[Chem. Pharm. Bull. 16(1) 82—91 (1968)]

UDC 547.94 : 582.38

Serratinine; A Novel Skeletal Lycopodium Alkaloid[1,2]

YASUO INUBUSHI, HISASHI ISHII, BOMPEI YASUI,
MASASHI HASHIMOTO,[3a] and TAKASHI HARAYAMA

Faculty of Pharmaceutical Sciences, Osaka University[3]

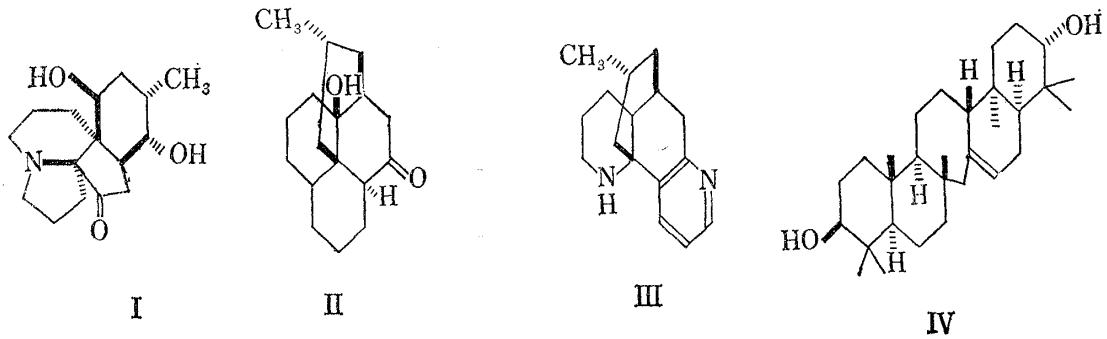
(Received March 31, 1967)

Serratinine, one of major alkaloids of *Lycopodium serratum* THUNB. var. *Thunbergii* MAKINO, is a tetracyclic new alkaloid possessing an expanded formula, $C_{10}H_{15}$(>CH–OH) (>CH–OH) (>CH–CH_3) (–$COCH_2$–) (–N–). On the basis of chemical and spectroscopic data, the partial structure (C) for serratinine was proposed.

The great advance in our knowledge of the chemistry of the lycopodium alkaloids has been recorded mainly by the Canadian research groups during the past ten years. These significant advances in this field prompted us to examine the constituents of domestic lycopodium genus plants. Thus, our interest in the constituents of these plants was first aroused by the alkaloid chemistry but subsequently, we directed our attention not only to the alkaloids but also to the triterpenoid constituents.

In a prevous paper,[4] we described the isolation of five triterpenoids with unknown structures and three new alkaloids, serratinine (I), serratine and serratanine as well as two known alkaloids, lycodoline (II)[5] and lycodine (III)[6] from *Lycopodium serratum* THUNB. var. *Thunbergii* MAKINO.

The structures of serratenediol (IV), a unique skeletal triterpenoid containing a seven membered ring and four other related triterpenoids have been established.[7] On the alkaloid constituents, we have reported the structure establishment of serratinine (I), a major alkaloid



I II III IV

Chart 1

1) Studies on the Constituents of Domestic Lycopodium Genus Plants; Part IV. Part III: *Chem. Pharm. Bull.* (Tokyo), **15**, 1153 (1967).
2) The preliminary report of this work appeared in *Tetrahedron Letters*, **1966**, 1537.
3) Location : *6–5 Toneyama, Toyonaka, Osaka-fu* ; *a*) Present address: *Research Laboratories Fujisawa Pharmaceutical Co., Ltd., 1–52, Kashima-cho, Higashiyodogawa-ku, Osaka.*
4) Y. Inubushi, Y. Tsuda, H. Ishii, T. Sano, M. Hosokawa, and T. Harayama, *Yakugaku Zasshi*, **84**, 1108 (1964).
5) W.A. Ayer and G.G. Iverach, *Tetrahedron Letters*, **1962**, 87; W.A. Ayer and G.G. Iverach, *Can. J. Chem.*, **42**, 2514 (1964).
6) F.A.L. Anet and M.V. Rao, *Tetrahedron Letters*, **1960**, 9; W.A. Ayer and G.G. Iverach, *Can. J. Chem.*, **38**, 1823 (1960).
7) Y. Inubushi, T. Sano, and Y. Tsuda, *Tetrahedron Letters*, **1964**, 1303; Y. Tsuda, T. Sano, K. Kawaguchi, and Y. Inubushi, *Tetrahedron Letters*, **1964**, 1279; Y. Inubushi, Y. Tsuda, T. Sano, and R. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 104 (1965); Y. Inubushi, Y. Tsuda, and T. Sano, *Chem. Pharm. Bull.* (Tokyo), **13**, 750 (1965).

of this plant, in a preliminary communication.[8] In the series of the present papers, we wish to give a full detail of the experiments which permited to establish firmly the structure and stereochemistry of this unique lycopodium alkaloid.

Serratinine was obtained as colorless prisms or needles, mp 244—245°, $[\alpha]_D^8$ −27.8°(c=1.44, EtOH) and the molecular formula $C_{16}H_{25}O_3N$ was fixed on the basis of the analytical and mass spectral data. The IR spectrum showed the presence of hydroxyl groups by absorption bands at 3472, 3436 and 3185 cm^{-1} and a ketonic group at 1724 cm^{-1}.

Acetylation of serratinine with acetic anhydride in pyridine at 98° for 3.5 hours, gave diacetylserratinine (V), $C_{16}H_{23}ON(OCOCH_3)_2$, which showed no hydroxyl absorption in the IR spectrum. The NMR spectrum of diacetylserratinine showed two multiplets, each corresponding to one proton at 5.06 and 5.39τ together with two 3H singlets attributable to two acetyl methyls at 7.98 and 8.11τ. A signal (3H, d., J=6 cps) at 9.10τ in the NMR spectrum coupled with a result of Kuhn–Roth C–methyl determination of serratinine suggested the presence of a secondary C–methyl group.

The presence of an active methylene group in serratinine was confirmend not only by an absorption band at 1427 cm^{-1} in the IR spectrum but the formation of benzylidene serratinine (VI), $C_{23}H_{29}O_3N$. In addition to these facts, lack of any signal due to an olefinic proton and N-methyl group in the NMR spectrum of diacetylserratinine led us to a conclusion that serratinine must be a tetracylic alkaloid having an expanded formula, $C_{10}H_{15}$ (>CH–OH)(>CH OH)(>CH–CH_3)(–CO–CH_2–)(>N–).

For the sake of comparison of the framework of serratinine with that of lycopodane[9] (X) which is the common ring system to a large majority of lycopodium alkaloids, serratinine was converted to serratinane methiodide (IX) by the following manner. Wolff–Kishner reduction of serratinine by Nagata's variation[10] afforded deoxoserratinine (VII), $C_{16}H_{27}O_2N$, in good yield whose IR spectrum showed no absorption in the carbonyl region. Oxidation of the compound (VII) with chromium trioxide in aq. 90% acetic acid furnished a diketone, bisdehydrodeoxoserratinine (VIII), $C_{16}H_{23}O_2N$, which showed no hydroxyl absorption in the IR spectrum but carbonyl bands at 1709 and 1692 cm^{-1}. Wolff–Kishner reduction of the diketone (VIII) followed by treatment with methyl iodide gave serratinane methiodide (IX), mp 249—250°. The IR spectrum of the methiodide definitely differed from that of

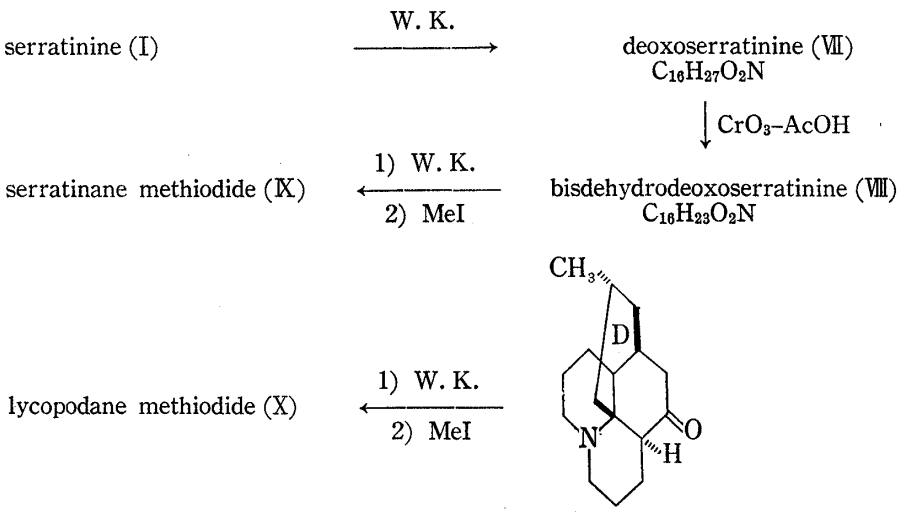


Chart 2 lycopodine

8) Y. Inubushi, H. Ishii, B. Yasui, M. Hashimoto, and T. Harayama, *Tetrahedron Letters*, **1966**, 1537; Y. Inubushi, H. Ishii, B. Yasui, and T. Harayama, *Tetrahedron Letters*, **1966**, 1551.
9) R.H. Burnell and D.R. Taylor, *Tetrahedron*, **15**, 173 (1961).
10) W. Nagata and H. Itazaki, *Chem. Ind.* (London), 1194 (1964).

lycopodane methiodide (X), mp 288—290°, prepared from lycopodine[11] by Wolff–Kishner reduction in our laboratory. This finding proves clearly that serratinine does not belong to the lycopodine type alkaloids, if no rearrangement occurred during the reduction process. Furthermore, mass spectral observation showed rigorously that serratinine is not a member of lycopidium alkaloids possessing the known ring system.

Recently, MacLean[13] has suggested that the loss of ring D of lycopodine type nucleus (refer to lycopodine in Chart 2) is the dominant feature in the mass spectra of this type alkaloids. In the mass spectrum of serratinine,[14] however, there was not found any peak corresponding to this characteristic fragmentation but three characteristic peaks at M^+-28, *m/e* 152 and *m/e* 150 were observed. Since it was found that the appearance of these three peaks in the mass spectra of serratinine and its derivatives is characteristic of this type alkaloids, mass spectrometry is available for the diagnostic determination of these alkaloids.[15] From these facts, it is obvious that serratinine is a unique skeletal lycopodium alkaloid.

In order to obtain a clue for the interrelationship among oxygen functions, serratinine was oxidized with Jones' reagent[16] to give a triketone, bisdehydroserratinine (XI), which showed carbonyl bands at 1736 and 1693 cm^{-1} and no hydroxyl band in the IR spectrum and only end absorption in the UV spectrum, suggesting that three carbonyl groups would be isolated one another by at least two carbon atoms.

Acetylation of serratinine with acetic anhydride in pyridine at room temperature furnished monoacetylserratinine I (XII), $C_{16}H_{24}O_2N(OCOCH_3)$, whereas hydrolysis of diacetylserratinine (V) in aq. 10% HCl gave another monoacetyl-derivative, monoacetylserratinine II (XIII),

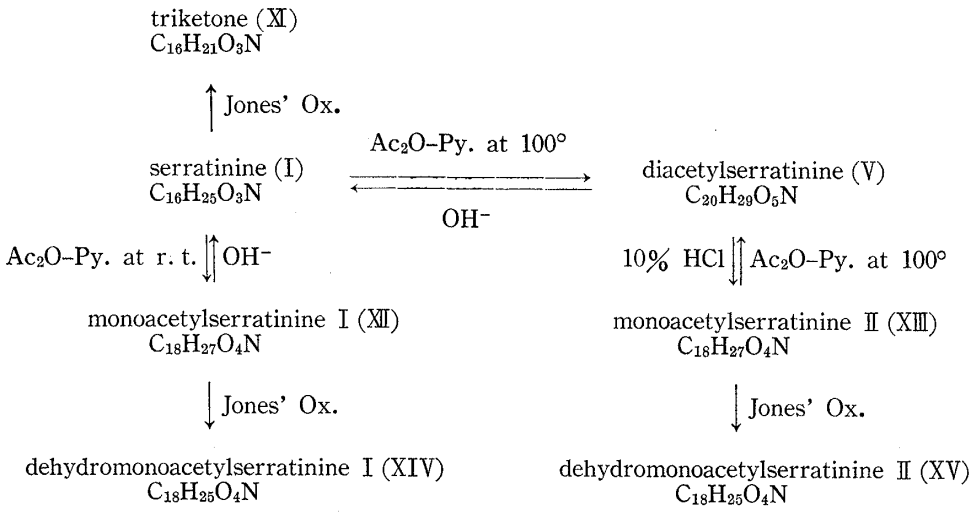


Chart 3

$C_{16}H_{24}O_2N(OCOCH_3)$. Although both monoacetates happened to show the same melting point, the dissimilarity of these two monoacetates was realized by the depression of the mixed melting point and the discrepancy of *Rf* values on thin-layer chromatography.[17] Since acetylation of both monoacetates under the same condition as described for diacetylser-

11) This experiment has been performed by using lycopodine isolated from *Lycopodium clavatum* L.[12]
12) Y. Inubushi, Y. Tsuda, and T. Sano, *Yakugaku Zasshi*, **82**, 1535 (1962).
13) D.B. MacLean, *Can. J. Chem.*, **41**, 2654 (1963).
14) Mass spectrum was obtained on a Hitachi mass spectrometer Model RMU 6C equipped with a heated inlet system.
15) The discussion on the mass spectra of serratinine type alkaloids will be published in elsewhere.
16) K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.L. Weedon, *J. Chem. Soc.*, **1946**, 39.
17) Thin-layer chromatography was performed on Silica gel G, and a solution of 1% $Ce(SO_4)_2$ in aq. 10% H_2SO_4 as a detection reagent and a solvent system; $CHCl_3$–cyclohexane–diethylanine (4:5:1) were employed.

ratinine afforded the same product, diacetylserratinine, the difference between monoacetylseratinine I and monoacetylserratinine II can be ascribed to the position of a free hydroxyl group. That no rearrangement had occurred during acetylation was certain because both monoacetylserratinines and diacetylserratinine were hydrolyzed to afford serratinine.

Oxidation of monoacetylserratinine I and monoacetylserratinine II with Jones' reagent gave the corresponding keto acetates, dehydromonoacetylserratinine I (XIV) and dehydromonoacetylserratinine II (XV), respectively. In the IR spectrum, the former showed an absorption due to the ketonic group newly created at 1701 cm^{-1} and the latter at 1695 cm^{-1}. From these spectral data, we can conclude that both hydroxyl groups in serratinine would be situated on a six membered or larger ring.

The environment of the carbonyl group in serratinine was defined in the following experiments. The nature of the immediate vicinity of the nitrogen to a carbonyl group was suggested by the striking difference of pK_a' values between serratinine (pK_a' 7.0)[18] and deoxoserratinine (VII) (pK_a' 10.9).[18] The vicinal relationship of these two functions was then confirmed chemically by the finding that reduction of serratinine with zinc dust in acetic anhydride afforded a neutral amide, O,O,N-triacetylchanodihydroserratinine (XVI), $C_{22}H_{33}O_6N$, which showed an amide band at 1641 cm^{-1} in the IR spectrum and a 6H singlet at 7.92τ and a 3H singlet at 8.10τ attributable to two O-acetyl and one N-acetyl methyls in the NMR spectrum. The formation of an amide with this reduction is formulated in Chart 4. Reduction of the amide with lithium aluminum hydride furnished a tertiary amine, N-ethylchanotetrahydroserratinine (XVII), $C_{18}H_{33}O_3N$. On the other hand, careful reduction of the amide with sodium borohydride produced an alcohol, O,O,N-triacetylchanotetrahydroserratinine (XVIII), whose IR spectrum showed an absorption at 3436 cm^{-1} due to a hydroxyl group. Dehydration of the alcohol (XVIII) with phosphorus oxychloride in pyridine afforded an anhydride, O,O,N-triacetylanhydrochanotetrahydroserratinien (XIX) as an oil which showed only one olefinic

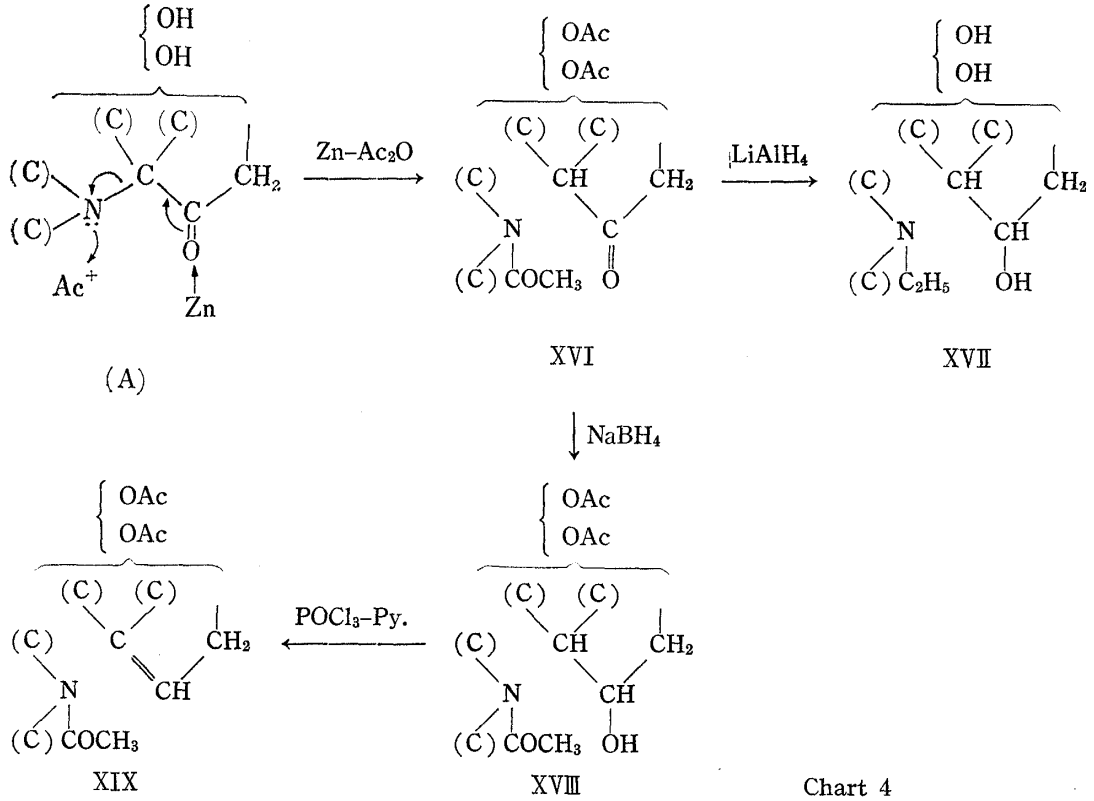


Chart 4

18) The pK_a' values were measured in 1/10 N H_2SO_4 (1 ml)–EtOH (5 ml)–H_2O (4 ml) solvent system by titration with 1/10 N NaOH solution.

proton as a 1H multiplet at 4.37τ in the NMR spectrum. This finding indicates that the carbon being situated between the nitrogen and carbonyl group in serratinine would be quaternary as shown by the partial structure A.

Refluxing of diacetylserratinine (V) with selenium dioxide in dioxane gave an α,β–unsaturated ketone (XX), $C_{20}H_{27}O_5N$ which showed a maximum at 228 mμ (log ε 4.02) in the UV spectrum and bands attributable to an α,β–unsaturated ketonic group at 1686 and 1631 cm^{-1} in the IR spectrum. A comparison of the NMR spectrum of the α,β–unsaturated ketone (XX) with that of diacetylserratinine(V) provided a striking information on the structure of serratinine. The signals due to protons geminal to an acetoxyl group of serratinine derivatives always appeared between 5.0—5.4τ. For instance, diacetylserratinine showed two 1H signals due to protons concerned at 5.06 and 5.39τ, respectively. In contrast with these observations, one of these signals in the α,β–unsaturated ketone moved to a lower field and appeared at 4.28τ as a 1H doublet. This down field shift of the signal in the case of α,β–unsaturated ketone could be rationalized by assuming that one of two acetoxyl groups is located at the allylic position of the double bond. The change of the signal pattern due to a proton concerned from a multiplet in diacetylserratinine to a doublet in α,β–unsaturated ketone (XX) may be ascribed to the loss of one of two protons adjacent to the proton geminal to an acetoxyl group. Moreover, the fact that the NMR spectrum of the α,β,–unsaturated ketone showed a 1H singlet due to the olefinic proton at 3.77τ might imply the presence of a trisubstituted double bond as shown in formula (XX). If this assumption is correct, serratinine may have the partial structure B.

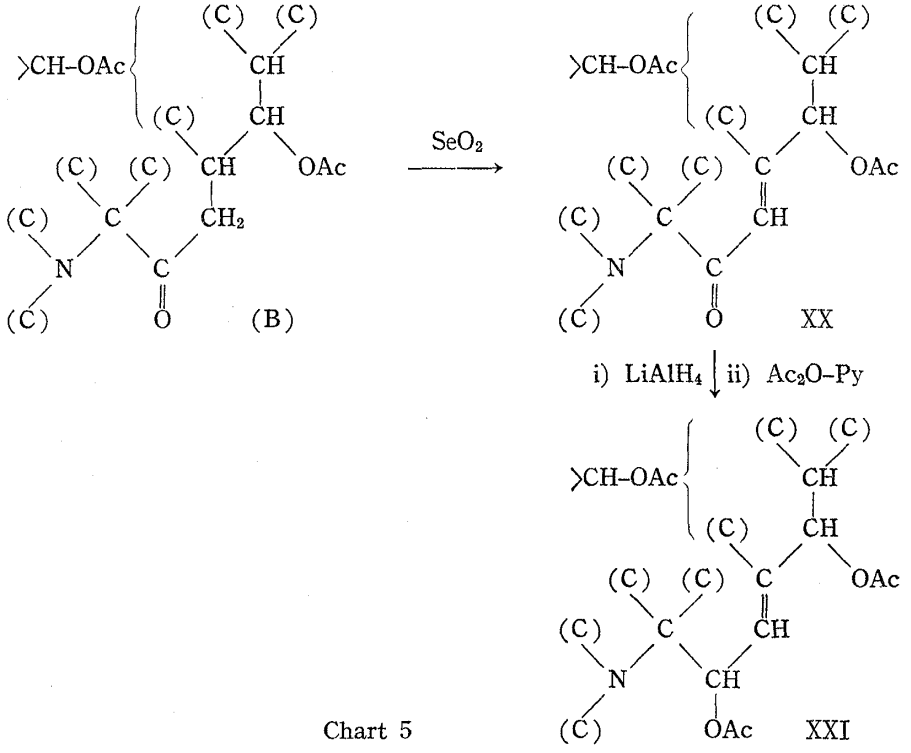


Chart 5

Reduction of the α,β–unsaturated ketone (XX) with lithium aluminum hydride followed by reacetylation afforded a triacetate (XXI). In the NMR spectrum of this compound, the signal pattern of olefinic proton changed from a singlet in the α,β–unsaturated ketone to a doublet (J=2 cps). In addition to this observation, there was found an additional 1H doublet (J=2 cps) at 4.52τ which should be assigned to the proton geminal to an acetoxyl group derived from the ketonic group in serratinine. This observation supports the previous deduction that there is no hydrogen atom on the carbon ato mintervened between the nitrogen and ketone group.

It is now of considerable importance to confirm which of two hydroxyl groups in serratinine is located at the allylic position of double bond in the α,β-unsaturated ketone. Dehydration of monoacetlyserratinine II (XIII) with phosphorus oxychloride in pyridine gave anhydro-monoacetylserratinine II (XXII). In the NMR spectrum of this compound, the τ value of a signal due to a methyl group at 8.33τ exhibited the presence of vinyl methyl group in this compound. Accordingly, if no rearrangement took place during dehydration process, a free hydroxyl group in monoacetylserratinine II (XIII) is defined to be located on the carbon atom adjacent to the C-methyl group. This hydroxyl group is more easily acetylated hydroxyl group than another one because monoacetylserratinine II was prepared by the partial hydrolysis of diacetylserratinine (refer to the Chart 2). Treatment of this compound (XXII) with selenium dioxide in dioxane furnished a yellow compound (XXIII), $C_{18}H_{23}O_3N$, which was characterized as a conjugated dienone derivative because its UV spectrum showed a maximum at 288 mμ (log ε 4.33). In the NMR spectrum of this compound, a 3H singlet due to the vinyl methyl group which was observed at 8.33τ in the compound (XXII) appeared again at 8.11τ and two 1H singlets due to two olefinic protons were observed at 3.65 and 4.12τ, respectively.

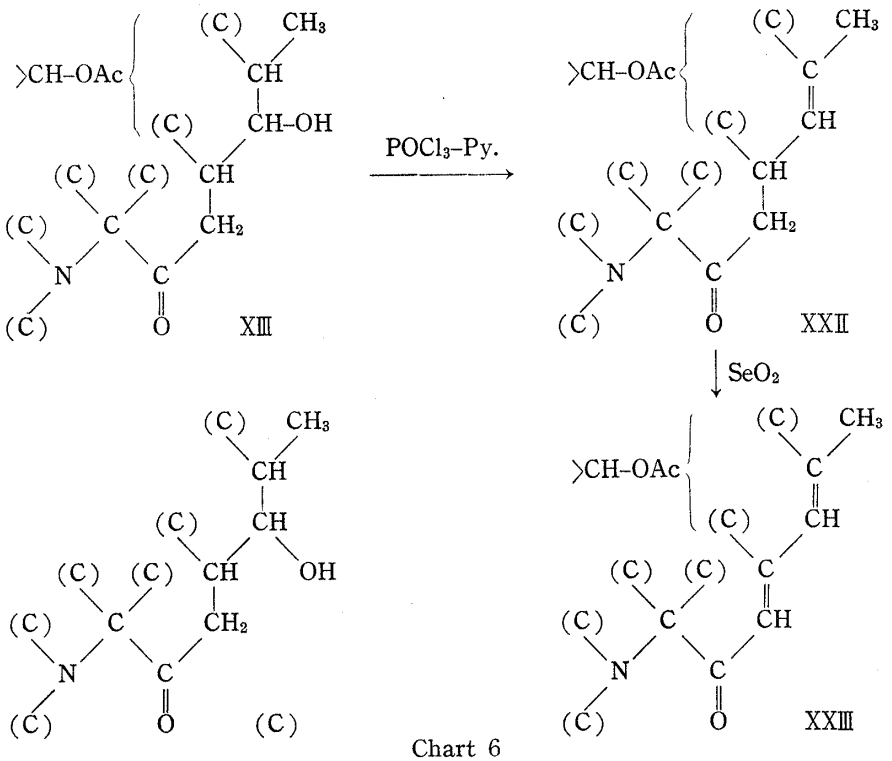


Chart 6

Thus, we may summarize the sequence of reactions as shown in Chart 6 and this formulation is compatible with the chemical and spectroscopic data presented so far.

Consequently, we may conclude that serratinine should have a partial formula C in its structure.

Experimental[19]

Isolation and Preliminary Characterization of the Alkaloids——Lycodine, lycodoline, serratinine and serratine were isolated from *Lycopodium serratum* Thunb. var. *Thunbergii* Makino (from Mt. Hira) by the

19) 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 Associates A-60 recording spectrometer. Aluminumoxyd Woelm, neutral, grade II, was available for column chromatography, unless any comment was added. Unless otherwise noted, IR spectra were measured on Nujol mull and UV spectra were taken in EtOH solution.

procedure reported in an earlier paper.[5] Each alkaloid was recrystallized to give material of the same melting point and infrared spectrum as that reported earlier.

Serratinine (I)——The alkaloid was recrystallized from acetone or ethyl acetate, colorless prisms or needles, mp 244—245°, $[\alpha]_D^8$ −27.8° (c=1.44, EtOH). *Anal.* Calcd. for $C_{16}H_{25}O_3N$: C, 68.78; H, 9.02; N, 5.01; (C)–CH_3, 5.38. Found: C, 68.82; H, 9.17; N, 5.04; (C)–CH_3, 6.06. IR cm^{-1}: ν_{O-H} 3472, 3436, 3185 (broad); $\nu_{C=O}$ 1724; δ_{C-H} 1427. IR cm^{-1}: ν_{O-H} 3597; $\nu_{C=O}$ 1736 ($CHCl_3$); IR cm^{-1}: $\nu_{C=O}$ 1735; δ_{C-H} 1435 (KBr). pK_a' 7.0[18]; M^+: 279.

Diacetylserratinine (V)——A solution of 100 mg of serratine (I) in 1 ml of acetic anhydride and 1 ml of pyridine was heated at 98° for 3.5 hr, cooled, and evaporated to dryness *in vacuo*. The residue was dissolved in water, made alkaline with NH_4OH and then extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue in benzene was chromatographed on alumina. Elution with benzene afforded colorless needles. Recrystallization from n-hexane gave 55 mg of pure diacetylserratinine (V), mp 157—158°; $[\alpha]_D^{10}$ −21.7° (c=1.65, EtOH). *Anal.* Calcd. for $C_{16}H_{23}ON(OCOCH_3)_2$: C, 66.09; H, 8.04; N, 3.85. Found: C, 66.00; H, 8.05; N, 3.63. IR cm^{-1}: $\nu_{C=O}$ 1736, 1727; ν_{C-O} 1242, 1229. NMR τ: 5.06 (1H, m., >C<u>H</u>OAc); 5.39 (1H, m., >C<u>H</u>OAc); 7.98 (3H, s., –OCOC<u>H</u>$_3$); 8.11 (3H, s., –OCOC<u>H</u>$_3$); 9.10 (3H, d., J=6 cps, >CH–C<u>H</u>$_3$). pK_a': 5.3.[18] M^+: 363.

Hydrolysis of Diacetylserratinine (V)——To a solution of 100 mg of diacetylserratinine (V) in 5 ml of methanol was added a solution of 2.5 g of NaOH in 5 ml of water. The reaction mixture was allowed to stand overnight at room temperature, diluted with water and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. Recrystallization of the residue from ethyl acetate afforded 70 mg of colorless prisms which were identified with an authentic sample of serratinine (I) by comparison of the infrared spectra and the mixed melting point determinations.

Benzylidene Serratinine (VI)——To a solution of 2 g of serratinine (I) in 200 ml of methanol dissolving 2 g of sodium, was added 2 ml of benzaldehyde. The mixture was heated under reflux for 1 hr, cooled, and concentrated *in vacuo*, diluted with water and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3, evaporated, and the residue in benzene was chromatographed on alumina. Elution with benzene followed by ether afforded 2.0 g of crystalline mass which was recrystallized from the mixture of ethyl acetate and n-hexane to give colorless needles (VI), mp 204—205°. *Anal.* Calcd. for $C_{23}H_{29}O_3N$; C, 75.17; H, 7.95. Found: C, 75.21; H, 7.94. IR cm^{-1}: ν_{O-H} 3425 (broad); $\nu_{C=O}$ 1698; $\nu_{aromatic}$ 1629, 1597, 1570. UV λ_{max} mμ (log ε): 225 (3.81), 295.5 (4.18).

Deoxoserratinine (VII)——To a solution of 2 g of serratinine (I) in 200 ml of triethyleneglycol was added 100 ml of anhydrous hydrazine and 30 g of hydrazine hydrochloride. The reaction mixture was heated at 100° for 7 hr. After addition of 60 g of potassium hydroxide, the temperature was gradually raised to 220° by distilling the excess of hydrazine off and the mixture was then kept at this temperature for further 5 hr. After cooling, the mixture was diluted with a large quantity of 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 and elution with benezne followed by ether gave 1.6 g of solid mass which was recrystallized from ether to give colorless needles (VII), mp 127—128°. $[\alpha]_D^{23}$ +15.8° (c=1.01, EtOH). *Anal.* Calcd. for $C_{16}H_{27}O_2N$: C, 72.41; H, 10.26. Found: C, 72.39; H, 10.39. IR cm^{-1}: ν_{O-H} 3448, 3175. pK_a': 10.9.[18]

Methiodide——Prepared from deoxoserratinine as usual and recrystallized from the mixture of methanol and acetone. The methiodide formed colorless needles, mp 242—244°. *Anal.* Calcd. for $C_{16}H_{27}O_2N\cdot CH_3I$: C, 50.12; H, 7.42. Found: C, 49.86; H, 7.39. IR cm^{-1}: ν_{O-H} 3356.

Bisdehydrodeoxoserratinine (VIII)——A solution of 140 mg of deoxoserratinine (VII) in 6 ml of aq. 90% AcOH was added slowly to a stirred solution of 70 mg of chromium trioxide in 1 ml of aq. 90% AcOH at room temperature. The mixture was stirred at room temperature for 15 hr. The excess of reagent was destroyed with methanol, and the mxiture was evaporated to dryness *in vacuo*. The residue was dissolved in water, 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 and elution with benzene followed by ether gave 60 mg of crystals which were recrystallized from n-hexane to give colorless needles, (VIII), mp 142.5—144°. $[\alpha]_D^{21}$ +150.1° (c=1.02, EtOH). *Anal.* Calcd. for $C_{16}H_{23}O_2N$: C, 73.53; H, 8.87. Found: C, 73.27; H, 9.02. IR cm^{-1}: $\nu_{C=O}$ 1709, 1692.

Serratinane Methiodide (IX)——A solution of 0.2 g of sodium in 10 ml of triethyleneglycol was heated at 180° and completely anhydrous hydrazine was distilled into the solution until it refluxed freely at 180°. To a cooled solution was added 100 mg of bisdehydrodeoxoserratinine (VIII) and the mixture was then refluxed for 16 hr at 180°. Excess hydrazine was distilled off from the reaction mixture until the temperature of the solution had raised to 220° and refluxing was then continued for further 24 hr. The cooled solution was diluted with a large quantity of water and extracted with $CHCl_3$. The extract was washed with aq. 30% NaOH and dried over anhydr. K_2CO_3. The solvent was evaporated and the residue in n-hexane was chromatographed on alumina. Elution with n-hexane gave colorless oil. Treatment of this oil with methyl iodide in acetone afforded 18 mg. of crystals which were recrystallized from acetone–ether to give colorless pillars, (IX), mp 249—250°; $[\alpha]_D^{24}$ −7.4 (c=0.54, EtOH). *Anal.* Calcd. for $C_{16}H_{27}N\cdot CH_3I$: C, 54.40; H, 8.06. Found: C, 54.11; H, 8.14.

Lycopodane Methiodide (X)[9]——Wolff-Kishner reduction of 300 mg of lycopodine[11] in the same method as described for serratinane afforded colorless oil. Treatment of this oil with methyl iodide in acetone gave 150 mg of crude methiodide which was recrystallized from MeOH–acetone to give colorless leaflets, (X), mp 288—290°. *Anal.* Calcd. for $C_{16}H_{27}N \cdot CH_3I$: C, 54.40; H, 8.06; N, 3.73. Found: C, 54.14; H, 8.05; N, 3.94.

Bisdehydroserratinine (XI)——To a solution of 90 mg of serratinine (I) in 40 ml of acetone was added dropwise 2.5 ml of Jones' reagent at 5°. The reaction mixture was stirred at room temperature for 20 minutes. After decomposition of the excess of reagent with methanol, the reaction mixture was concentrated *in vacuo*, 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 was purified by sublimation at 180° (2 mm Hg) to give 40 mg of distillate (XI). Recrystallization of the distillate from ether gave 15 mg of colorless prisms (XI), mp 157—160°. *Anal.* Calcd. for $C_{16}H_{21}O_3N$: C, 69.79; H, 7.69. Found: C, 69.79; H, 7.98. IR cm^{-1}: $\nu_{C=O}$ 1736, 1693.

Monoacetylserratinine I (XII)——A solution of 1 g of serratinine (I) in 15 ml of pyridine and 15 ml of acetic anhydride was allowed to stand overnight in a refrigerator. The solution was poured into ice water, made alkaline with NH_4OH and extracted with $CHCl_3$. The chloroform extract was dried over anhydr. K_2CO_3 and evaporated to dryness *in vacuo*. The residue was chromatographed on alumina and elution with $CHCl_3$ gave 0.21 g of diacetylserratinine (*vide ante*) and continued elution with $CHCl_3$ gave 0.74 g of monoacetylserratinine I (XII) as colorless needles, which were recrystallized from acetone to give colorless prisms, mp 244—245°, $[\alpha]_D^{12}$ −46.3° (c=1.60, EtOH). *Anal.* Calcd. for $C_{16}H_{24}O_2N(OCOCH_3)$: C, 67.26; H, 8.47; N, 4.36. Found: C, 67.28; H, 8.44; N, 4.22. IR cm^{-1}: ν_{O-H} 3215 (broad); $\nu_{C=O}$ 1736; ν_{C-O} 1248. NMR τ: 4.94 (1H, m., >C<u>H</u>–OAc); 6.30 (1H, m., >C<u>H</u>–OH); 7.94 (3H, s., –OCOC<u>H</u>$_3$); 9.08 (3H, d., J=6 cps, >CH–C<u>H</u>$_3$).

Monoacetylserratinine II (XIII)——A solution of 500 mg deacetylserratinine (V) in 80 ml of aq. 10% HCl was refluxed at 130° for 1.5 hr. The reaction mixture was made alkaline with NH_4OH and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3 and evaporated. Recrystallization of the residue from benzene gave 390 mg of colorless pillars (XIII), mp 240—242°, $[\alpha]_D^{23}$ −25.6° (c=1.41, EtOH). *Anal.* Calcd. for $C_{16}H_{24}O_2N(OCOCH_3)$: C, 67.26; H, 8.47. Found: C, 67.02; H, 8.74. IR cm^{-1}: ν_{O-H} 3185; $\nu_{C=O}$ 1733; ν_{C-O} 1252, 1238. Acetylation of both monoacetylserratinine I (XII) and monoacetylserratinine II (XIII) with the same method as mentioned for diacetylserratinine gave diacetylserratinine (V) quantitatively. Hydrolysis of both (XII) and (XIII) with alkali regenerated serratinine (III), quantitatively.

Dehydromonoacetylserratinine I (XIV)——To a stirred solution of 500 mg of monoacetylserratinine I (XII) in 35 ml of acetone was added dropwise 2 ml of Jones' reagent under cooling. The reaction mixture was stirred at room temperature (20°) for 35 minutes. After decomposition of the excess of reagent with methanol, 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 gave 460 mg of crystals which were recrystallized from *n*-hexane to give colorless pillars (XIV), mp 96—97°, $[\alpha]_D^{24}$ −55.0° (c=1.09, EtOH). *Anal.* Calcd. for $C_{18}H_{25}O_4N$: C, 67.69; H, 7.89. Found: C, 67.99; H, 8.00. IR cm^{-1}: $\nu_{C=O}$ 1735, 1701; ν_{C-O} 1230. NMR τ: 4.86 (1H, m., >C<u>H</u>–OAc); 7.86 (3H, s., –OCOC<u>H</u>$_3$); 8.98 (3H, d., J=6 cps, >CH–C<u>H</u>$_3$).

Dehydromonoacetylserratinine II (XV)——To a stirred solution of 620 mg of monoacetylserratinine II (XIII) in 43 ml of acetone was added dropwise 2.5 ml of Jones' reagent under cooling and the reaction mixture was stirred at room temperature for 30 minutes. Treatment of the reaction mixture in the same manner as mentioned for dehydromonoacetate I (XIV) gave crystals, quantitatively, which were recrystallized from ether to give colorless prisms (XV), mp 187.5—188°, $[\alpha]_D^{20}$ +43.1° (c=1.44, EtOH). *Anal.* Calcd. for $C_{18}H_{25}O_4N$: C, 67.69; H, 7.89. Found: C, 67.97; H, 8.10. IR cm^{-1}: $\nu_{C=O}$ 1730, 1695; ν_{C-O} 1252, 1238. NMR τ: 5.21 (1H, m., >C<u>H</u>–OAc); 7.98(3H, s., –OCOC<u>H</u>$_3$); 8.94(3H, d., J=7 cps., >CH–C<u>H</u>$_3$).

O,O,N-Triacetylchanodihydroserratinine (XVI)——To a stirred solution of 50 mg of serratinine (I) in 10 ml of acetic anhydride was added 200 mg of zinc dust under reflux. After 2 hr, a second portion of 200 mg of zinc dust was added and the solution was heated for further 5 hr. The remaining zinc dust was filtered off and the filtrate was evaporated to dryness *in vacuo*. The residue was taken up in water, made acidic with conc. HCl and extracted with $CHCl_3$. The extract was dried over anhydr. K_2CO_3. Removal of the solvent gave 35 mg of crystalline mass, which was recrystallized from acetone–ether to afford colorless prisms (XVI), mp 198—201°. *Anal.* Calcd. for $C_{22}H_{33}O_6N$: C, 64.86; H, 8.16. Found: C, 65.01; H, 8.45. IR cm^{-1}: $\nu_{C=O}$ 1735, 1726, 1641; ν_{C-O} 1241. NMR τ: 4.94(1H, m., >C<u>H</u>–OAc); 5.03(1H, m., >C<u>H</u>–OAc); 7.92(6H, s., 2×–COC<u>H</u>$_3$); 8.10(3H, s., –COC<u>H</u>$_3$); 9.08(3H, d., J=6 cps, >CH–C<u>H</u>$_3$).

N-Ethylchanotetrahydroserratinine (XVII)——To a solution of 350 mg of O,O,N–triacetylchanodihydroserratinine (XVI) in 30 ml of dry tetrahydrofuran was added 350 mg of $LiAlH_4$. The mixture was refluxed on a steam bath for 8 hr. Decomposition of excess hydride was effected by the addition of wet ether. The precipitate formed was separated by decantation and washed with ether. The combined ethereal solution was dried over anhydr. K_2CO_3 and evaporated. Recrystallization of the residue from acetone gave 260 mg of colorless prisms (XVII) mp 194°. *Anal.* Calcd. for $C_{18}H_{33}O_3N$: C, 69.41; H, 10.68; N, 4.50. Found: C, 69.55; H, 10.58; N, 4.38. IR cm^{-1}: ν_{O-H} 3356, 3175.

O,O, N-Triacetyltetrahydroserratinine (XVIII)——To a solution of 200 mg of O,O,N-triacetylchanodihydroserratinine (XVI) in 30 ml of EtOH was added 150 mg of $NaBH_4$. The mixture was allowed to stand at room temperature overnight. A second portion of 70 mg of $NaBH_4$ was added and then the mixture was left one more night at room temperature. A few drops of AcOH were added to the reaction mixture for decomposing the excess of reagent. The solution was condensed to about 5 ml *in vacuo*, diluted with water and extracted with $CHCl_3$. The extract was dried over anhydr. $MgSO_4$ and the solvent was evaporated. The residue in benzene was chromatographed on alumina, and elution with benzene followed by ether gave 90 mg of crude crystals which were recrystallized from ether to give colorless prisms (XVIII), mp 188—189°. *Anal.* Calcd. for $C_{22}H_{35}O_6N$: C, 64.52; H, 8.62. Found: C, 64.56; H, 8.61. IR cm^{-1}: ν_{O-H} 3436; $\nu_{C=O}$ 1724, 1631; ν_{C-O} 1238.

O,O,N-Triacetylanhydrotetrahydroserratinine (XIX)——To a solution of 20 mg of O,O,N-triacetylchanotetrahydroserratinine (XVIII) in 2 ml of dry pyridine was added two drops of $POCl_3$ and the mixture was allowed to stand overnight at room temperature. The reaction mixture was evaporated to dryness *in vacuo*, diluted with water and extracted with ether. The extract was dried over anhydr. $MgSO_4$ and evaporated. The residue in *n*-hexane was chromatographed on alumina and elution with *n*-hexane gave 10 mg of colorless oil (XIX) which gave one spot on thin-layer chromatography and the homogeneity of this sample was also shown by its NMR spectrum. *Anal.* Calcd. for $C_{22}H_{33}O_5N$: C, 67.49; H, 8.50. Found: C, 66.46; H, 8.35. IR cm^{-1};: $\nu_{C=O}$ 1735, 1645 ν_{C-O} 1230 (film). NMR τ: 4.37 (1H, m., olefinic proton); 4.97(1H, m., >C<u>H</u>–OAc); 5.08(1H, m., >C<u>H</u>–OAc); 7.93(6H, s., 2×–COC<u>H</u>$_3$); 8.05(3H, s., –COC<u>H</u>$_3$); 9.08(3H, d., J=6.5 cps, >CH–C<u>H</u>$_3$).

***α,β*-Unsaturated Ketone (XX)**——To a solution of 300 mg of selenium dioxide in 40 ml of dioxane was added 670 mg of diacetylserratinine (V). The mixture was heated under reflux for 4 hr. After excess selenium dioxide was decomposed with sulfur dioxide, the precipitated metal selenium was filtered off. The filtrate was diluted with 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 in benzene was chromatographed on alumina. Elution with benzene gave 340 mg of crystals, which were recrystallized from *n*-hexane to give colorless plates (XX), mp 139—141°. Occasionally, this substance crystallized as pale yellow needles (XX), mp 121—123°. Each specimen of these two crystal forms was interchangeable with another form by the cross seeding method and IR spectra of these specimens in solution were completely identical. *Anal.* Calcd. for $C_{20}H_{27}O_5N$: C, 66.46; H, 7.53. Found: C, 66.38; H, 7.54. IR cm^{-1}: $\nu_{C=O}$ 1733, 1686; $\nu_{C=C}$ 1631; ν_{C-O} 1235. IR cm^{-1}: $\nu_{C=O}$ 1739, 1698; $\nu_{C=C}$ 1634; ν_{C-O} 1230 (CCl_4). UV λ_{max} mμ (log ε): 228 (4.02); $\lambda_{max}^{EtOH-HCl}$ mμ (log ε): 239 (3.93). NMR τ: 3.77 (1H, s., olefinic proton); 4.28 (1H, d. , J=3 cps, >C<u>H</u>–OAc); 5.03 (1H, m., >C<u>H</u>–OAc); 7.92 (3H, s., –OCOC<u>H</u>$_3$); 8.04 (3H, s., –OCOC<u>H</u>$_3$); 8.99 (3H, d., J= 6 cps, >CH–C<u>H</u>$_3$).

Triacetate (XXI)——A solution of 300 mg of the α,β-unsaturated ketone (XX) in 70 ml of dry ether was added dropwise to a stirred solution of 600 mg of $LiAlH_4$ in 100 ml of dry ether. The mixture was heated at 50° for 3.5 hr, and the excess hydride was decomposed by addition of ethyl acetate to the cooled solution. A saturated sodium sulfate solution was then added until the precipitate had adhered to the wall of the flask. The ethereal solution was separated from the precipitate by decantation and the precipitate was washed with ether. The combined ethereal solution was dried over anhydr. K_2CO_3 and evaporated. Recrystallization of the residue from MeOH–acetone gave 195 mg of the triol as colorless pillars, mp 248—250°. *Anal.* Calcd. for $C_{16}H_{25}O_3N$: C, 68.78; H, 9.02. Found: C, 68.70; H, 9.22. IR cm^{-1}: ν_{O-H} 3390, 3268; $\nu_{C=C}$ 1661.

To a solution of 250 mg of the triol in 2.5 ml of pyridine was added 2.5 ml of acetic anhydride. The mixture was heated at 100° for 4.5 hr, cooled, and evaporated to dryness *in vacuo*. The residue was taken up in water, 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 and elution with benzene afforded 180 mg of crude crystals which were recrystallized from *n*-hexane to give colorless pillars (XXI), mp 124°. *Anal.* Calcd. for $C_{22}H_{31}O_6N$: C, 65.16; H, 7.71; N, 3.45. Found: C, 65.06; H, 7.75; N, 3.42. IR cm^{-1}: $\nu_{C=O}$ 1727; $\nu_{C=C}$ 1667; ν_{C-O} 1233. NMR τ: 4.09 (1H, d., J=2 cps, olefinic proton); 4.45 (1H, d., J=3 cps, >C<u>H</u>–OAc); 4.52 (1H, d., J=2 cps, >C<u>H</u>–OAc); 5.07 (1H, m., >C<u>H</u>–OAc); 7.94 (3H, s., –OCOC<u>H</u>$_3$); 7.95 (3H, s., –OCOC<u>H</u>$_3$); 7.97 (3H, s., –OCOC<u>H</u>$_3$); 9.07 (3H, d., J=6 cps, >CH–C<u>H</u>$_3$).

Anhydromonoacetylserratinine II (XXII)——Two drops of $POCl_3$ were added to a solution of 100 mg of monoacetylserratinine II (XIII) in 2 ml of pyridine. The mixture was allowed to stand at room temperature overnight 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. $MgSO_4$ and the solvent was evaporated. The residue in benzene was chromatographed on alumina and elution with benzene gave 75 mg of solid mass, which was recrystallized from ether to afford colorless prisms (XXII), mp 188—189°. *Anal.* Calcd. for $C_{18}H_{25}O_3N$: C, 71.25; H, 8.31. Found: C, 71.54; H, 8.26. IR cm^{-1}: $\nu_{C=O}$ 1724; ν_{C-O} 1229. NMR τ: 4.44 (1H, m., olefinic proton); 5.25 (1H, t., J=2.5 cps, >C<u>H</u>OAc); 8.14 (3H, s., –OCOC<u>H</u>$_3$); 8.33 (3H, s., vinyl methyl).

Dienone (XXIII)——A solution of 700 mg of anhydromonoacetylserratinine II (XXII) in 20 ml of dioxane containing 560 mg of selenium dioxide was heated for 30 minutes at 130°, cooled, and evaporated

to dryness *in vacuo*. The residue was dissolved in aq. 3% HCl and washed with ether. The aqueous solution was made alkaline with NH_4OH and extracted with ether. The extract was dried over anhydr. K_2CO_3 and evaporated. The residue was chromatographed on alumina, and elution with ether gave 130 mg of yellow oil which was distilled at 145° *in vacuo* (3 mm Hg). Recrystallization of the distillate from ether gave 120 mg of yellow plates (XXIII), mp 166—169°. *Anal.* Calcd. for $C_{18}H_{23}O_3N$; C, 71.73; H, 7.69. Found: C, 71.91; H, 7.74. IR cm^{-1}: $\nu_{C=O}$ 1728, 1680; $\nu_{C=C}$ 1625, 1583; ν_{C-O} 1246, 1234. UV λ_{max} mμ (log ε); 288 (4.33). NMR τ: 3.65 (1H, s., olefinic proton); 4.12 (1H, s., olefinic proton); 4.86 (1H, t., J=2.5 cps, >C<u>H</u>–OAc); 8.03 (3H, s., –OCOC<u>H</u>$_3$); 8.11 (3H, s., vinyl methyl).

Acknowledgement We are grateful to Mr. Nakaba, Dainippon Seiyaku Pharmaceutical Co., Ltd., Osaka and Miss Y. Mano, Kyoto University, for elementary analyses and to Dr. T. Shingu, Kyoto University, for NMR spectral measurements. Thanks are also to Dr. F. Kusuda, Nihonshinyaku Pharmaceutical Co., Ltd., Kyoto, for the extraction of materials.