforded anhydromonoacetylserratine (XI), mp 180—181°, which was proved to be identical with 8-anhydro-13-acetylserratinine[6] by comparison of IR spectrum mixed melting point determination and specific rotation. Moreover, oxidation of serratine with Jones' reagent gave a mixture which showed two spots on thin-layer chromatography. When chromatographed on alumina the first eluate was found to be identical with the α,β-unsaturated-ketone (XII)[6] derived from serratinine, and the successive elution provided dehydroserratine (XIII), $C_{16}H_{23}O_3N$, whose IR spectrum showed a hydroxyl band at 3500 cm^{-1} and ketonic bands at 1740 and 1705 cm^{-1}.



(A)

These facts established the structure of serratine as the formula (VI) except the stereochemistry at C_{15}. The information of the configuration at C_{15} was obtained from behavior of serratine to acetylation. Thus, acetylation of serratine with acetic anhydride in pyridine at room temperature gave a small amount of diacetylserratine (X) together with monoacetyl-

7) Y. Inubushi, H. Ishii and T. Harayama, *Chem. Pharm. Bull.* (Tokyo), **15**, 250(1967).
8) Y. Inubushi, T. Harayama, M. Akatsu, H. Ishii and Y. Nakahara, *Chem. Pharm. Bull.* (Tokyo), **16**, 561 (1968).
9) B. Yasui, H. Ishii, T. Harayama, R. Nishino and Y. Inubushi, *Tetrahedron Letters*, **1966**, 3967.
10) Y. Inubushi, T. Ibuka, T. Harayama and H. Ishii, *Tetrahedron*, **24**, 3541 (1968).

serratine (IX). The facile acetylation of the tertiary hydroxyl group in serratine at room temperature, which may be caused by the acyl migration as shown in Chart 2, suggests that serratine has the *cis* 1,3-diaxial glycol system in its molecule. This presumption, the *cis* relationship of the C_{13} hydroxyl group to the C_{15} hydroxyl group, was definitely confirmed by the following experiment. Treatment of serratine with phosgene in pyridine gave serratine carbonate (XIV), $C_{17}H_{23}O_4N$, which in the IR spectrum showed the carbonyl band at 1738 cm^{-1} but no hydroxyl band.

Consequently, serratine should be represented by the formula (VI) including an absolute configuration.

Serratanidine (VII), mp 210—211°, $[\alpha]_D^{12.5}$ −52.0° (c=1.01, EtOH) was analyzed for $C_{16}H_{25}O_4N$ and showed IR bands for hydroxyl groups at 3510, 3470 and 3150 cm^{-1}, a ketonic group at 1720 cm^{-1} and an active methylene group at 1415 cm^{-1}. Acetylation of serratanidine with acetic anhydride in pyridine at room temperature afforded diacetylserratanidine (XV), whose IR spectrum still showed an absorption band of a hydroxyl group at 3510 cm^{-1}. Its NMR spectrum showed a 3H singlet attributable to a tertiary methyl group at 8.82τ and two signals at 5.18τ (1H, t., J=3 cps,) and 4.92τ (1H, br. s.,) together with two 3H singlets at 8.06 and 7.93τ due to acetoxyl groups. The proton geminal to a hydroxyl group was not observed. From the foregoing results, serratanidine could be represented by the expanded

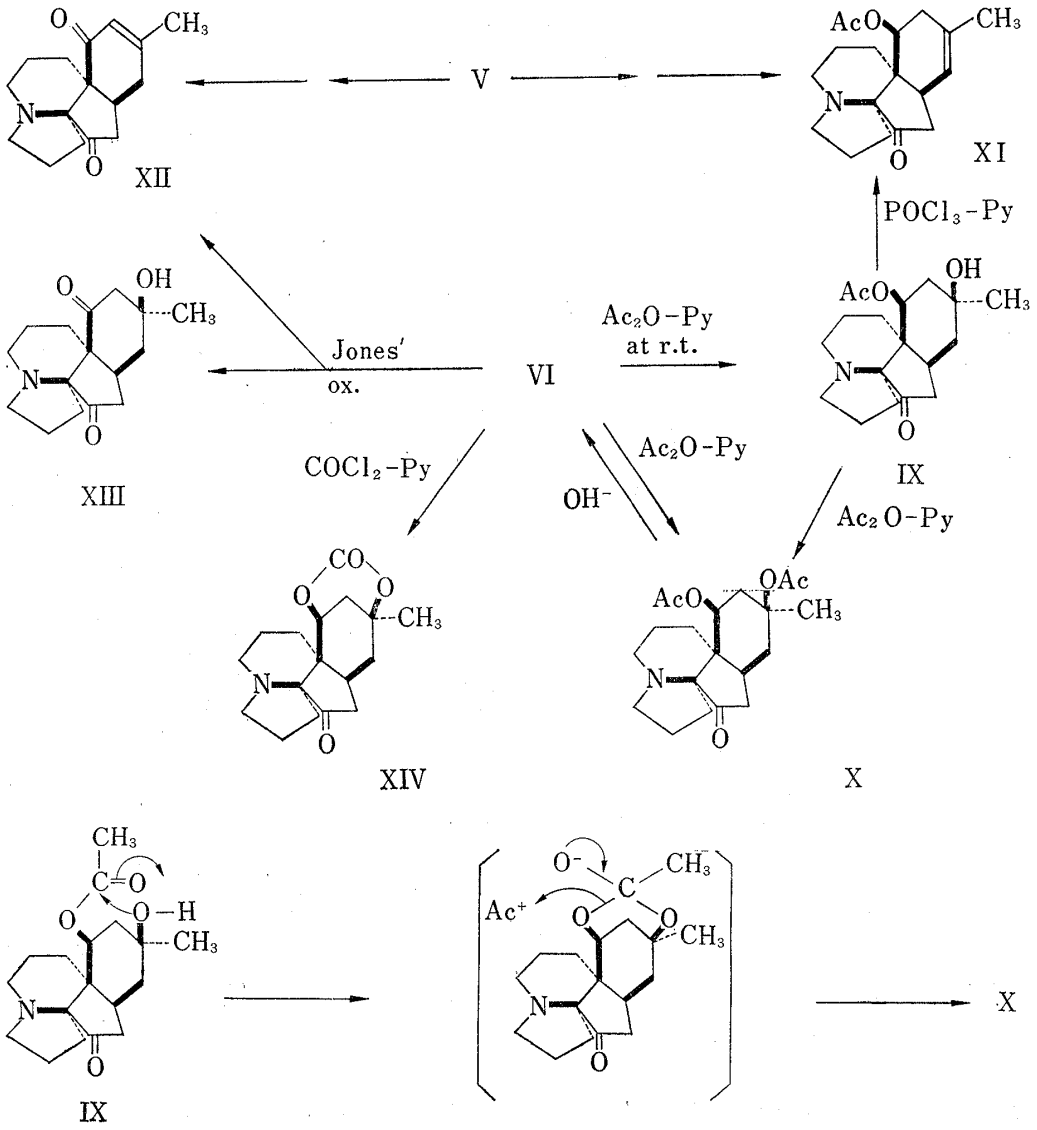


Chart 2

molecular formula C_9H_{15} (>N–) (–CH_2–CO–) (≥C–CH_3) (≥C–OH) (>CH–OH)$_2$. The mass spectrum of diacetylserratanidine gave the useful information on the framework of serratanidine. Its mass spectrum showed four characteristic fragment ions at M-28, M-87, *m/e* 194, *m/e* 152, which are diagnostically important for the serratinine type alkaloid carrying an acetoxyl group at C_{13}.[10] This observation that serratanidine possesses serratinine skeleton as well as serratine was supported by chemical correlation of serratanidine derivative with serratinine derivative.

Hydrolysis of diacetylserratanidine (XV) with aq. 10% HCl unexpectedly afforded a ketonic compound (XVI), $C_{16}H_{23}O_3N$, which showed a hydroxyl band at 3100 cm^{-1} and ketonic bands at 1735 and 1700 cm^{-1} in the IR spectrum. The identity of this ketone with 8-dehydroserratinine was demonstrated by the following experiments. Thus, hydrolysis of 8-dehydro-13-acetylserratinine (XVII) derived from serratinine, with acid provided 8-dehydroserratinine (XVI) which regenerated the acetate (XVII) by acetylation. 8-Dehydroserratinine thus obtained was proved to be identical with the ketonic compound (XVI) by comparison of IR spectrum and mixed melting point determination. These experimental data suggest that serratanidine should be represented by the plane structure (VII).

The remaining problem to be settled is the configurations of two hydroxyl groups at C_8 and C_{15}. Osmolation of 8-anhydro-13-acetylserratinine (XI) with osmium tetroxide, followed by decomposition of the osmate with hydrogen sulfide furnished *cis*–diol-A (XVIII), $C_{18}H_{27}O_5N$, whose IR spectrum indicated a hydroxyl band at 3450 cm^{-1}. Its NMR spectrum showed a 3H singlet due to a tertiary methyl group at 8.65τ and a 1H doublet (J=4 cps,) attributable to a proton geminal to a hydroxyl group at 6.30τ. From these spectral data, it is obvious that both C_8 and C_{15} in the anhydro compound (XI) are hydroxylated. *cis*–Diol-A (XVIII) was hydrolyzed by alkali to give *cis*–triol-A (XIX), $C_{16}H_{25}O_4N$, which was not identical with natural serratanidine. On the other hand, decomposition of the osmate by aq. sodium sulfite solution afforded both *cis*–diol-A (XVIII) and *cis*–triol-A (XIX). Attempt to obtain diastereoisomeric *cis*–diol by Woodward's–*cis*–hydroxylation[11] of (XI) with silver acetate–iodine failed to recover the starting material. Next, derivation of an epimer at C_8 of *cis*–diol-A was tried. Jones' oxidation of *cis*–diol-A afforded a dehydro derivative (XX), $C_{18}H_{25}O_5N$, which showed a hydroxyl band at 3430 cm^{-1} and a carbonyl band at 1720 cm^{-1}. Reduction of this compound (XX) with $NaBH_4$, followed by hydrolysis afforded *trans*–triol-A (XXI), $C_{16}H_{25}O_4N$, whose IR spectrum showed hydroxyl bands at 3460, 3320 and 3220 cm^{-1} and a ketonic band at 1720 cm^{-1}, and this compound was proved to be not identical with *cis*–triol-A (XIX) and serratanidine. It is apparent that two triol (XIX) and (XXI) are epimer with respect to the hydroxyl group at C_8, because the carbonyl group at C_5 of serratinine type alkaloid carrying a β-hydroxyl group at C_{13} was shown to be inactive to $NaBH_4$ even under the forced condition.[6] This conclusion was also supported by the mass spectrum of *trans*–triol-A (XXI), since this triol (XXI) showed three diagnostic fragment ion peaks, M-28, *m/e* 152 and *m/e* 150 which are always observed in all serratinine derivatives with the original ketone group at C_5 intact.

Oxidation with H_2O_2–HCOOH[11] which has been well known to give *trans*–diol derivative from the olefinic compound was applied to 8-anhydro-13-acetylserratinine (XI). Oxidation product, *trans*–triol-B (VII), $[\alpha]_D^{27}$ −44.0° (c=1.0 in EtOH) was identical with natural serratanidine in all respects. This observation indicates that the C_{13} hydroxyl group in serratanidine is β-configuration and the configurational relationship between two hydroxyl groups at C_8 and C_{15} is *trans*. Which of these two hydroxyl groups is β and the other is α or *vice versa* was settled by the following experiment. Treatment of serratanidine with phosgene in pyridine provided serratanidine carbonate (XXII), $C_{17}H_{23}O_5N$, which showed the presence of a hydroxyl group at 3050 cm^{-1} and carbonyl groups at 1747 cm^{-1} in the IR spectrum.

11) "Advances in Organic Chemistry," ed. Raphael, Taylor and Wynberg, Vol. 1, Interscience Publ. Inc., New York, 1960, p. 117.

These IR spectral data indicate that the carbonyl group of ester is situated on the six membered ring or the larger. The stereostructure XXII and XXIII for serratanidine carbonate are still possible but the conclusive evidence which eliminates the latter XXIII, was obtained by the NMR spectrum measurement of this carbonate in DMSO. The NMR spectrum of the carbonate (XXII) showed a br. doublet (J=4 cps) attributable to a proton geminal to a hydroxyl group at 6.28τ, a br. doublet (J=4.3 cps) due to a proton geminal to a carbonate ester at 5.55τ and a clean doublet (J=4 cps) attributable to a proton of hydroxyl group at 4.26τ. The disappearance of the peak at 4.26τ and the change of a br. doublet at 6.28τ to a br. singlet by addition of D_2O showed the existence of a free secondary hydroxyl group in the

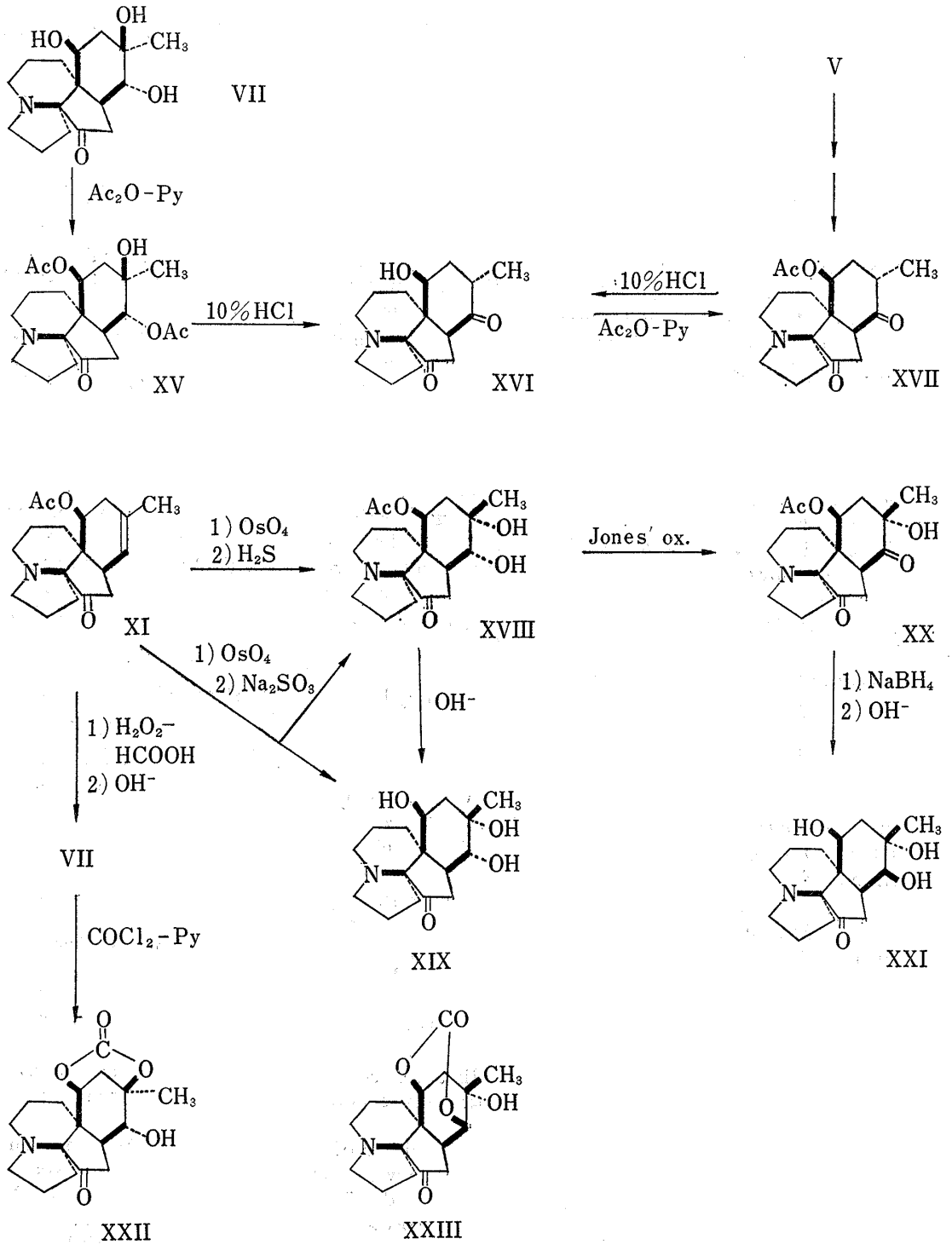


Chart 3

carbonate and the double resonance technique supported also the correctness of this assignment.

These findings led us to conclude that serratanidine carbonate should be represented by the stereostructure (XXII) suggesting *cis* diaxial relationship between the C_{13} and C_{15} hydroxyl groups in serratanidine.

Since the absolute configuration of serratinine (V) which was correlated with serratanidine through the compound (XI) has been firmly established, the absolute stereostructure of serratanidine should be represented by the formula (VII). Moreover, *cis*-diol-A which seems to arise from the compound (XI) by the attack of OsO_4 from the less hindered α side, is depicted by the formula (XVIII) because *trans*-triol-A (XXI) is not identical with natural serratanidine (VII) and two hydroxyl groups at C_{13} and C_{15} in serratanidine have been proved to be situated in the *cis* relationship.

Experimental

All melting points were observed on a microscopic hotstage and are uncorrected. All NMR spectra were obtained in $CDCl_3$ solution with tetramethylsilane as an internal standard on a Varian Associate A-60 recording spectrometer, unless otherwise noted. Aluminumoxide Woelm was available for column chromatography and IR spectra were measured on Nujol mull. Mass spectra were recorded on Hitachi mass spectrometer Model RMU 6C at an ionizing potential of 80 eV and at an evaporating temperature of 180—200°. Samples were injected directly into the ion source by using a vacuum lock system.

Serratine (VI)——The alkaloid was isolated from *Lycopodium serratum* Thunb. var. *serratum* f. *serratum* (from Mt. Hira) by the procedure reported in an earlier paper[4,5] and recrystallized from Me_2CO or AcOEt, colorless pillars, mp 253°, $[\alpha]_D^{22}$ −15.0° (c=1.02, EtOH). *Anal.* Calcd. for $C_{16}H_{25}O_3N$: C, 68.78; H, 9.02; N, 5.01. Found: C, 68.79; H, 9.25; N, 4.91. IR cm^{-1}: ν_{O-H} 3185; $\nu_{C=O}$ 1730. NMR τ: 8.69 (3H, s., $\geqslant$C–C$\underline{H}_3$) (pyridine); pKa′ 6.54; M^+: 279.

Monoacetylserratine (IX)——A solution of 444 mg of serratine (VI) in 10 ml of pyridine and 9 ml of acetic anhydride was allowed to stand at room temperature for six days. The solution was poured into ice water, made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated to dryness *in vacuo*. The residue was chromatographed on alumina (grade II) and elution with benzene, followed by ether afforded 360 mg of monoacetylserratine (IX) which was recrystallized from acetone to give colorless plates, mp 264—265.5°. *Anal.* Calcd. for $C_{16}H_{24}O_2N(OCOCH_3)$: C, 67.26; H, 8.47. Found: C, 67.27; H, 8.73. IR cm^{-1}: ν_{O-H} 3550; $\nu_{C=O}$ 1718; ν_{C-O} 1278, 1265. NMR τ: 8.79 (3H, s., $\geqslant$C–C$\underline{H}_3$); 8.05 (3H, s., –OCOC$\underline{H}_3$); 5.21 (1H, m., $\geqslant$C$\underline{H}$–OAc).

Diacetylserratine (X)——i) Acetylation with Acetic Anhydride in Pyridine: A mixture of 140 mg of serratine (VI), 2 ml of pyridine and 2 ml of acetic anhydride was heated for 5 hours at 100°. The excess reagents were evaporated to dryness *in vacuo* and the residue was diluted with water, made alkaline with NH_4OH and extracted. The residue from the extract in benzene was chromatographed on alumina (grade I) and elution with benzene gave 50 mg of diacetylserratine (X) as colorless needles, which were recrystallized from n-hexane, mp 212—214°. *Anal.* Calcd. for $C_{16}H_{23}ON(OCOCH_3)_2$: C, 66.09; H, 8.04. Found: C, 65.83; H, 8.18. IR cm^{-1}: $\nu_{C=O}$ 1740; ν_{C-O} 1250, 1240. NMR τ: 8.51 (3H, s., $\geqslant$C–C$\underline{H}_3$); 8.10 (3H, s., –OCOC$\underline{H}_3$); 8.04 (3H, s., –OCOC$\underline{H}_3$); 5.23 (1H, t., J=2.5 cps, $>$C$\underline{H}$–OAc). Elution with ether afforded 50 mg of monoacetylserratine (IX) (*vide ante*).

ii) Acetylation with Ac_2O–AcONa: A mixture of 100 mg of serratine (VI), 5 ml of Ac_2O and 200 mg of anhydr. sodium acetate was heated on water bath for 4 hours. Decomposition of excess reagent was effected by addition of K_2CO_3 little by little and then, a large quantity of water was added. The reaction mixture was basified and extracted with ether and after drying over anhydr. K_2CO_3, the solvent was evaporated off to leave the residue. Chromatography of the residue in benzene on alumina (grade I) and elution with benzene, followed by ether afforded a crystalline mass which was recrystallized from n-hexane to give 70 mg of diacetylserratine (X), mp 211—213°.

Acetylation of monoacetylserratine (IX) with the same method as mentioned for diacetylserratine (X) gave diacetylserratine (X). Hydrolysis of both monoacetylserratine (IX) and diacetylserratine (X) with alkali regenerated serratine (VI), quantitatively.

Dehydration of Monoacetylserratine (IX)——Ten drops of $POCl_3$ were added to a solution of 80 mg of monoacetylserratine in 2 ml of pyridine. The mixture was allowed to stand overnight at room temperature and poured into ice water. The aqueous solution was made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and the solvent was evaporated. The residue in benzene was chromatographed on alumina (grade I) and elution with benzene gave 50 mg of solid mass, which was recrystallized from n-pentane to afford colorless prisms, mp 180—181°, $[\alpha]_D^{22}$ +4.4° (c=1.04, EtOH). This

compound was identical with 8-anhydro-13-acetylserratine (XI) by comparison of IR spectra, specific rotation and mixed melting point determination.

Oxidation of Serratine (VI) with Jones' Reagent——To a stirred solution of 100 mg of serratine (VI) in 9 ml of acetone was added 0.9 ml of Jones' reagent. The mixture was stirred and allowed to stand at room temperature for two days. The excess reagent was decomposed with MeOH and the mixture was diluted with water, made alkaline with NH_4OH and extracted with ether. Ether extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina (grade II) and elution with benzene gave 48 mg of colorless needles, mp 116—117°. whose IR spectrum was identical with that of the α,β-unsaturated ketone (XII) derived from serratinine. Further elution with AcOEt afforded 40 mg of crystalline mass which was recrystallized from ether to give 40 mg of dehydroserratine (XIII), mp 140—142°. *Anal.* Calcd. for $C_{16}H_{23}O_3N$: C, 69.28; H, 8.36. Found: C, 69.32; H, 8.42. IR cm^{-1}: ν_{O-H} 3500; $\nu_{C=O}$ 1740, 1705.

Serratine Carbonate (XIV)——To a solution of 80 mg of serratine (VI) in 2 ml of pyridine and 1 ml of $CHCl_3$ was added 1 ml of toluene solution containing phosgene (15 g/58 g). After being kept at room temperature overnight, the mixture was poured into ice water, made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue in $CHCl_3$ was chromatographed on alumina (grade II) and the eluate from AcOEt was recrystallized from AcOEt to give 40 mg serratine carbonate (XIV) as colorless plates, mp 295—297°. *Anal.* Calcd. for $C_{17}H_{23}O_4N$: C, 66.86; H, 7.59. Found: C, 66.98; H, 7.70. IR cm^{-1}: $\nu_{C=O}$ 1738.

Hydrolysis of Diacetylserratanidine (XV) with aq. 10% HCl——A solution of 55 mg of diacetylserratanidine (XV)[5] in 10 ml of aq. 10% HCl was heated on water bath for 4 hours. The reaction mixture was basified with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina (grade IV) and elution with benzene afforded 13 mg of a ketonic compound (XVI) mp 201—204° which was identical with 8-dehydroserratinine (*vide ante*). Elution with $CHCl_3$, followed by AcOEt gave 17 mg of serratanidine (VII).

8-Dehydroserratinine——A solution of 100 mg of 8-dehydro-13-acetylserratinine (XVII) in 10 ml of aq. 10% HCl was heated at 130° for 9 hours. After cooling, the mixture was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina (grade I) and elution with benzene gave 8-dehydro-13-acetylserratinine (XVII). Elution with ether and $CHCl_3$ afforded 50 mg of 8-dehydroserratinine (XVI) which was recrystallized from ether to give colorless plates, mp 201—204°. *Anal.* Calcd. for $C_{16}H_{23}O_3N$: C, 69.28; H, 8.36. Found: C, 69.33; H. 8.33. IR cm^{-1}: ν_{O-H} 3100; $\nu_{C=O}$ 1735, 1700. Acetylation of 8-dehydroacetylserratinine (XVI) with Ac_2O–pyridine regenerated 8-dehydro-13-acetylserratinine (XVII).

Osmolation of 8-Anhydro-13-acetylserratinine (XI)——i) To a solution of 100 mg of 8-anhydro-13-acetylserratinine (XI) in 2 ml abs. benzene was added a solution of 110 mg of OsO_4 in 1 ml of pyridine. The mixture was allowed to stand at room temperature overnight and evaporated to dryness at room temperature *in vacuo* and the residue was dissolved in 10 ml of EtOH. After the addition of 800 mg of sodium sulfite in 20 ml of water, the solution was boiled, cooled and filtered. The filtrate diluted with water was made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina (grade IV) and the eluate from benzene was recrystallized from AcOEt to give 25 mg of *cis*-diol-A (XVIII), mp 222—223°, colorless prisms. *Anal.* Calcd. for $C_{18}H_{27}O_5N$: C, 64.07; H, 8.06; N, 4.15. Found: C, 63.78; H, 8.25, N, 4.33. IR cm^{-1}: ν_{O-H} 3450; $\nu_{C=O}$ 1735; 1720. NMR τ: 8.65 (3H, s., $\geqslant$C–C$\underline{H}_3$); 8.08 (3H, s., –OCOC$\underline{H}_3$); 6.30 (1H, *a.*, J=4 cps, >C$\underline{H}$–OH); 5.20 (1H, t., J=3 cps, >C$\underline{H}$–OAc). Elution with $CHCl_3$, followed by AcOEt gave 10 mg of *cis*-triol-A (XIX), which was recrystallized from acetone to give colorless needles, mp 232—233°. *Anal.* Calcd. for $C_{16}H_{25}O_4N$: C, 65.06; H, 8.53. Found: C, 64.94; H, 8.74. IR cm^{-1}: ν_{O-H} 3400; $\nu_{C=O}$ 1735. Hydrolysis of *cis*-diol-A (XVIII) afforded *cis*-triol-A (XIX), quantitatively.

ii) A solution of 230 mg of osmium tetroxide in 2 ml of pyridine was added to a solution of 200 mg of 8-anhydro-13-acetylserratinine (XI) in 2 ml of abs. dioxane and the mixture was allowed to stand overnight at room temperature. After decomposition of osmate with hydrogen sulfide, the solution was filtrated and the precipitate was washed with ethanol. The combined solution was evaporated to dryness *in vacuo*. The residue in benzene was chromatographed on alumina (grade I). Elution with ether, $CHCl_3$ and AcOEt gave 160 mg of crystalline mass which was identified with *cis*-diol-A (XVIII).

Oxidation of *cis*-Diol-A (XVIII) with Jones' Reagent——To a stirred solution of 120 mg of *cis*-diol-A (XVIII) in 20 ml of acetone was added dropwise 0.16 ml of Jones' reagent at 0°. The reaction mixture was stirred at 0° for eight minutes. After decomposition of the excess of reagent with MeOH, the reaction mixture was diluted with water, made alkaline with NH_4OH and extracted with ether. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina and elution with benzene, followed by ether afforded 40 mg of crystals (XX) which were recrystallized from ether to give colorless prisms, mp 230—232°. *Anal.* Calcd. for $C_{18}H_{25}O_5N$: C, 64.46; H, 7.51. Found: C, 64.26; H, 7.49. IR cm^{-1}: ν_{O-H} 3430; $\nu_{C=O}$ 1720.

***trans*-Ttriol-A (XXI)**——To a solution of 90 mg of the ketonic compound (XX) in 20 ml of EtOH was gradually added 200 mg of $NaBH_4$ in small portions under reflux during 4 hours. After addition of 5 ml

of aq. 5% NaOH solution to the reaction mixture, the mixture was heated for further 20 minutes, diluted with water and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and the solvent was distilled off. The residue was recrystallized from acetone to give 20 mg of *trans*-triol-A (XXI), colorless prisms, mp 273—274°. *Anal.* Calcd. for $C_{16}H_{25}O_4N$: C, 65.06; H, 8.53. Found: C, 65.03; H, 8.48. IR cm^{-1}: ν_{O-H} 3460, 3320, 3220; $\nu_{C=O}$ 1720.

***trans*-Triol-B (VII)**——To a stirred solution of 200 mg of 8-anhydro-13-acetylserratinine in 4 ml of formic acid was added 0.5 ml of H_2O_2 (30%) at 50° and the mixture was stirred at the same temperature for 4 hours. After addition of 1 ml of aq. 50% NaOH solution to the reaction mixture, the mixture was stirred for further 10 minutes, diluted with water and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina (grade IV) and elution with $CHCl_3$, AcOEt afforded 40 mg of *trans*-triol-B, mp 209—212°, $[\alpha]_D^{27}$ −44.0° (c=1.0, EtOH), which was identical with natural serratanidine in all respects.

Serratanidine Carbonate (XXII)——To a solution of 100 mg of serratanidine (VII) in 4 ml of pyridine and 6 ml of $CHCl_3$ was added dropwise 3 ml of toluene saturated with phosgene at 0°. The mixture was allowed to stand at room temperature overnight, poured into ice water, made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. $MgSO_4$ and evaporated. Chromatography of the residue in $CHCl_3$ on alumina (grade III) and elution with AcOEt afforded a crystalline mass which was recrystallized from a mixture of AcOEt and acetone to give 25 mg of serratanidine carbonate (XXII), mp 277—278°, colorless needles. *Anal.* Calcd. for $C_{17}H_{23}O_5N$: C, 63.53; H, 7.21. Found: C, 63.37; H, 7.16. IR cm^{-1}: ν_{O-H} 3050; $\nu_{C=O}$ 1747. NMR τ: 8.65 (3H, s., $\geqslant$C–C$\underline{H}_3$); 6.28 (1H, br. d., J=4 cps, >C$\underline{H}$–OH); 5.55 (1H, br. d., J=4.3 cps, >C$\underline{H}$–OCO–); 4.26 (1H, d., J=4 cps, –O$\underline{H}$) (in DMSO).