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MASS SPECTRA OF SERRATININE AND ITS DERIVATIVES[1]

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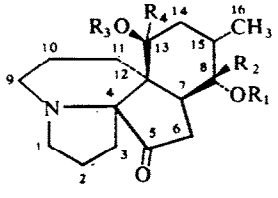
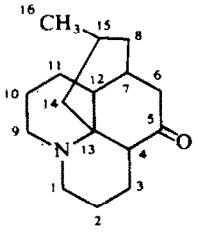
Abstract—The mass spectra of serratinine and its derivatives have been determined. Interpretation of the mechanism for the resulting ions are shown and the diagnostic value of this technique for the serratinine skeleton is also indicated.

MASS spectrometry of Lycopodium alkaloids was first reported by MacLean *et al.*[2] and application of this technique to the structure determination of these alkaloids has been recorded.[3,4] In these papers, it has been pointed out that alkaloids related in skeletal structure to lycopodine (II) undergo an exactly analogous fragmentation of bridge atoms, for instance the C_8, C_{14} and C_{15} atoms of lycopodine (II), together with their attached substituents.

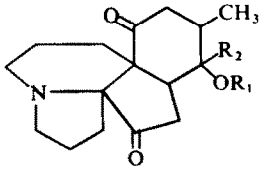
Serratinine was first isolated from a kind of lycopodium species[5] growing in Japan and its structure (I) has been completely established.[6]



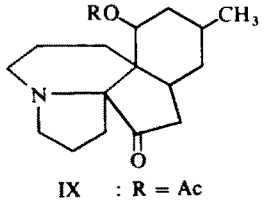
I : $R_1 = R_2 = R_3 = R_4 = H$
VI : $R_1 = Ac$, $R_2 = R_3 = R_4 = H$
VII : $R_1 = R_2 = R_4 = H$, $R_3 = Ac$
VIII : $R_1 = R_3 = Ac$, $R_2 = R_4 = H$
XIV : $R_1 = R_2 = R_3 = H$, $R_4 = D$
XVIII: $R_1 = Ac$, $R_4 = D$, $R_2 = R_3 = H$
XIX : $R_1 = R_2 = H$, $R_3 = Ac$, $R_4 = D$
XXIV: $R_1 = C_6H_5CO$, $R_2 = R_4 = H$, $R_3 = Ac$
XXV : $R_1 = R_3 = Ac$, $R_2 = D$, $R_4 = H$
XXVI: $R_1 = R_3 = Ac$, $R_2 = H$, $R_4 = D$



II



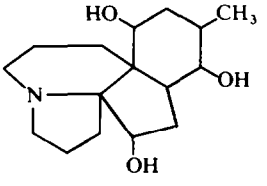
III : $R_1 = R_2 = H$
XII : $R_1 = Ac$, $R_2 = H$
XXIX: $R_1 = Ac$, $R_2 = D$



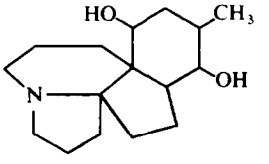
IX : R = Ac
XV : R = H

Since serratinine possesses a novel skeleton entirely different from those of lycopodium alkaloids hitherto reported, interpretation of a possible mechanism of fragmentation of this alkaloid is important since the fragmentation pattern of serratinine should be quite different and mass spectrometry, therefore, will provide a rapid and convenient method of elucidating the structures of serratinine type alkaloids, especially the minor alkaloids which have been isolated from the plant, and for which the available chemical method is extremely costly in both time and material.

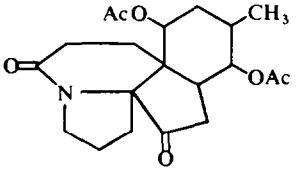
In this paper, we propose a reasonable mechanism for the fragmentation of serratinine and give possible structures for the resulting ions.



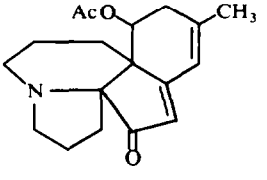
IV



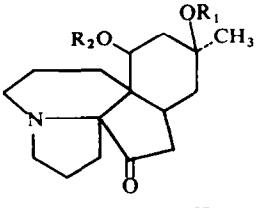
V



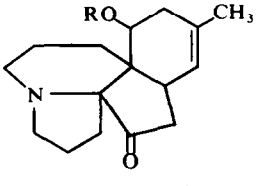
X



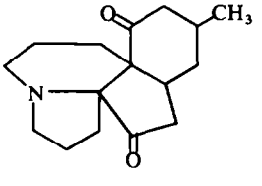
XI



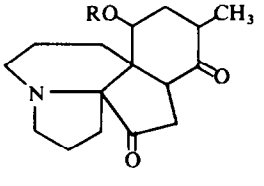
XVI* : $R_1 = R_2 = H$
XXI : $R_1 = H$, $R_2 = Ac$
XXII : $R_1 = R_2 = Ac$



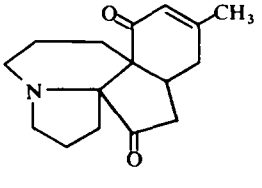
XVII : R = H
XXIII: R = Ac



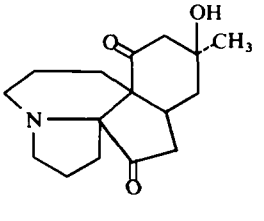
XIII



XX : R = Ac



XXVII



XXVIII

* This alkaloid was isolated from the same plant together with serratinine and designated as serratine.

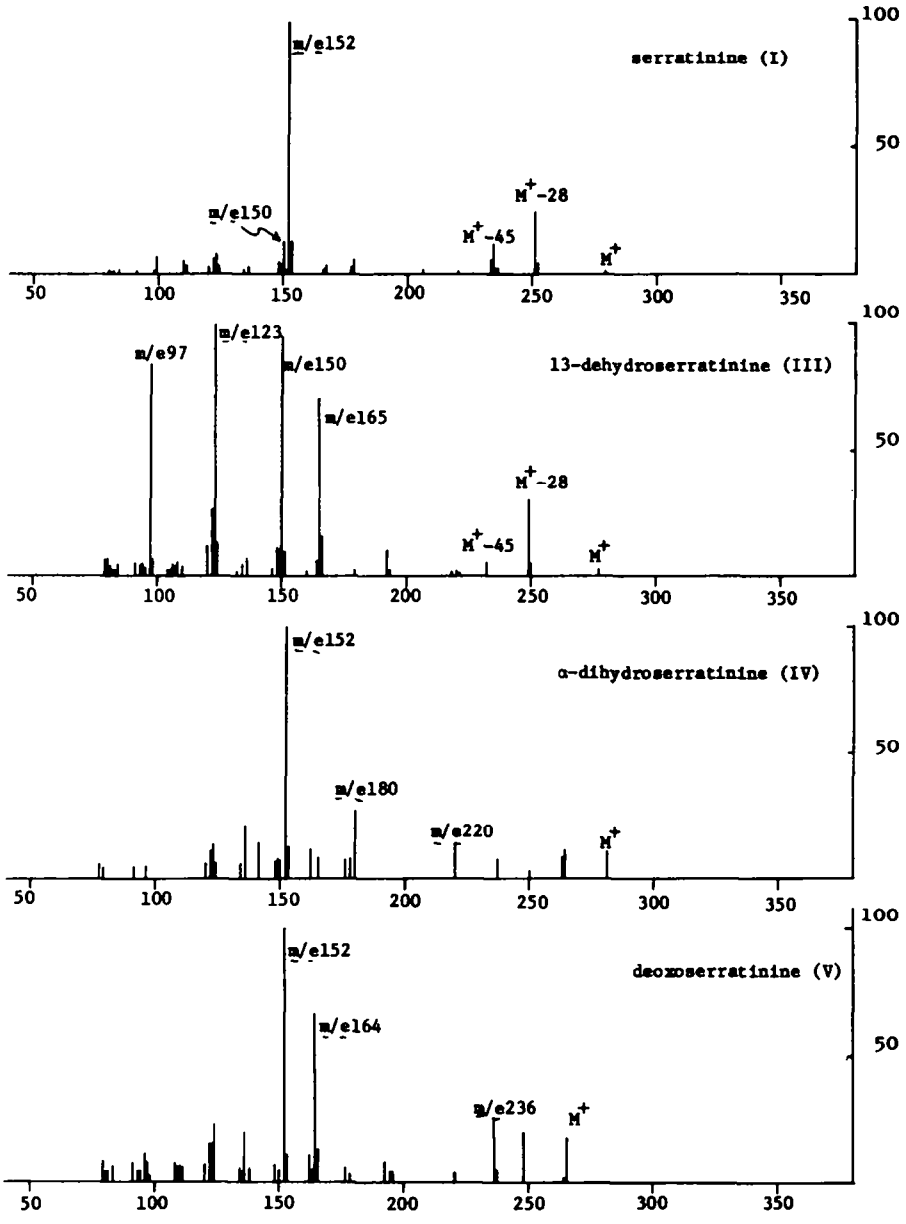


FIG. 1.

In previous papers,[6] we suggested that three fragment ions at M^+-28, *m/e* 152 and *m/e* 150 may be diagnostically important for the serratinine skeleton. The results of analyses of the high-resolution spectra of serratinine (I) and 13-dehydroserratinine (III) are shown in Table 1. The peak at *m/e* 152 in serratinine reveals a singlet with the elemental composition of $C_9H_{14}NO$ which corresponds to the ion (an ion D) proposed in the fragmentation mechanism (Scheme 1). The ion at *m/e* 150, however,

TABLE 1. ACCURATE MASS MEASUREMENTS OF *m/e* 152 AND *m/e* 150 PEAKS IN THE SPECTRA OF SERRATININE (I) AND 13-DEHYDROSERRATININE (III)

	Measured mass	Error (obs–calc) in milli mass units	Elemental composition
Serratinine (I)	152·1074	−0·1	$C_9H_{14}NO$
	150·1019	−2·5	$C_{10}H_{14}O$
	150·1322	+4·9	$C_{10}H_{16}N$
13-Dehydroserratinine (III)	150·0945	−2·5	$C_{10}H_{16}N$
	150·1263	−2·0	$C_9H_{12}NO$
	97·0898	+0·7	$C_6H_{11}N$

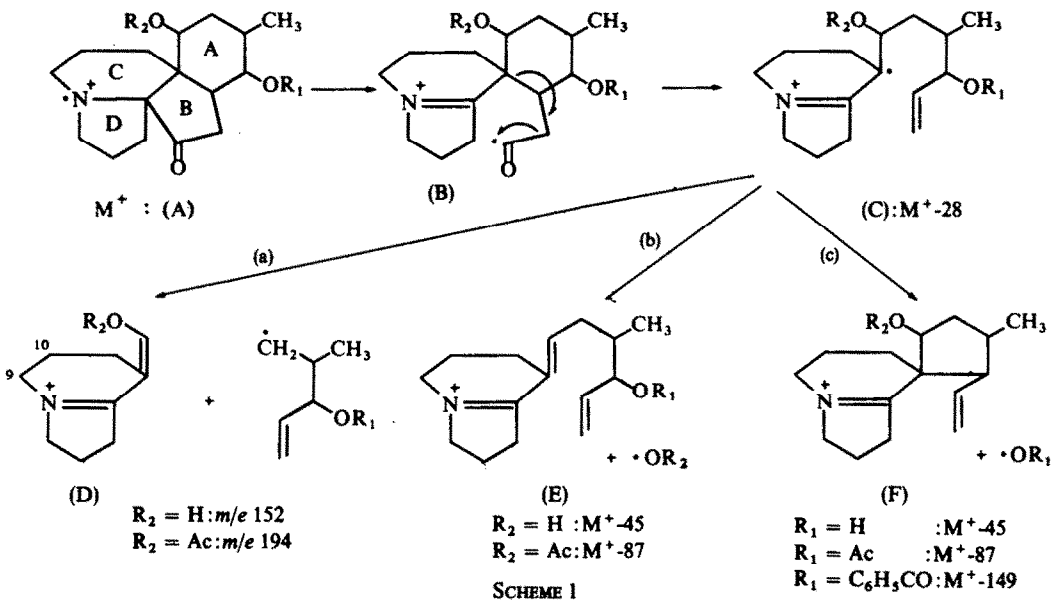
shows a doublet, each corresponding in composition to $C_{10}H_{16}N$ and $C_{10}H_{14}O$, respectively and the intensity of the peak corresponding to the former is about four times as intense as that of the peak corresponding to the latter. This finding shows that the peak at *m/e* 150 arises from at least two different fragmentation processes, although their origin and structures are still obscure.

The genesis of M^+-28 ion can be visualized as occurring by loss of CO in the molecular ion because an α-amino ketone structure may be expected to suffer the facile fission of ring B owing to the simultaneous generation of immonium ion (an ion B in Scheme 1) and to give rise to an ion (M^+-28) by loss of CO. The result of analysis of the high-resolution spectrum of 13-dehydroserratinine (III) is shown in Table 2.

TABLE 2. ACCURATE MASS MEASUREMENT OF M^+-28 (*m/e* 249) PEAK IN THE SPECTRUM OF 13-DEHYDROSERRATININE (III)

	Elemental composition	Calc mass	Observed mass	Error (obs–calc) in milli mass units
Standard subst	$C_{16}H_{27}NO$	249·2093	—	—
M^+-28	—	—	249·1751	—
M^+-CO	$C_{15}H_{23}NO_2$	249·1728	—	+ 2·3
M^+-C_2H_4	$C_{14}H_{19}NO_3$	249·1364	—	+38·7
M^+-CH_2N	$C_{15}H_{21}O_3$	249·1491	—	+26·0

The peak at M^+-28 actually reveals a singlet with the elemental composition of $C_{15}H_{23}NO_2$ which corresponds to the ion arising from the molecular ion by loss of CO. In accord with this observation, the general fragmentation patterns of α-dihydroserratinine (IV) and deoxoserratinine (V) which lack the original ketone group at C_5 of serratinine, are entirely different from those of serratinine and its derivatives leaving the original ketone intact. The fragmentation, $M^+ \rightarrow M^+$-28, is supported by the presence of metastable peak corresponding to this process as shown in Table 3.



SCHEME 1

TABLE 3. METASTABLE PEAKS CORRESPONDING TO THE $M^+ \rightarrow M^+$-28 FRAGMENTATION IN THE SPECTRA OF SERRATININE AND ITS DERIVATIVES

	m^*_{obs}	m^*_{calc}	
		M^+ M^+-28	
Serratinine (I)	226·0	279 → 251	225·8
8-Acetylserratinine (VI)	267·5	321 → 293	267·4
13-Acetylserratinine (VII)	267·5	321 → 293	267·4
8,13-Diacetylserratinine (VIII)	309·5	363 → 335	309·2
13-Acetyl-8-deoxyserratinine (IX)	252·0	305 → 277	251·6
8,13-Diacetyl-9-oxo-serratinine (X)	324·0	377 → 349	323·1
13-Acetyl-6,7-dehydro-8(15)-anhydroserratinine (XI)	247·0	301 → 273	247·6
13-Dehydroserratinine (III)	224·0	277 → 249	223·8
8-Acetyl-13-dehydroserratinine (XII)	265·0	319 → 291	265·5
13-Dehydro-8-deoxyserratinine (XIII)	208·0	261 → 233	208·0

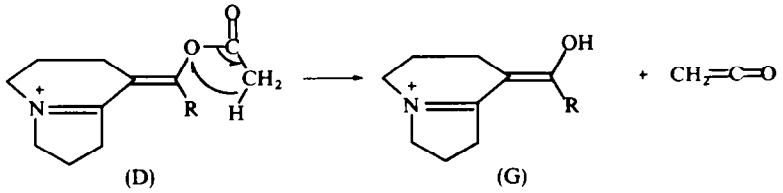
Thus, the fragment ions at M^+-28 and *m/e* 152 are indeed important indicators for the presence of the serratinine skeleton. The fragmentation sequence initiated by the $M^+ \rightarrow M^+$-28 transformation is formulated in Scheme 1. The ion at *m/e* 152 no doubt arises directly from the ion M^+-28 since the metastable peak associated with this transformation was observed as shown in Table 4. The substantial peak at *m/e* 152 must include the C_{13} carbon atom since it is shifted to *m/e* 153 in the spectrum of 13-d_1-serratinine (XIV). Further evidence for the proposed process was provided by inspection of the spectra of acetylserratinine (VII) and 13-dehydroserratinine (III). In the former compound, the peak of fairly relative intensity at *m/e* 194 (which is not present in the spectra of serratinine and its derivatives possessing the C_{13} hydroxyl

group) and the peak at *m/e* 152 are observed, whereas in the corresponding deuterium-labelling compound, 13-d_1-13-acetylserratinine (XIX), these two peaks shift to *m/e* 195 and *m/e* 153, respectively (Scheme 2). Appearance of these peaks could be explained by assuming a fragmentation pattern analogous to that of alkaloids bearing a C_{13} hydroxyl group. In this process, the first step involves loss of CO, giving rise to an ion

TABLE 4. METASTABLE PEAKS ASSOCIATED WITH THE M^+-28 → *m/e* 152 ION (*m/e* 153) TRANSFORMATION IN THE SPECTRA OF SERRATININE AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}
		M^+-28	
Serratinine (I)	92·0	251 → 152	92·0
8-Acetylserratinine (VI)	78·8	293 → 152	78·9
8-Deoxyserratinine (XV)	98·5	235 → 152	98·3
Serratine (XVI)	92·0	251 → 152	92·0
8(15)-Anhydroserratinine (XVII)	99·2	233 → 152	99·2
13-d_1-Serratinine (XIV)	92·8	252 → 153	92·9
13-d_1-8-Acetylserratinine (XVIII)	79·5	294 → 153	79·6

M^+-28 which then undergoes further fragmentation to give an ion of *m/e* 194 (D), and in turn, loss of ketene gives rise to an ion (G) of *m/e* 152 (Scheme 2). The values of metastable peaks calculated for these two fragmentations, M^+-28 → *m/e* 194 → *m/e* 152, respectively are listed in Table 5. The fact that a metastable peak corresponding to M^+-28 → *m/e* 152 fragmentation which has been observed in the spectra of serratinine and its derivatives possessing the C_{13} hydroxyl group, is not present in the spectra of 13-acetyl derivatives, gives further support to this stepwise fragmentation process.



(D) (G)

R = H:*m/e* 194 R = H:*m/e* 152
R = D:*m/e* 195 R = D:*m/e* 153

SCHEME 2

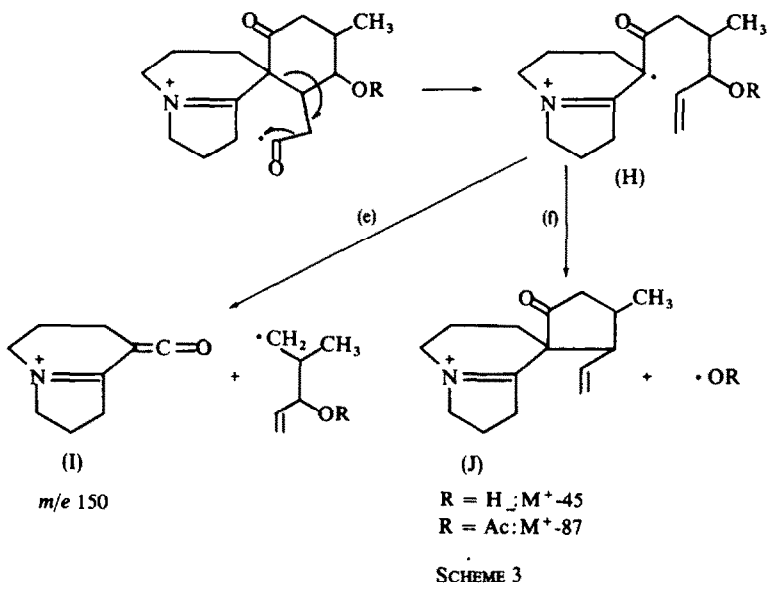
In the mass spectrum of 13-dehydroserratinine (III) [C_{13} ketone in place of C_{13} hydroxyl group] an intense peak (a base peak) is observed at *m/e* 150 without an accompanying peak at *m/e* 152. This ion may be formed by a fragmentation mechanism similar to that in the formation of the ion at *m/e* 152 appearing in the spectrum of serratinine [(a) process in Scheme 1]. As the ion at *m/e* 152 in serratinine is always accompanied by *m/e* 150 and as the latter peak has been proved to be a doublet in the high-resolution spectrum the origin of which has not been settled, the high-resolution spectrum of 13-dehydroserratinine was also carried out (Table 1). Although the ion at *m/e* 150 is actually a doublet, in this case, the major component (ca. 85%) of the doublet corresponds in composition, $C_9H_{12}NO$, to the ion (I) proposed in the frag-

TABLE 5. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → *m/e* 194 (*m/e* 195) → *m/e* 152 (*m/e* 153) FRAGMENTATIONS IN THE SPECTRA OF 13-ACETYLSERRATININE (XIII) AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}
13-Acetylserratinine (VII)	128·5	293 → 194	128·5
	119·2	194 → 152	119·1
8,13-Diacetylserratinine (VIII)	112·5	335 → 194	112·3
	119·2	194 → 152	119·1
13-Acetyl-8-deoxyserratinine (IX)	136·0	277 → 194	135·9
	119·3	194 → 152	119·1
13-Acetyl-8-dehydroserratinine (XX)	129·5	291 → 194	129·3
	119·1	194 → 152	119·1
13-Acetylserratine (XXI)	not obs	293 → 194	128·5
	119·0	194 → 152	119·1
13,15-Diacetylserratine (XXII)	not obs	335 → 194	112·3
	119·0	194 → 152	119·1
13-Acetyl-8(15)-anhydroserratinine (XXIII)	137·0	275 → 194	136·9
	119·2	194 → 152	119·1
13-Acetyl-6,7-dehydro-8(15)-anhydroserratinine (XI)	not obs	273 → 194	137·9
	119·2	194 → 152	119·1
13-Acetyl-8-benzoylserratinine (XXIV)	94·8	397 → 194	94·8
	119·2	194 → 152	119·1
8-d_1-8,13-Diacetylserratinine (XXV)	113·1	336 → 194	112·0
	119·3	194 → 152	119·1
13-d_1-13-Acetylserratinine (XIX)	129·3	294 → 195	129·3
	not obs	195 → 153	120·0
13-d_1-8,13-Diacetylserratinine (XXVI)	113·2	336 → 195	113·2
	120·1	195 → 153	120·0
8,13-Diacetyl-9-oxo-serratinine (X)	124·0	349 → 208	124·0
	132·8	208 → 166	132·5

mentation mechanism (Scheme 3). This fragmentation (M^+-28 → *m/e* 150) is supported by the presence of metastable peak associated with the fragmentation proposed as shown in Table 6.

A study of the modification of structures reveals clearly that both an ion (D) in Scheme 1 and an ion (I) in Scheme 3 do not comprise the carbon atoms, C_6, C_7, C_8, C_{14} and C_{15}(C_{16}) in the serratinine molecule. Thus, in the mass spectrum of 13-acetyl-6,7-dehydro-8(15)-anhydroserratinine (XI) in which two double bonds, $C_6{=}C_7$ and $C_8{=}C_{15}$, are involved, two characteristic peaks at *m/e* 194 and *m/e* 152 being common to 13-acetylserratinine derivatives still can be observed, and the peak at



SCHEME 3

TABLE 6. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → *m/e* 150 FRAGMENTATION IN THE SPECTRA OF 13-DEHYDROSERRATININE (III) AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}
		M^+-28	
13-Dehydroserratinine (III)	90·5	249 → 150	90·4
13-Dehydro-8-deoxyserratinine (XIII)	97·0	233 → 150	96·6
13-Dehydro-14(15)-anhydroserratine (XXVII)	97·0	231 → 150	97·4

m/e 150 which is common to 13-dehydroserratinine derivatives, is detected also in the mass spectrum of 13-dehydro-14(15)-anhydroserratine (XXVII). In addition, the mass spectrum of 8,13-diacetyl-9-oxoserratinine (X) which differs from 8,13-diacetylserratinine in bearing a C=O group in place of CH_2 at the C_9 position, was examined (Fig. 2). The breakdown of this substance should proceed according to the fragmentation process of C_{13}-acetylserratinine derivatives ($M^+ \rightarrow M^+$-28 (*m/e* 335) → *m/e* 194 → *m/e* 152). The relatively intense ions at *m/e* 349 (M^+-28), *m/e* 208 and *m/e* 166 each appear 14 mass units above the corresponding ions in 13-acetylserratinine (Fig. 2), and detection of appropriate metastable peaks (Table 1 and 5) provide sufficient evidence for this fragmentation.

These findings together with the results from high-resolution mass spectral measurements and deuterium-labelling experiments mentioned previously, lend strong support not only to the proposed fragmentation mechanism but also to the proposal of structure D (R_2 = H) for the prominent fragment ion at *m/e* 152.

All serratinine derivatives possessing the C_{13} hydroxyl group reveal the base peak at *m/e* 152, whereas the C_{13} acetyl derivatives exhibit the base peak at M^+-87 together with peaks at M^+-28, *m/e* 194 and *m/e* 152, or instead a very intense peak

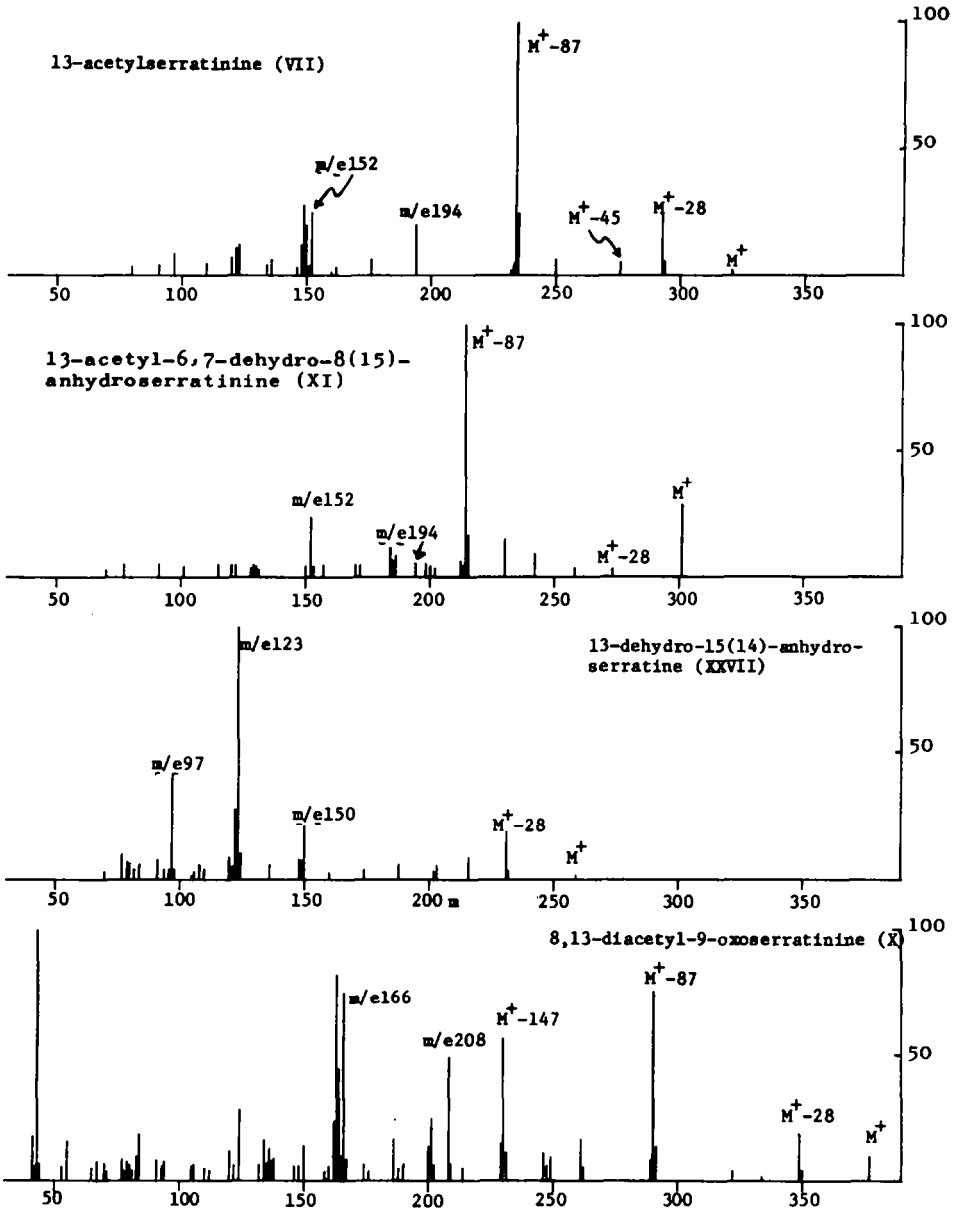


FIG. 2.

at M^+-87 is observed. The formation of this intense ion at M^+-87 can be considered to arise by loss of an acetoxyl radical from M^+-28 ion [an ion (E), (b) process in Scheme 1]. This fragmentation is supported by the detection of an appropriate metastable peak (Table 7).

An analogous fragmentation would be expected also in the serratinine derivatives possessing the C_{13} hydroxyl group. In fact, these compounds reveal the peak at M^+-45

TABLE 7. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → M^+-87 FRAGMENTATION IN THE SPECTRA OF ACETYLSERRATININES

	m^*_{obs}		m^*_{calc}	Intensity of M^+-87/ base peak
		M^+-28 M^+-87		
13-Acetylserratinine (VII)	187·0	293 → 234	186·9	base peak
13-Acetyl-8-dehydro-serratinine (XX)	185·0	291 → 232	185·0	base peak
13-Acetyl-8-deoxyserratinine (IX)	171·7	277 → 218	171·6	base peak
13-Acetylserratinine (XXI)	186·9	293 → 234	186·9	base peak
8,13-Diacetylserratinine (VIII)	227·8	335 → 276	227·4	base peak
13,15-Diacetylserratine (XXII)	227·0	335 → 276	227·4	89%
8,13-Diacetyl-9-oxo-serratinine (X)	240·5	349 → 290	241·0	66%
13-Acetyl-8(15)-anhydro-serratinine (XXIII)	169·0	275 → 216	169·7	29%
13-Acetyl-8(15)-anhydro-6,7-dehydroserratinine (XI)	168·0	273 → 214	167·8	base peak
8-Acetyl-13-dehydro-serratinine (XII)	185·1	291 → 232	185·0	base peak

(an ion, E; R_2 = H) which can be considered to arise by loss of a hydroxyl radical from the M^+-28 ion although its intensity is rather weak (Table 8).

So far, we have directed our attention only to the fragmentation processes in which the C_{13} hydroxyl group or the C_{13} acetoxyl group participates in the fragmentation. In order to inspect the fragmentation pattern in which the C_8 hydroxyl or the C_8 acetoxyl group participates, the spectra of 8-acetyl-13-dehydroserratinine (XII)

TABLE 8. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → M^+-45 FRAGMENTATION IN THE SPECTRA OF SERRATININE (I) AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}	Intensity of M^+-45/ base peak
		M^+-28 M^+-45		
8-Deoxyserratinine (XV)	not obs	235 → 218	202·2	3%
8-Acetylserratinine (VI)	not obs	293 → 276	260·0	2%
8(15)-Anhydroserratinine (XVII)	200·3	233 → 216	200·2	4%
Serratinine (I)	218·0	251 → 234	218·2	12·5%

and 8-acetylserratinine (VI) were examined These two compounds, especially the former, exhibit the ion at M^+-87 in their spectra, this peak appearing as a base peak. This peak may directly result from M^+-28 ion and this fragmentation is supported by the presence of metastable peak. On the other hand, the compound possessing the C_8 hydroxyl group shows the peak at M^+-45, although its intensity is rather weak. These results indicate that the most probable structure for M^+-87 or M^+-45 which is formed by loss of an acetoxyl radical or a hydroxyl radical at C_8, respectively, is depicted by the formula (F; Scheme 1). This fragmentation pattern seems to operate to a considerable extent in the derivatives possessing the acetoxyl group at the C_8 position.

In serratinine derivatives possessing two oxygen functions at both C_8 and C_{13}, the two fragmentations discussed [(b) and (c) process in Scheme 1] will occur simultaneously and this situation is revealed in the mass spectrum of 13-acetyl-8-benzoylserratinine (XXIV). This is shown in Fig. 3 where besides three peaks at M^+-28 (*m/e* 397), *m/e* 194 (D) and *m/e* 152 (G), both M^+-87 (*m/e* 338, E, a base peak) associated with the (b) fragmentation and M^+-149 (*m/e* 276, F) arising by loss of benzoyloxy radical from M^+-28 ion through the (c) fragmentation are observed. Metastable

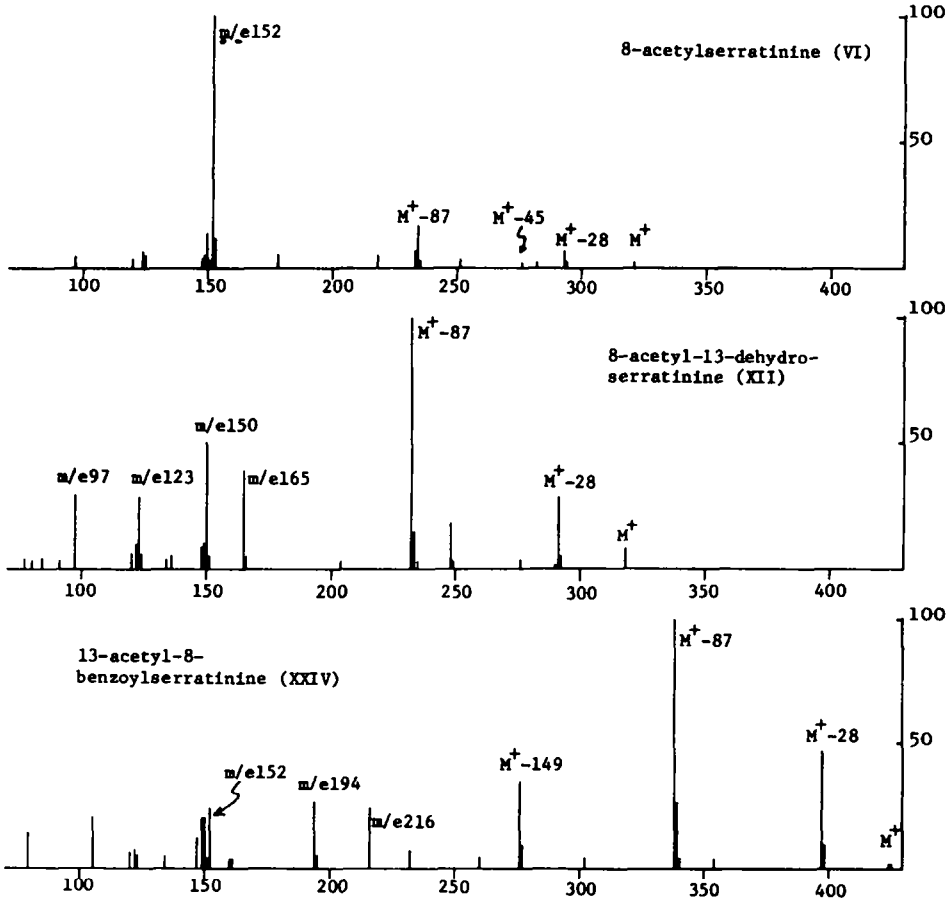
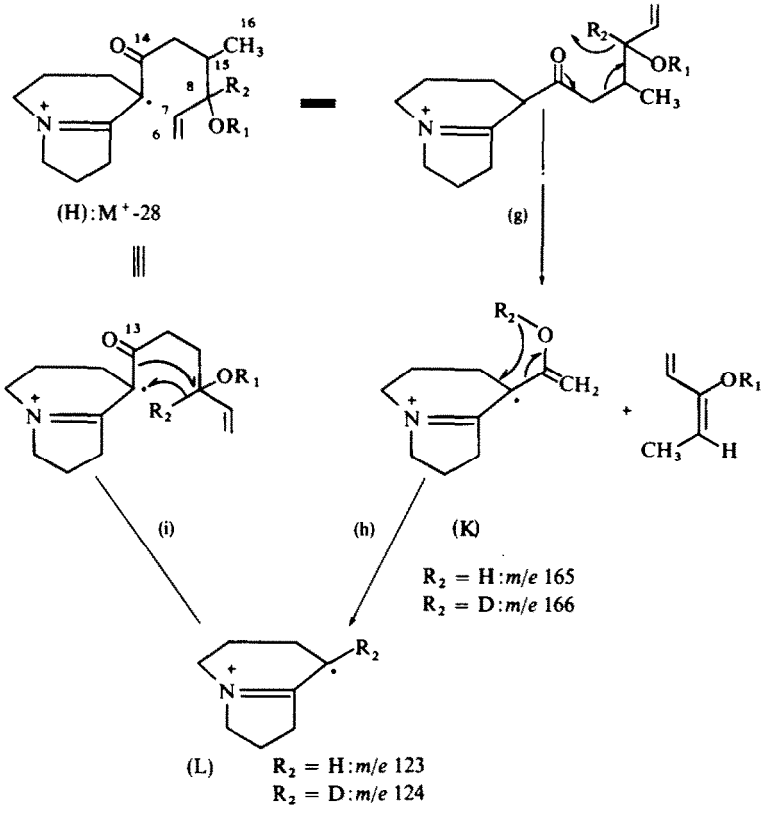


FIG. 3.

peaks are observed at *m/e* 288·0 and 192·5, corresponding, respectively, to the transformation *m/e* 397 → *m/e* 338 (m* Calc. = 287·8) and *m/e* 397 → *m/e* 276 (m* Calc. = 191·9). A relatively intense peak at *m/e* 216 may be formed concurrently by loss of benzoic acid from M^+-87 (*m/e* 338) and loss of acetic acid from M^+-149 (*m/e* 276), respectively. These mechanisms are supported by the presence of metastable peaks at *m/e* 137·8 (m*, *m/e* 338 → *m/e* 216 = 138·0) and *m/e* 169·0 (m*, *m/e* 276 → *m/e* 216 = 169·0).

Thus, all strong peaks appearing in the spectrum of compound (XXIV) are now interpreted on the basis of the fragmentation mechanisms discussed.

Other fragmentation sequences which occur in some 13-dehydroserratinine derivatives will be considered next.



SCHEME 4

In the spectrum of 13-dehydroserratinine (III) (Fig. 1), three relatively intense peaks at *m/e* 165, *m/e* 123 (a base peak) and *m/e* 97 together with the peaks associated with the transformation stated heretofore, are observed. Among these peaks, the geneses of the peaks at *m/e* 165 and *m/e* 123 can be accounted for by the fragmentation shown in Scheme 4. The fragment ion at *m/e* 165 (an ion K) may arise by McLafferty rearrangement and loss of the C_6, C_7, C_8 and C_{15} (C_{16}) atoms together with their attached substituents from M^+-28 (an ion H) and the resulting ion in turn loses the

ketene molecule to afford an ion at *m/e* 123 (an ion L). Unfortunately, the metastable peaks corresponding to these two fragmentations, especially the first step, are not always detected (Table 9). However, the participation of the hydrogen atom attached to the C_8 atom in these transformations was proved by the following deuterium-labelling experiment. Thus, in the spectrum of 8-d_1-8-acetyl-13-dehydroserratinine (XXIX), the peaks concerned are shifted one mass unit above the corresponding peaks, each appear at *m/e* 166 and *m/e* 124.

TABLE 9. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → *m/e* 165 → *m/e* 123 FRAGMENTATIONS IN THE SPECTRA OF 13-DEHYDROSERRATININE AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}
13-Dehydroserratinine (III)	not obs	249 → 165	109·3
	91·5	165 → 123	91·7
13-Dehydro-8-deoxyserratinine (XIII)	not obs	233 → 165	116·8
	91·5	165 → 123	91·7
8-d_1-8-Acetyl-13-dehydroserratinine (XXIX)	94·0	292 → 166	94·3
	92·8	166 → 124	92·6
13-Dehydroserratine (XXVIII)	109·9	249 → 165	109·3
	91·8	165 → 123	91·7

With regard to the genesis of an ion at *m/e* 123 (an ion L), an alternative fragmentation process may be considered. In this the cleavage of the C_{12}—C_{13} bond is accompanied by the intramolecular transfer of the C_8 hydrogen atom in an M^+-28 ion leading directly to an ion *m/e* 123. In accord with this, a metastable peak corresponding to the transformation, M^+-28 → *m/e* 123, is actually observed in the spectrum of

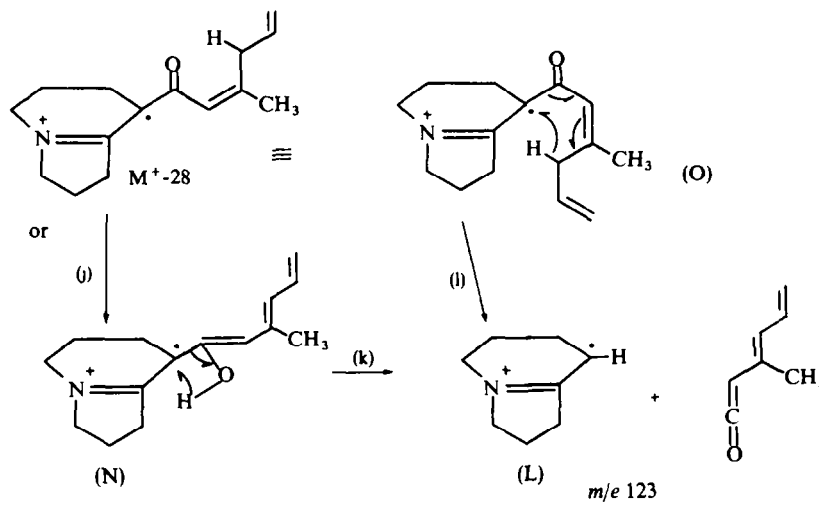
TABLE 10. METASTABLE PEAKS CORRESPONDING TO THE M^+-28 → *m/e* 123 (*m/e* 124) FRAGMENTATION IN THE SPECTRA OF 13-DEHYDROSERRATININE AND ITS DERIVATIVES

	m^*_{obs}		m^*_{calc}
		M^+-28	
13-Dehydroserratinine (III)	not obs	249 → 123	60·7
8-Acetyl-13-dehydroserratinine (XII)	not obs	291 → 123	52·0
13-Dehydro-8-deoxyserratinine (XIII)	65·0	233 → 123	64·9
8-d_1-8-Acetyl-13-dehydroserratinine (XXIX)	52·0	292 → 124	52·6
13-Dehydro-14(15)-anhydroserratine (XXVII)	65·5	231 → 123	65·5

13-dehydro-8-deoxyserratinine (XIII), (Table 10). Moreover, the spectrum of 13-dehydro-14(15)-anhydroserratine (XXVII) in which the fission of the C_{14}—C_{15} bond caused by McLafferty rearrangement is not likely because of its unsaturation, still exhibits an intense peak at *m/e* 123 (a base peak) although an intermediate ion (*m/e* 165) could not be detected (Scheme 5). These findings reveal that a direct mechanism takes part in the formation of the fragment ion at *m/e* 123.

The result of the analysis of the high-resolution spectrum shows that the ion at *m/e* 97 is a singlet corresponding in composition to $C_6H_{11}N$ but its structure and genesis have not been settled yet.

In summary, the most characteristic feature of the mass spectra of serratinine and its derivatives leaving the original ketone group at the C_5 position intact, is the ready



SCHEME 5

loss of CO from the molecular ion to give a key intermediate (an ion C) which seeks further stabilization in a number of directions through decomposition.

The course of further decomposition from an ion C is mainly influenced by the two oxygen functions at both C_{13} and C_8 position, especially by the function at the former position. In the fragmentation of serratinine and its derivatives bearing a hydroxyl group at C_{13}, the cleavage of the C_{13}—C_{14} bond is the dominant feature and the resulting ion at *m/e* 152 (an ion D) is by far the most intense in the spectrum.

In the mass spectra of serratinine derivatives carrying an acetoxyl group at C_{13}, an ion at M^+-87 (an ion E) which arises by loss of an acetoxyl radical from a key intermediate is observed as a base peak together with the ion at *m/e* 194 (an ion D) being characteristic of this type of derivative.

Comparing the spectra of derivatives bearing an acetoxyl group at C_{13} with those of derivatives carrying an acetoxyl group at C_8, differences as well as similarities are found. The latter exhibit equally the peaks at M^+-87 and *m/e* 152 (a base peak) which are also observed in the spectra of the former. The differences, however, are as follows. The reasonable structure of the ion M^+-87 appearing in the latter compounds, is distinctly different from that of the ion in the spectra of the former compounds, although both ions have the same mass units as shown in the Scheme 1. Moreover, the peak at *m/e* 194 which in all cases is present in the spectra of the former compounds can not be detected in the spectra of the latter compounds.

There are other characteristic fragmentation sequences observed in some C_{13}-dehydroserratinine derivatives. Two characteristic peaks at *m/e* 165 (an ion K) and

m/e 123 (an ion L) are detected in the spectra of this type of derivative and their geneses and structures are shown in Schemes 4 and 5.

EXPERIMENTAL

All m.ps are uncorrected. TLC was performed on Silica gel G, and a soln of 1% $Ce(SO_4)_2$ in 10% H_2SO_4 as a detection reagent and a solvent system ($CHCl_3$–cyclohexane–diethylamine; 4:5:1) was employed.

Apparatus, methods and material. The low-resolution spectra were recorded on Hitachi mass spectrometer Model RMU 6C at an ionizing potential of 80 eV, an ion accelation voltage of 1800 V and at an evaporating temperature of 180–200°. Samples were injected directly into the ion source by using a vacuum lock system. All compounds used in this investigation were reported in the previous papers[6] except deuterium labelling compounds.

13-d_1-*Serratinine* (XIV). To a soln of 435 mg of 8-acetyl-13-dehydroserratinine (m.p. 96–97°) in 20 ml of abs EtOH was added little by little 114 mg of $NaBD_4$ under reflux for 4 hr. After adding a soln of 1 g of NaOH in 5 ml water to the reaction mixture, the mixture was heated for further 1 hr. A large quantity of water was then added and the mixture was extracted with $CHCl_3$. After drying over anhyd K_2CO_3, the solvent was evaporated off and the residue in $CHCl_3$ was chromatographed over alumina (17 g; Woelm, Neutral, Grade I). Elution of the column with EtOAc gave a solid mass which on recrystallizations from acetone furnished 317 mg of colorless prisms, m.p. 244–245°. On admixture with an authentic sample of serratinine, this substance showed a single spot on TLC.

13-d_1-8-*Acetylserratinine* (XVIII). A mixture of 135 mg of 13-d_1-serratinine, 2 ml of Ac_2O and 2 ml of pyridine kept on standing at room temp for 2 days. Then, the mixture was poured into water, basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over anhyd K_2CO_3, and evaporation of the solvent and pyridine left the residue which upon trituration with acetone afforded crystals. Recrystallizations from acetone afforded 130 mg of colorless needles, m.p. 244·5–245°. On admixture with an authentic sample of 8-acetylserratinine, this product showed a single spot on TLC.

13-d_1-8,13-*Diacetylserratinine* (XXVI). The mixture of 212 mg of XIV, 3 ml of Ac_2O and 3 ml of pyridine was heated at 100° for 3·5 hr. Excess of reagents was removed by distillation and water was added to the residue. The mixture was made alkaline with NH_4OH and extracted with $CHCl_3$. After drying over anhyd K_2CO_3, the solvent was distilled off and the residue in benzene was chromatographed on alumina (3 g; Woelm, Neutral, Grade III). Elution of the column with benzene afforded a solid mass which was recrystallized from n-hexane to give 202 mg of needles, m.p. 155–158°. On admixture with an authentic sample of 8,13-diacetylserratinine, this substance exhibited a single spot on TLC.

13-d_1-13-*Acetylserratinine* (XIX). A soln of 190 mg of 13-d_1-8,13-diacetylserratinine in 20 ml of 10% HClaq was heated on an oil bath at 130° under reflux for 1·5 hr. After cooling, the mixture was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extracts were combined, dried over anhyd K_2CO_3 and evaporated. The residue in benzene was then chromatographed on alumina (3 g; Woelm, Neutral, Grade II). Elution of the column with benzene and ether gave a solid mass which on recrystallization from benzene afforded 140 mg of cubical crystals. This substance showed a single spot on TLC plate on admixture with an authentic sample of 13-acetylserratinine.

8-d_1-8,13-*Diacetylserratinine* (XXV). To a soln of 420 mg of 8-dehydro-13-acetylserratinine (m.p. 187·5–188°) in 20 ml of abs EtOH was added little by little 114 mg of $NaBD_4$ under reflux for 3 hr. Decomposition of excess hydride was effected by addition of AcOH and the reaction mixture was made alkaline with 10% NaOHaq and heated for 15 min. Then, the reaction mixture was diluted with water and extracted with $CHCl_3$. After drying over anhyd K_2CO_3, the solvent was removed by evaporation. The residue was then dissolved in $CHCl_3$ and chromatographed on alumina (14·5 g; Woelm, Neutral, Grade II). Elution of the column with $CHCl_3$ gave 126 mg of the product. Recrystallizations from EtOAc afforded colorless prisms, m.p. 244–245° which on admixture with an authentic sample of serratinine showed a single spot on TLC.

Further elution of the column with EtOAc gave 121 mg of the crude 8-d_1-8-episerratinine which was recrystallized from EtOAc to give colorless needles, m.p. 235–237°. This base showed a single spot on TLC on admixture with an authentic sample of 8-episerratinine.

A mixture of 40 mg of 8-d_1-serratinine obtained above, 1 ml of Ac_2O and 1 ml of pyridine was heated at 100° for 2·5 hr. The reagents were evaporated off under reduced press and the residue was diluted with water. The aqueous soln was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ extract

was dried over anhyd K_2CO_3 and the solvent was evaporated off to leave the residue. The benzene soluble substance was obtained by reextraction of the residue with benzene and evaporation of benzene left the crude product which was recrystallized from n-hexane to give 40 mg of colorless needles, m.p. 157–158°. This substance showed a single spot on TLC on admixture with an authentic sample of 8,13-diacetylserratinine.

8-d_1-*Acetyl*-13-*dehydroserratinine* (XXIX). A soln of 86 mg of 8-d_1-serratinine dissolved in a mixture of 2 ml of Ac_2O and 2 ml of pyridine was kept standing at room temp for 2 days. After no starting material had been detected by TLC, the excess Ac_2O was decomposed by addition of ice-water. The mixture was made alkaline with NH_4OH and extracted with $CHCl_3$. After drying over anhyd K_2CO_3, the solvent and pyridine were removed by evaporation under reduced press. The residue was recrystallized several times from acetone to give 75 mg of colorless leaflets, m.p. 244–245°. This substance showed a single spot on TLC on admixture with an authentic sample of 8-acetylserratinine. To a soln 33 mg of 8-d_1-8-acetylserratinine in 2·3 ml of acetone was added 0·13 ml of freshly prepared Jones' reagent and the mixture was stirred at room temp for 40 min. Decomposition of excess oxidizing reagent was effected by addition of MeOH and then, a large quantity of water was added. The reaction mixture was basified with NH_4OH and extracted with ether and after drying over anhyd K_2CO_3, the solvent was evaporated off to leave the residue. Chromatography of the residue in benzene over alumina (900 mg; Woelm, Neutral, Grade III) and elution of the column with benzene afforded a crystalline mass which was recrystallized several times from n-hexane to give 25 mg of colorless prisms, m.p. 96–97°. This substance showed a single spot on TLC on admixture with an authentic sample of 8-acetyl-13-dehydroserratinine.

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