Tetrahedron Letters No.14, pp. 1537-1549, 1966. Pergamon Press Ltd.
Printed in Great Britain.

SERRATININE:
A NOVEL SKELETAL LYCOPODIUM ALKALOID

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(Received 29 January 1966)

RECENTLY, the triterpenoid components of Lycopodium serratum THUNB. var. Thunbergii MAKINO (Japanese name Hosoba-Tohogeshiba) have been examined and the structures of serratenediol, a new skeletal triterpenoid containing a seven membered ring and several related triterpenoids have been established$^{1)}$. The present authors also investigated the basic constituents of this plant and three new alkaloids, serratinine (I)$^{2)}$, serratanine and serratine, and two known alkaloids, lycodoline (II)$^{3)}$ and lycodine (III)$^{4)}$ were isolated.

This communication deals with the structure elucidation of serratinine whose skeleton is entirely different from those of lycopodium alkaloids reported hitherto.

Serratinine (I), m.p. 244-245° *1, $C_{16}H_{25}O_3N$*1, $[\alpha]_D^8$ -27.8° (c,1.44 in EtOH), ν_{max}*2 3472, 3436, 3185 (OH) and 1724 cm^{-1}

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*1 All melting points were observed on a Kofler type microscope hotstage and are uncorrected. All compounds given by formulae in this communication gave correct elementary analyses.

*2 Unless otherwise noted, IR spectra were measured on Nujol mulls and UV spectra were obtained in ethanol solution.

($>$C=O), δ_{max} 1427 cm^{-1} (-CH$_2$-CO-) crystallized as colorless prisms from acetone. Acetylation of (I) with Ac$_2$O-pyridine at 100° afforded diacetylserratinine (Ic), m.p. 157-158°, $C_{20}H_{29}O_5N$ (M$^+$363), IR no OH band, NMR[*3] 5.06 (1H, m., $>$C$\underline{H}$-OAc), 5.39 (1H, m., $>$C$\underline{H}$-OAc), 7.98 (3H, s., -CO-C$\underline{H}_3$), 8.11 (3H, s., -CO-C$\underline{H}_3$), 9.10 τ (3H, d., J= 6 c.p.s., $>$CH-C$\underline{H}_3$). Oxidation of serratinine with Jones' reagent furnished a triketone, m.p. 157-160° $C_{16}H_{21}O_3N$, ν_{max} 1736 and 1693 cm^{-1} ($>$C=O), no hydroxyl band. For lack of the characteristic shift in the UV spectrum of triketone by alkali addition it seems that three carbonyl groups are separated one another by at least two carbon atoms. The presence of an active methylene group was verified by formation of benzylidene serratinine, m.p. 204-205°, $C_{23}H_{29}O_3N$, ν_{max} 1698 (conjugated carbonyl), 1629, 1597 and 1570 cm^{-1}(aromatic), λ_{max}[*2] 225 mμ (log ϵ 3.81), and 295.5 mμ (logϵ 4.18) ; its diacetyl derivative, m.p. 184-185°, $C_{27}H_{33}O_5N$, NMR 9.43 τ (3H, diffused d., J= 6 c.p.s., $>$CH-C$\underline{H}_3$). In addition to the facts described above, no signals due to olefinic proton and N-methyl group in the NMR spectra indicated that serratinine should be a tetracyclic alkaloid possessing the expanded molecular formula, $C_{10}H_{15}$ ($>$CH-CH$_3$), (-CH$_2$-CO-), ($>$CH-OH)$_2$, ($>$N-).

[*3] All NMR spectra were taken on a Varian Associates recording Spectrometer (A-60) at 60 Mc. in CDCl$_3$. Chemical shiftsare reported in τ values, using tetramethylsilane as an internal reference. Abbreviations used for the multiplicity of the signals: s.=singlet, d.=doublet, t.=triplet, q.=quartet, m.=multiplet.

Serratinine (pKa' 7.00)[*4] is less basic than deoxoserratinine (pKa' 10.9), m.p. 127-128°, $C_{16}H_{27}O_{2}N$, ν_{max} 3448 and 3175 cm^{-1}(OH), no carbonyl band, obtained by modified Wolff-Kishner reduction[5] of (I). Thus, it seems quite likely that a carbonyl group is situated in the proximity of the basic nitrogen. The relative position of these two functions was firmly established by the chemical transformations. Reduction of (I) with Zn-Ac_2O gave a neutral substance, O,O,N-triacetylchanodihydroserratinine (IV), m.p. 198-201°, $C_{22}H_{33}O_{6}N$, ν_{max} 1641 cm^{-1}(>N-CO-), NMR 7.92 (6H, s., 2x-CO-$C\underline{H}_3$), 8.10 τ (3H, s., -CO-$C\underline{H}_3$) which on reduction with $NaBH_4$ afforded an amido-alcohol (V), m.p. 188-189°, $C_{22}H_{35}O_{6}N$, ν_{max} 3436 cm^{-1}(OH) . Dehydration of (V) with $POCl_3$-pyridine gave a trisubstituted olefinic compound (VI), oil, $C_{22}H_{33}O_{5}N$, ν_{max} 1735 (OAc) and 1645 cm^{-1}(>N-CO-), no OH band. The NMR spectrum of (VI) revealed an additional signal due to an olefinic proton at 4.37 τ (m.) together with other signals properly expected in (VI). On the other hand, oxidation of (VII=Ic) with SeO_2 provided an α,β-unsaturated ketone (VIII), m.p. 121-123°[*5], $C_{20}H_{27}O_{5}N$, ν_{max} 1686 and 1631 cm^{-1}(α,β-unsaturated ketone), λ_{max} 228 mμ (logε 4.02), NMR 3.77 (1H, s., olefinic proton), 4.28 (1H, d., J=3 c.p.s., >C$\underline{H}$-OAc), 5.03 (1H, m., >C$\underline{H}$-OAc), 7.92 (3H, s., OAc), 8.04 τ (3H, s., OAc). Reduction of (VIII) with $LiAlH_4$, followed by acetylation

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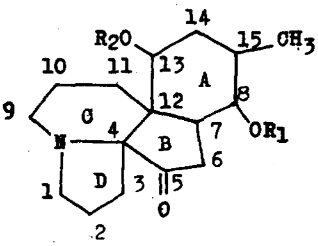
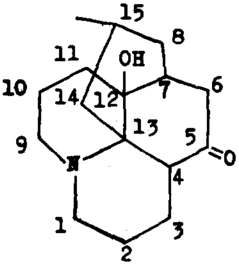
[*4] pKa' values were measured in 1/10 N-H_2SO_4 (1 ml)-EtOH (5 ml)-H_2O (4 ml) solvent system by titration with 1/10 N-NaOH solution.

[*5] This substance has two m.p.s (121-123°,and 139-141°) and that these are dimorphism was shown by the cross seeding method and the identity of IR spectra in CCl_4 solution.



(I) $R_1 = R_2 = H$
serratinine

(Ia) $R_1 = Ac$, $R_2 = H$
(Ib) $R_1 = H$, $R_2 = Ac$
(Ic) $R_1 = Ac$, $R_2 = Ac$



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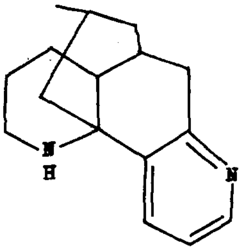

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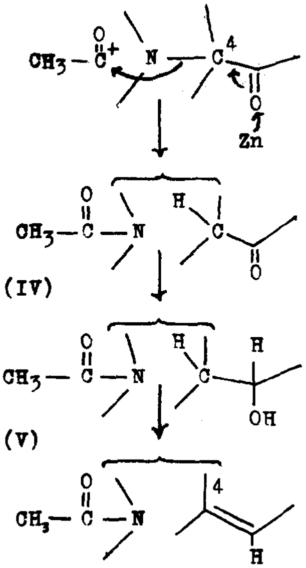
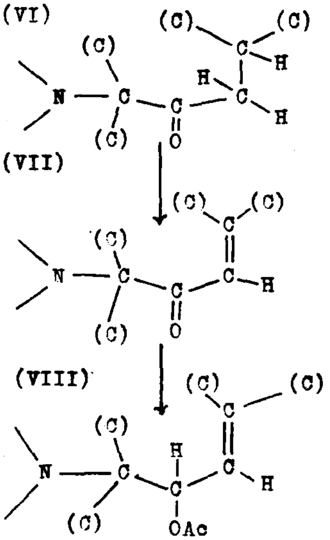


(IV)

(V)

(VI)



(VII)

(VIII)

(IX)

furnished a triacetyl derivative (IX), m.p. 124°, $C_{22}H_{31}O_6N$, ν_{max} 1727 ($>$C=O) and 1667 cm^{-1}($>$C=C$<$). In the NMR spectrum below 7.00 τ, the triacetyl derivative (IX) gave rise to two additional signals at 4.09 (1H,d.,J=2 cps., olefinic proton) and 4.52 τ(1H,d.,J=2 c.p.s.,$>$C$\underline{H}$-OAc) besides two signals at 4.45 (1H, d.,J=3 c.p.s.,$>$C$\underline{H}$-OAc) and 5.07 τ(1H,m.,$>$C$\underline{H}$-OAc) as constrasted to diacetylserratinine (Ic) in which two multiplet signals appeared at 5.06 and 5.39 τ. From these observations it could be deduced that there is no hydrogen atom on another side carbon atom (C_4) of carbonyl group and that the partial structure (VII) would be given for serratinine. In the NMR spectrum of the compound (VIII), the signal due to one of two protons geminal to an acetoxyl group caused the down field shift to 4.28 τ and appeared as a doublet (J=3 c.p.s.). This finding indicates that one acetoxyl group in the compound (VIII) would be situated in the allylic position (C_8;refer to the formula (X)). Since a singlet peak of an olefinic proton suggests no appreciable allylic coupling and moreover the signal due to C_8-H appeared as a doublet, only one proton would be present on the C_{15} carbon atom. Thus, the partial structure (X) with the exception of C_{15} methyl group could be presumed for serratinine and this was firmly established by the subsequent experiments.

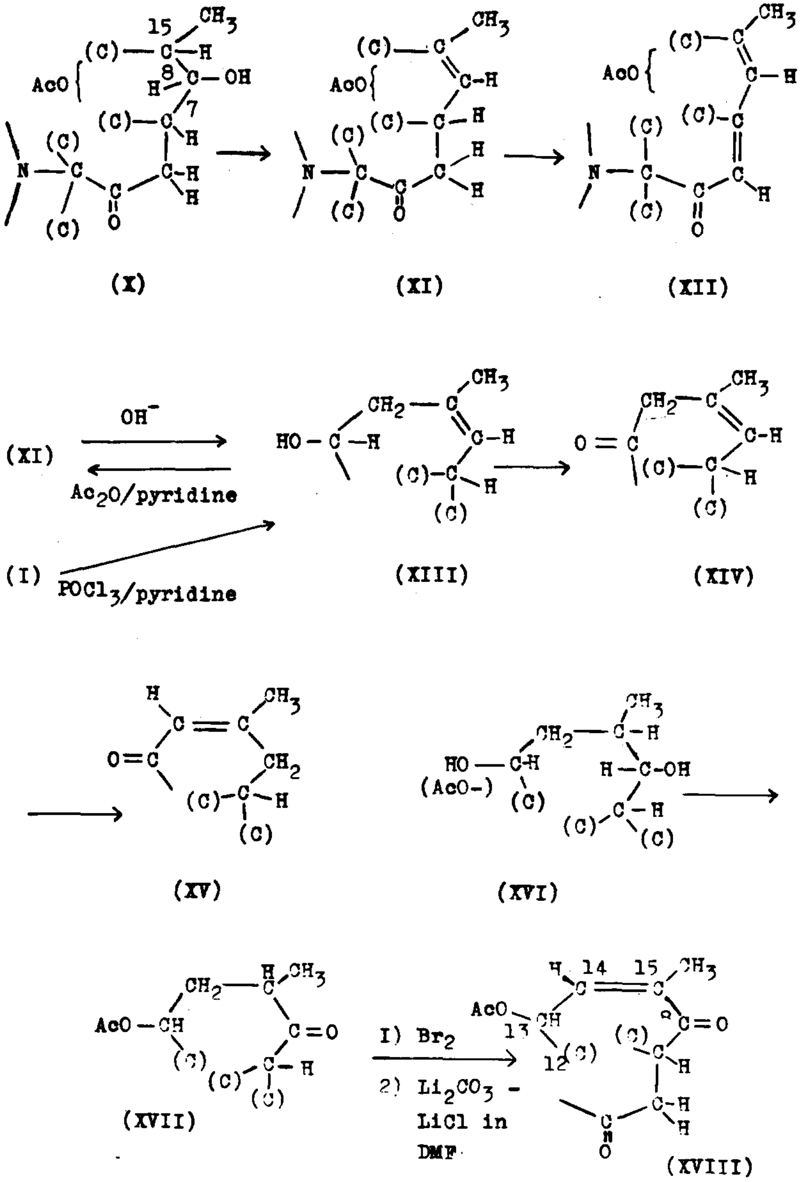
Acetylation of (I) with Ac_2O-pyridine at room temperature gave monoacetylserratinine I (Ia), m.p. 244-245°, $C_{18}H_{27}O_4N$, ν_{max} 3215 (OH) and 1736 cm^{-1} ($>$C=O), NMR 4.94 (1H, m.,$>$C$\underline{H}$-OAc), 6.30 (1H, m., C$\underline{H}$-OH), 7.94 τ (3H, s., OAc). In the meantime, hydrolysis of diacetylserratinine with aqueous 10% HCl solution afforded another monoacetyl compound, monoacetylserratinine II (Ib), m.p. 240-242°, $C_{18}H_{27}O_4N$, ν_{max} 3185 (OH) and 1733 cm^{-1} ($>C=O$). Since on further acetylation, both (Ia) and (Ib) gave the same product diacetylserratinine (Ic),the difference between them should be ascribed to the position of free hydroxyl group.

Dehydration of (Ib) with $POCl_3$-pyridine at room temperature gave anhydromonoacetylserratinine II (XI), m.p. 188-189° $C_{18}H_{25}O_3N$, ν_{max} 1724 cm^{-1} ($>C=O$), UV only end absorption, NMR 4.44 (1H, m., olefinic proton), 8.33 τ (3H, s., vinyl methyl). Oxidation of (XI) with SeO_2 afforded a dienone compound (XII), m.p. 166-169°, $C_{18}H_{23}O_3N$, ν_{max} 1680, 1625 and 1583 cm^{-1} (dienone), λ_{max} 288 mμ (logε 4.33), NMR 3.65 (1H, broad s., olefinic proton), 4.12 (1H, s., olefinic proton)4.86 (1H, t., J=2.5 c.p.s.,$>C\underline{H}-OAc$), 8.03 (3H, s.,$-CO-C\underline{H}_3$) and 8.11 τ (3H, s., vinyl methyl). Thus, the partial structure of serratinine could be extended to the formula (X) and it is apparent that the free hydroxyl group in monoacetylserratinine II (Ib) must be located on the carbon atom (C_8) adjacent to the secondary methyl group.

Hydrolysis of (XI) provided anhydroserratinine II (XIII) m.p. 196-198° , $C_{16}H_{23}O_2N$, ν_{max} 3175 cm^{-1} (OH) which was also readily obtained by dehydration of (I) with $POCl_3$ in pyridine at room temperature. Oxidation of (XIII) with Jones' reagent afforded a ketone (XIV), m.p. 105-106°, $C_{16}H_{21}O_2N$, ν_{max} 1740 and 1700 cm^{-1} ($>C=O$), NMR 4.54 (1H, m., olefinic proton), 8.28 τ (3H, broad s., vinyl methyl), UV only end absorption. On vacuum distillation or chromatography on basic alumina, (XIV) isomerized to α,β-unsaturated ketone

(XV), m.p. 117-118.5°, $C_{16}H_{21}O_2N$, ν_{max} 1728 and 1642 cm^{-1} ($>C=O$), λ_{max} 238 mμ (log ε 4.04), NMR 4.04 (1H, m., olefinic proton), 8.02 τ (3H, s., vinyl methyl). Therefore, we can extend the partial structure of serratinine to the formula (XVI).

Oxidation of (XVI=Ib) with Jones' reagent gave dehydromonoacetylserratinine II (XVII), m.p. 187.5-188°, $C_{18}H_{25}O_4N$ ν_{max} 1695 cm^{-1}($>C=O$), NMR 5.21 (1H, m., $>C\underline{H}-OAc$), 8.94 τ (3H, d., J=7 c.p.s., $>CH-C\underline{H}_3$). Bromination of (XVII), followed by dehydrobromination with Li_2CO_3-LiCl in dimethyl formamide gave an α,β-unsaturated ketone (XVIII), m.p. 112-114°, $C_{18}H_{23}O_4N$, ν_{max} 1667 and 1650 cm^{-1} (αβ-unsaturated ketone) λ_{max} 232 mμ (log ε 3.99), NMR 3.18 (1H, a pair of quartets, J_1=1.8 c.p.s., J_2 = 6.2 c.p.s., olefinic proton), 5.02 (1H, d., J=6.2 c.p.s., $>C\underline{H}-OAc$), 8.12 τ (3H, d., J=1.8 c.p.s., vinyl methyl). Then, decoupling experiments were performed on the compound (XVIII). Irradiation of the signal due to the olefinic proton on C_{14} (3.18 τ) resulted in the changes of two doublets attributable to both C_{13}-H ($\underline{H}-\overset{|}{\underset{|}{C}}-OCOCH_3$) and $C_{15}-CH_3$ to a singlet, respectively. This finding suggested the absence of hydrogen on C_{12}. A second observation that in the NMR spectrum of dienone (XII), a signal of the proton geminal to the acetoxyl group appeared as a clean triplet (J=2.5 c.p.s.) (vide ante) provided more evidence on this deduction. On the basis of the above results, serratinine should have the partial structure of (XIX).

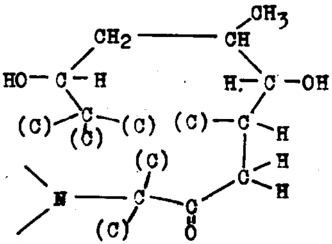

(X)
(XI)
(XII)
OH⁻
Ac2O/pyridine
POCl3/pyridine
(XIII)
(XIV)
(XV)
(XVI)
(XVII)
1) Br2
2) Li2CO3 - LiCl in DMF
(XVIII)

We now wish to refer to the ring system in serratinine.

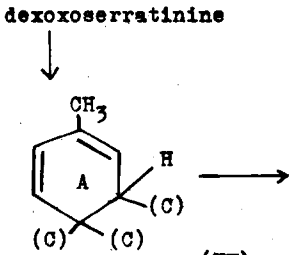
Dehydration of dexoserratinine with $POCl_3$-pyridine gave bisanhydrodeoxoserratinine (XX), oil, λ_{max} 266 mμ (logε 3.89) ; its picrate m.p. 145-147°, $C_{16}H_{23}N\cdot C_6H_3O_7N_3$. The reaction of (XX) with diethyl acetylenedicarboxylate afforded in one step diethyl 4-methylphthalate (XXI), oil which was identified with an authentic sample$^{6)}$ by comparison of IR and NMR spectra. According to Alder-Rickert rule$^{6)}$, this reaction is indicative that the ring A bearing the secondary methyl group is a six membered one.

That the B ring seems to be a five membered one was based on the IR absorption of serratinine and its derivatives at 1724-1748 cm^{-1}.

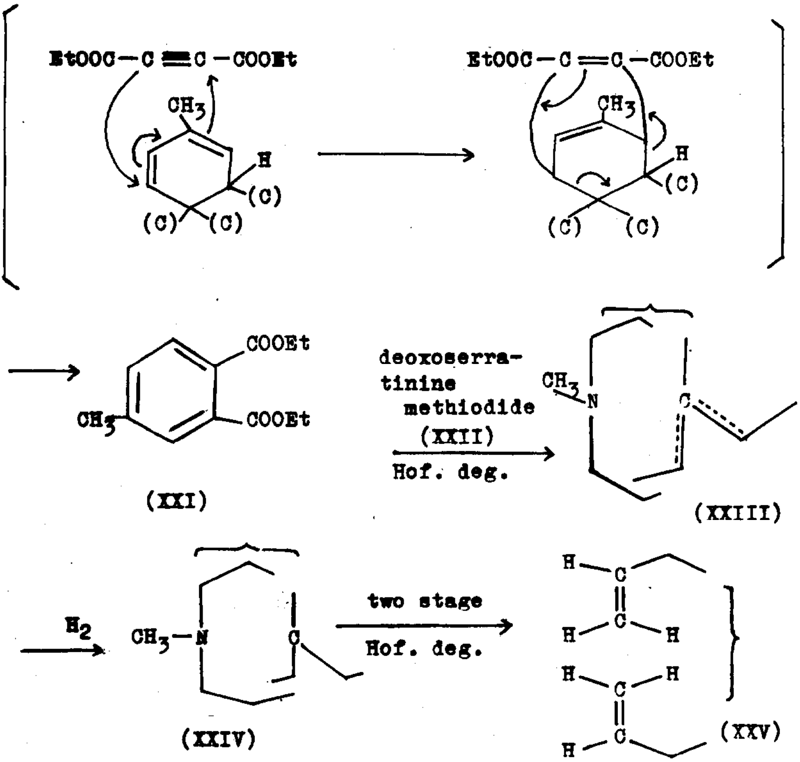
Hofmann degradation of deoxoserratinine methiodide (XXII), m.p.242-244°, $C_{16}H_{27}O_2N.MeI$ gave deoxoserratinine methine (XXIII) m.p. 105-106°, $C_{17}H_{29}O_2N$, ν_{max} 1626 cm^{-1}($>C=C<$), NMR 4.20 (1H, m., olefinic proton), 7.74 τ (3H, s., $\overset{|}{\underset{|}{N}}-C\underline{H}_3$) together with other signals. Catalytic hydrogenation of (XXIII) over platinum provided a dihydro methine (XXIV), m.p.86-88°, $C_{17}H_{31}O_2N$, NMR no olefinic proton. Successive treatments of (XXIV) with MeI, Ag_2O and pyrolysis gave an oily mixture which showed two spots on thin layer chromatography. This mixture seems to consist of two isomeric methines being produced by the C-N bond fissions in two different directions. This mixture, without separation, was subjected to the second stage Hofmann degradation to give the des-N compound (XXV), oil, which without purification



(XIX)



(XX)



(XXI)

(XXIII)

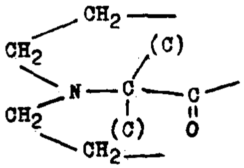
(XXIV)

(XXV)

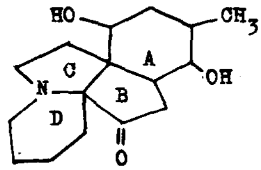
revealed virtually one spot on thin layer chromatography. For characterization, the product was purified by distillation to afford colorless oil, $C_{16}H_{26}O_2$, $\nu_{max}^{CHCl_3}$ 1634 cm^{-1} ($>C=C<$), $\delta_{max}^{CHCl_3}$ 997 and 917 cm^{-1} ($>CH=CH_2$), NMR 3.7-5.2 τ (6H, m., olefinic protons). Thus, the partial structure near to the nitrogen atom could be represented by the formula (XXVI).

Oxidation of diacetylserratinine (Ic) with $KMnO_4$-$MgSO_4$ in aqueous 80% acetone gave a neutral lactam compound (XXVII), m.p. 215-216°, $C_{20}H_{27}O_6N$ whose IR spectrum showed a band at 1640 cm^{-1}. This observation permits to assume that one of two rings containing a nitrogen atom would be a six membered one. Since reduction of (XXVII) with $LiAlH_4$ gave α-dihydroserratinine (XXVIII), m.p. 277-279°, $C_{16}H_{27}O_3N$, ν_{max} 3500 cm^{-1} (OH) which was also obtained by reduction of serratinine with the same reagent, the possibility of skeletal rearrangement during the oxidation process would be excluded. The structure of serratinine, now, can be represented by either the formula (I) or (XXIX).

Mass spectrometry would serve to prefer the formula (I) rather than another[7]. The base peak in the spectrum of diacetylserratinine (Ic) was at m/e 303 (M^+-60, loss of HAc) and the characteristic peak was found at m/e 336 (M^+-27). The latter peak seems to be due to cleavage of the bond beta to the carbonyl group with rearrangement of a hydrogen atom as dipicted. This assignment of the fragmentation pattern



(XXVI)



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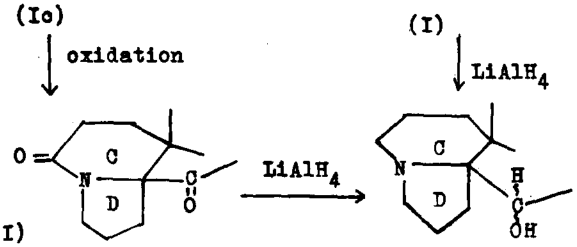
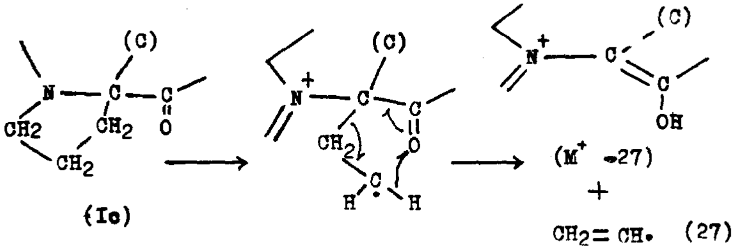


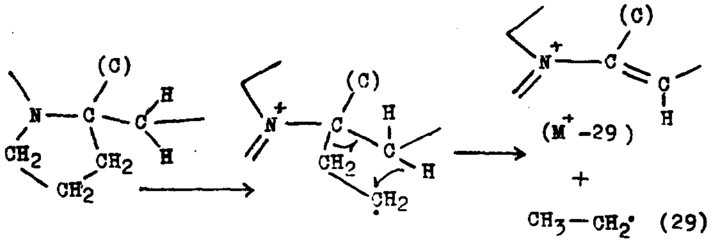
(XXVII)

(XXVIII)

or C ring is five mem. ring
and D ring is six mem.
ring

or C ring is five mem.
ring and D ring is
six mem. ring





was supported by the fact that no appreciable peak except a peak at m/e 336 was found in the region between M^+ (m/e 363) and M^+-60 (m/e 303) peaks and also by the presence of the expected metastable peak. In the spectrum of diacetyldeoxoserratinine showed a parent peak at m/e 349 and a peak at m/e 320 (M^+-29). Although the origin of the latter peak is somewhat uncertain, the probable fragmentation would be shown by the pattern cited in chart.

Finally, the formula (I) is the only satisfactory structure to all the structure requirementsfor serratinine.

Acknowledgement. The authors express their thanks to Drs. R. H. F. Manske and D. B. MacLean for providing authentic samples of lycodoline and lycodine, and to Dr. T. Shingu, Kyoto University for measuring NMR spectra.

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7. Mass spectra were measured by Dr. W. A. Ayer, Univ. of Alberta, Canada, to whom the authors are indebted. They also express their gratitude to Drs. T. Ibuka, Kyoto Univ. and Y. Tsuda, Osaka Univ. for their discussions on mass spectra. Details of spectra will be presented in other place.