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DETERMINATION OF THE STRUCTURES OF IMPERIALINE, EDUARDINE, AND EDPETILIDINE BY MASS SPECTROSCOPY

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Imperialine has been isolated from Fritillaria imperialis, [1, 2], F. sipeimu [3], Petilium raddeana, and P. eduardi [4, 5], and eduardine and edpetilidine have been isolated from P. eduardi [6, 7].

A structural formula has been proposed for imperialine [8-11] in which the position of the tertiary hydroxy group was not determined.

The mass spectra of imperialine (Fig. 1), imperialone, and deoxodihydroimperialone have the peaks of ions with m/e 98, 111, 112, 124, 125, 140, 150, 154, 155, 156, 162, 164, 180, 217, 234, 235, 236, $(M-71)^+$, $(M-57)^+$, $(M-45)^+$, $(M-43)^+$ (in imperialine and imperialone); $(M-47)^+$ (in deoxodihydroimperialone); $(M-18)^+$, $(M-15)^+$, and (M^+).

By comparing the spectra of imperialine and its conversion products with the spectra of alkaloids of known structure (zygacine [12] and isoverticine [13]) we have established, in addition to the ion peaks with m/e 98 and 112 that have been described for modified steroid alkaloids, characteristic peaks with m/e 124, 125, 138, 140, 150, 154, 155, 156, 162, and 164. In the spectrum of zygacine, the peak m/e 125 is strong and in the spectrum of imperialine, its conversion products, and isoverticine the peak with m/e 124 is strong. The spectrum of zygacine has a peak with m/e 138 and no peak with m/e 140; the spectrum of imperialine, its conversion products, and isoverticine have peaks with m/e

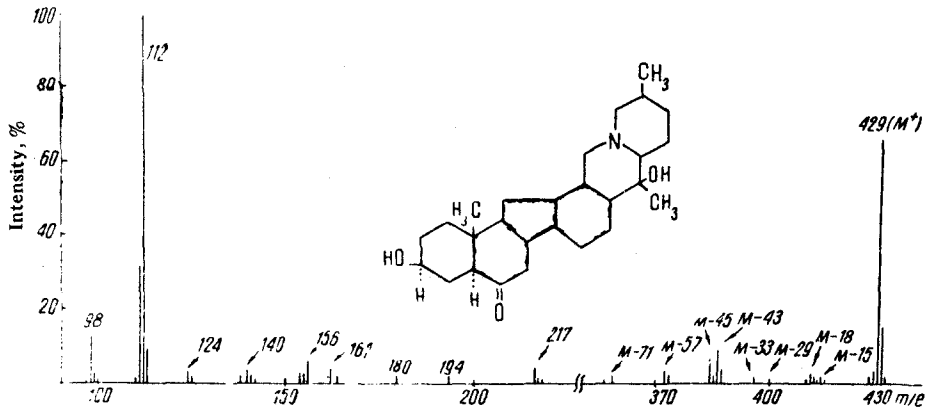
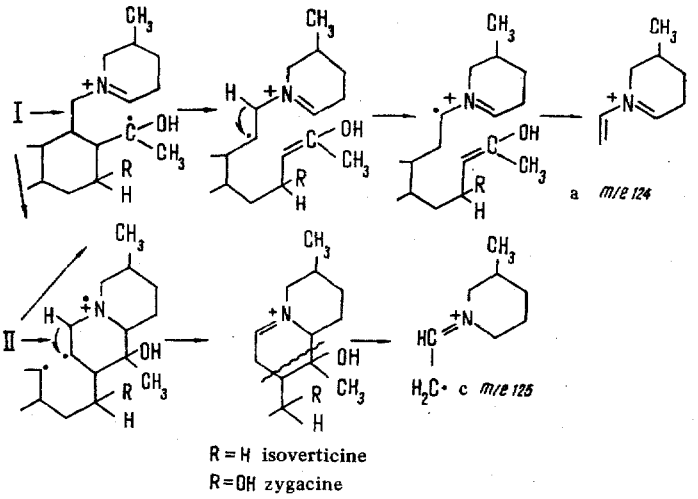


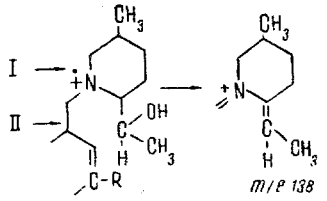
Fig. 1. Mass spectrum of imperialine.

138 and 140, the m/e 140 peak being the stronger. In the spectrum of zygacine, the peaks with m/e 155 and 156 are less strong and in the spectrum of isoverticine there is a peak with m/e 156. The spectra of imperialine and its conversion products exhibit peaks with m/e 154, 155, and 156. This difference in the peaks is apparently due to a steric difference of the parts of the molecule from which these ions are produced. Thus, the ions mentioned are formed from the structurally similar parts of the molecules of zygacine and imperialine, i.e., from rings E and F. We propose the most probable structure for the ions of zygacine and isoverticine with m/e 124, 125, 138, and 154.

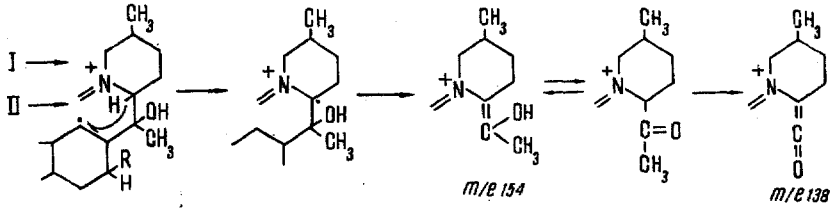
As a result of α- and β-cleavages of the molecular ion of zygacine (I) and isoverticine (II), a hydrogen atom migrates from C-18 to C-13, β-cleavage takes place, and an ion with m/e 124 (a) appears. β-Cleavage, migration of hydrogen from C-18 to C-13, α, β-cleavages, and the displacement of the hydrogen atom from the hydroxyl at C-22 lead to the formation of an ion with m/e 125 (c).



β-Cleavage of a bond with the migration of the hydrogen atom from C-16 to C-20 gives an unstable ion which, after dehydration and α-cleavage, becomes stabilized with the formation of an ion with m/e 138.



A fragment with m/e 154 (b) is obtained from the molecular ion as a result of α,α- and β-cleavages. The splitting out of a methyl radical and hydrogen from the fragment with m/e 154 (b) likewise leads to a fragment with m/e 138.



The characteristic peaks of ions with m/e 154, 155, and 156 present in the spectra of imperialine and its conversion products can be formed only if a hydroxy group is located at C-20. Consequently, structural formula (III) can be put forward for imperialine.

The results of a study of the spectra of imperialine (III), imperialone (IV), and deoxodihydroimperialone (V) support and confirm the structural formulas proposed for them and also permit the direction of their decomposition to be determined. In these compounds decomposition takes place by a single mechanism and they form almost similar ions with respect to mass number and intensity in the range of m/e values from 98 to 234. On the basis of this fact it may be stated that rings A and B do not participate in the production of such fragments. Consequently, no shifts by 2 or 16 mass units are found in these fragments.

The ions $(M-71)^+$– M^+ of imperialine and its conversion products are formed by the splitting off of similar molecular fragments. Here there are changes in the mass numbers by 2 or 16 units depending on the molecule of the compounds.

The fragments with m/e 98 and 112 from imperialine, imperialone, and deoxodihydroimperialone are obtained by the known scheme that has been described for zygacine. The ions with m/e 124 and 154 are also formed by the

scheme of decomposition proposed by us for zygacine and isoverticine. The fragment with m/e 111 appears as a result of α, α-cleavages without migration of a hydrogen atom in the manner of the formation of the fragment with m/e 112.

The ion with m/e 155 is obtained by α- and β-cleavages, the displacement of a hydrogen atom from C-16 to C-20 leading to a fragment with m/e 156. In the ion with m/e 155, after the migration of a hydrogen atom from the α-position to the β-position, a methyl group splits off and an ion with m/e 140 is formed.

The fragment with m/e 150 is apparently formed in the same way as the ion with m/e 180 with the subsequent splitting out of a methyl group from C-25 and migration of the hydrogen from C-22 to this carbon atom with the simultaneous splitting out of the methyl group from C-20. α-, β-, and γ-cleavages of the molecular ions lead to the appearance of a fragment with m/e 180 which, after the loss of water, forms an ion with m/e 162. After stepwise α-, γ-, and δ-cleavages, the dehydration ion gives a fragment with m/e 217. Similarly, fragmentation of the molecular ion gives a fragment with m/e 235.

In the spectra of imperialine, imperialone, and deoxodihydroimperialone, the peak of the $(M-57)^+$ ion can arise by the decomposition of rings A and F. Of the quinolizidine alkaloids, isosparteine [14], which has two rings (A and D) the decomposition of which does not differ markedly from the decomposition of ring F of imperialine and its conversion products, forms by α- and α-cleavages [sic] an intense peak of an ion $(M-41)^+$ and a comparatively very small peak of an ion $(M-43)^+$. The main fragment $(M-41)^+$ on decomposition in a hydrogen acceptor, and the radical of the hydrocarbon is a donor.

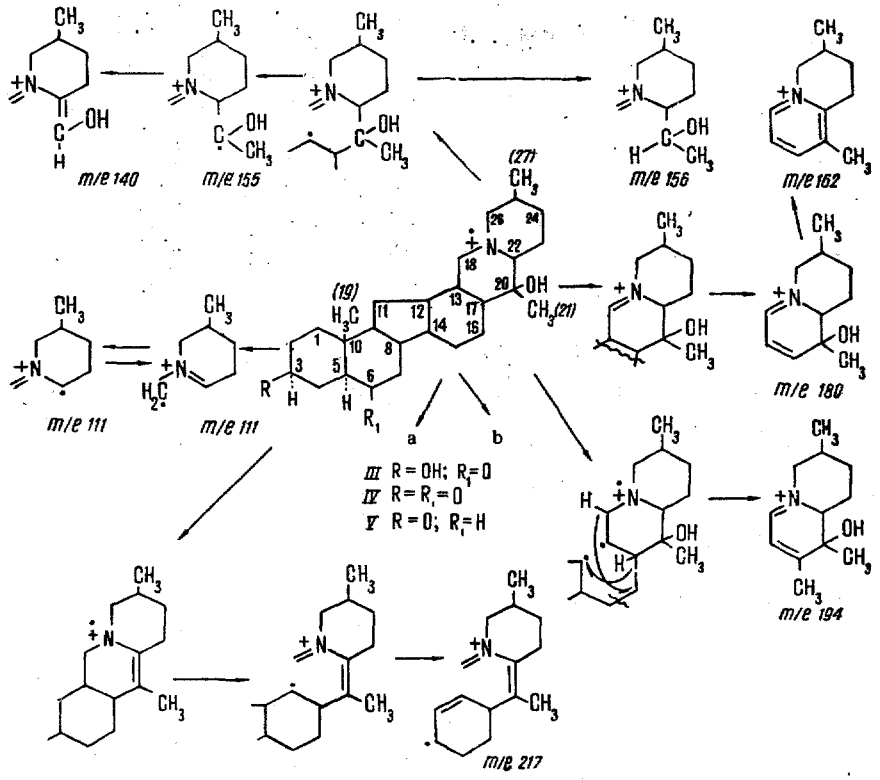
In the analogous decomposition of imperialine and its conversion products, a strong peak of the ion $(M-55)^+$ and a weak peak of the ion $(M-57)^+$ must be formed. In the mass spectrum, the peak of the ion $(M-27)^+$ is strong while the peak of the ion $(M-55)^+$ has a very low intensity. This shows that the fragment of the ion $(M-57)^+$ can appear through the decomposition of ring A with a carbonyl or hydroxy group present in position 3.

α-Cleavage between C-3 and C-4 and migration of the hydrogen atom from C-5 to C-3 following the α-cleavage because of the loss of a formyl group, leads to the formation of an ion $(M-29)^+$; the splitting off from the latter of ethylene leads to the fragment $(M-57)^+$ (in imperialone and deoxodihydroimperialone). The ion $(M-29)^+$ can also be obtained by the splitting off of an ethyl radical from ring F. As a result of α- and γ-cleavages and the migration of hydrogen from C-2 to C-4, imperialine forms an ion $(M-57)^+$, and α- and β-cleavages and the migration of hydrogen from C-5 to C-2 (in imperialone and deoxodihydroimperialone) and from C-4 to C-7 (in imperialine) give the ion $(M-43)^+$. Fragments with such mass numbers can be formed by the detachment of a propyl radical from ring F or an acetyl group from ring E. The spectrum of deoxodihydroimperialone has no peak of an ion $(M-29)^+$ and, in contrast to the spectra of imperialine and imperialone, has the peak of an ion $(M-47)^+$. The ion $(M-47)^+$ apparently corresponds to the fragment obtained by the detachment of a formyl residue from the dehydration ion. The ion $(M-33)^+$ is formed from the dehydration ion by the splitting out of one of the methyl groups, the ion $(M-18)^+$ by the splitting out of a hydroxy group in the form of water, and the ion $(M-15)^+$ after the elimination of a methyl group. The ion $(M-45)^+$ is formed by the splitting out of a methyl carbinol radical.

In spite of the presence in imperialone and deoxodihydroimperialone of a carbonyl group at C-3, the peaks of the ions $(M-55)^+$ and $(M-69)^+$ in their spectra are very weak. Here there is no loss of a fragment from the molecular ion, and the fragment is possibly eliminated from the dehydration ion. As a result, a peak of an ion $(M-73)^+$ is formed $(M-18-55)^+ = (M-73)^+$.

The proposed formula (III) is confirmed by the results of a study of the NMR spectra and its acetyl derivative. The NMR spectrum of imperialine has signals from the chemically equivalent protons of methyl groups: a singlet at 9.32 τ (3H, C-19 methyl group), a doublet at 900 τ (3H, C-27 methyl group), and a singlet at 9.01 τ (3H, C-21 methyl group) [16]. The NMR spectrum of acetylimperialine has a singlet at 9.3 τ (3H, C-19 methyl group), a singlet at 9.01 τ (3H, C-21 methyl group), a doublet at 9.00 τ (3H, C-27 methyl group) [15], and a singlet at 8.05 τ (3H, O-acetyl group).

In a routine study of the NMR spectra of the acetyl derivatives of the alkaloids of Veratrum it was found that in the group $HO-\underset{|}{C}(20)-CH_3(21)$ the protons of the C-21 methyl group have signals between 8.50 and 9 τ depending on the substituting groups [15]. Thus, in the NMR spectra of imperialine and its acetyl derivative, the singlets with a value of 9.01 τ confirm that the tertiary hydroxy groups and the methyl groups are located at C-20. In the spectrum of acetylimperialine there is a signal at 5.4 τ (C-3α H) [16, 17]. Consequently the C-3 OH has the β-configuration. In the NMR spectrum of imperialine the singlet at 9.32 τ is shifted after the introduction of an acetyl group into the molecule in the direction of weak fields, to 9.3 τ. This shows yet again that the secondary hydroxy group of imperialine [18] possesses the C-3β OH configuration.



Edpetilidine $C_{27}H_{45}O_2N$ and eduardine $C_{27}H_{43}O_2N$ each have three C-methyl groups, are not methylated by the Hess reaction, and do not contain N-methyl groups; they are tertiary bases.

The IR spectrum of edpetilidine has an absorption band of a hydroxy group (3425 cm^{-1}) and no absorption band of a carbonyl group and of an O bond. This shows that both oxygen atoms in this base are present in the form of hydroxy groups. The IR spectrum of eduardine shows absorption bands at 3530 and 1700 cm^{-1}. Consequently, in this case one oxygen atom is in the form of a hydroxy group and the second in the form of a carbonyl group. In actual fact, when edpetilidine is oxidized with chromic acid eduardine is obtained, and the reduction of eduardine gives edpetilidine. Thus, a structural relationship between these alkaloids and the presence of a tertiary hydroxy group in each of them has been established. The absorption curve of eduardine in the UV region of the spectrum has λ_{max} 290 mμ (log ε 1.7); it is identical with the corresponding absorption curve of imperialine [19].

The composition of the alkaloids mentioned (with 27 carbon atoms in the molecule), the presence of three C-methyl groups, the shape of the curve and the absorption maximum in the UV spectrum (eduardine), and also the characteristic peaks of ions with m/e 98, 111, and 112 [12] permit the assumption that edpetilidine and eduardine contain the heterocyclic skeleton of imperialine [8-10].

The results of a direct comparison of eduardine with deoxodihydroimperialone and of edpetilidine with deoxodihydroimperialine [10] shows that they are structurally different. The mass spectrum of edpetilidine has the principal characteristic peaks with m/e 98, 111, 112, 124, 139, 150, 164, 178, 218, $(M-71)^+$, $(M-70)^+$, $(M-57)^+$, $(M-56)^+$, $(M-47)^+$, $(M-45)^+$, $(M-43)^+$, $(M-19)^+$, $(M-18)^+$, $(M-15)^+$, 415 (M^+) (Fig. 2), and the mass spectrum of eduardine peaks with m/e 98, 111, 112, 124, 139, 150, 164, 178, 218, $(M-70)^+$, $(M-69)^+$, $(M-56)^+$, $(M-55)^+$, $(M-29)^+$, $(M-18)^+$, $(M-15)^+$, 413 (M^+). From the data given, it can be seen that certain ions of these two compounds, $(M-70)^+$, $(M-29)^+$, $(M-18)^+$, $(M-15)^+$, M^+, differ from one another by two units; they are formed by the splitting out of a structurally similar part of the molecule. The residual ions are formed with the retention of similar parts of the molecule. Moreover, the spectra differ in the intensities of the peaks. In eduardine, M^+ has the maximum intensity and in edpetilidine the peak with m/e 111. Furthermore, a characteristic difference between the spectra of eduardine and edpetilidine and those of imperialine and its derivatives has been noted. The spectra of eduardine and edpetilidine do not exhibit peaks of ions of m/e 154, 155, and 156, which shows the absence of a hydroxy group at C-20 in edpetilidine and eduardine, while the formation of a fragment with m/e 218 indicates that the hydroxy and carbonyl groups are not present in rings D, E, and F.

In the spectra of imperialine, imperialone, deoxodihydroimperialone, and zygacine, the peak of the ion with

m/e 112 has the maximum intensity, while in the spectra of eduardine and edpetilidine it is the peak of the ion with m/e 111. This is explained by the comparative difficulty of the migration of the hydrogen atom to the ion with m/e 111 formed in the decomposition of eduardine and edpetilidine.

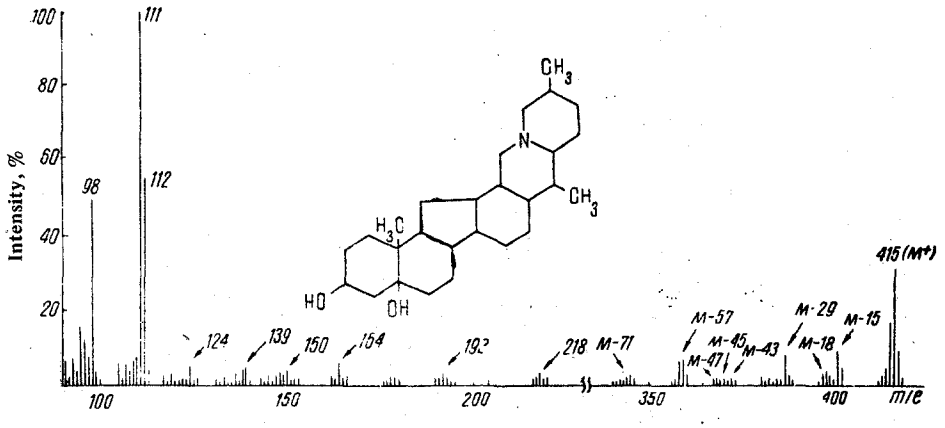


Fig. 2. Mass spectrum of edpetilidine.

Apparently, the ion with m/e 112 is formed from imperialine, zygacine, and isoverticine by the migration of the hydrogen from the hydroxyl more easily than from C-17 [12]. The absence of a hydroxy group at C-20 in edpetilidine and eduardine leads to an intense ion with m/e 111. The spectrum of edpetilidine has peaks of the ions $(M-71)^+$, $(M-70)^+$, $(M-57)^+$, and $(M-56)^+$. The $(M-70)^+$ fragment is obtained by the rupture of the F ring through C—N— and α-cleavages, and the $(M-56)^+$ fragment through α, α-cleavages. By comparing the characteristic peaks of the ions of eduardine $(M-69)^+$ and $(M-55)^+$ with the peaks of the ions $(M-71)^+$ and $(M-57)^+$ from edpetilidine it can be seen that the formation of the first two ions takes place through the loss of carbonyl-containing and two sec-hydroxyl-containing fragments.

The corresponding fragments can be formed where the secondary hydroxyl group of edpetilidine and the carbonyl group of eduardine are present at C-3. Positions at C-5, C-8, or C-9 remain for the tertiary hydroxy group. The results of a consideration of the biogenesis of these alkaloids enables us to assume that this group is located at C-5.

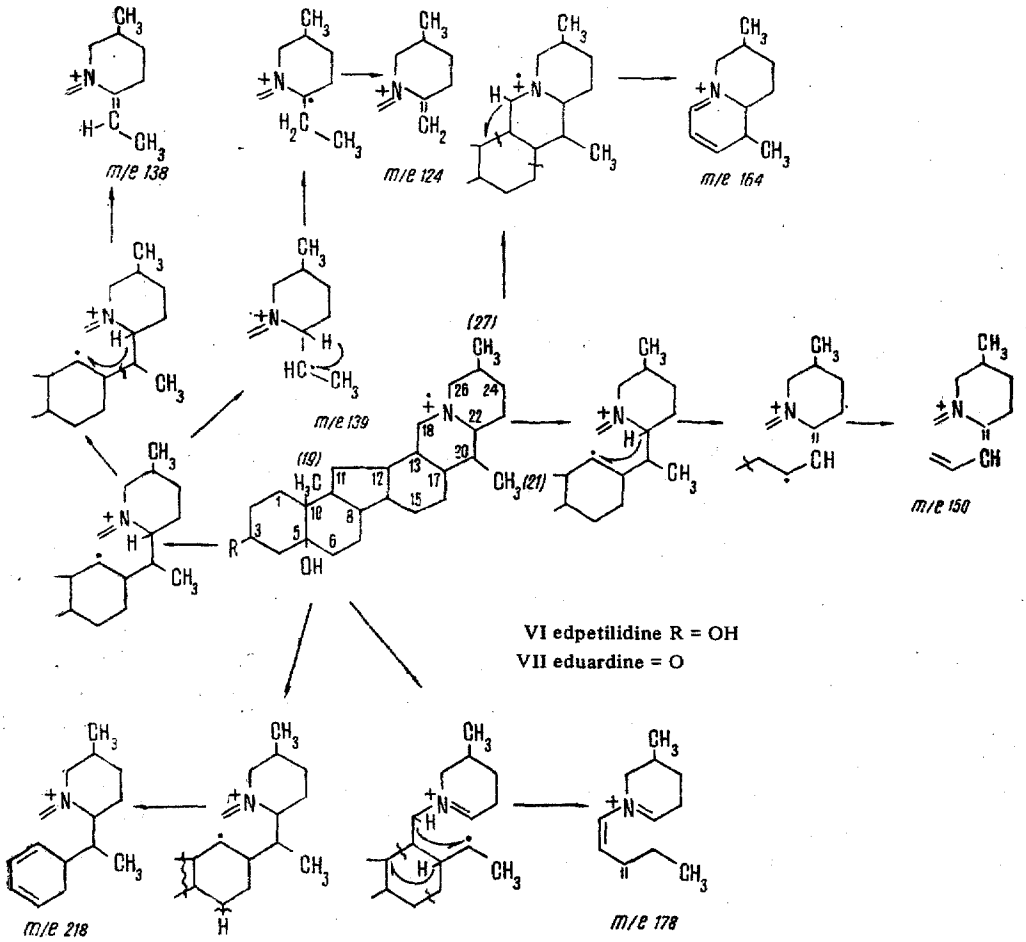
Consequently, (VI) and (VII), respectively, may be regarded as the most probable structures for edpetilidine and eduardine. They are confirmed by the fragments formed in the decomposition of these substances.

The fragments of the ions with m/e 98, 111, and 112 are obtained by the mechanism of bond cleavage in imperialine. α- and β-Cleavages lead to an ion with m/e 139; the splitting off of hydrogen forms a fragment with m/e 138. The fragment of the ion with m/e 139 loses a methyl group to give an ion with m/e 124. An ion with this mass number can be obtained by the mechanism for the fragmentation of imperialine.

A fragment with m/e 150 is formed by α, α-cleavages of the bond taking place after the loss of the methyl group at C-20 and subsequent cleavages in ring D. The fragment with this mass number from the molecular ions of imperialine, its conversion products, edpetilidine, and eduardine may also be formed by the rupture of the bond between C-20 and C-22, the migration of hydrogen from C-18 to C-20, and further ruptures of the bonds between C-13 and C-17, C-12 and C-14, and C-9 and C-11. The ion with m/e 164 is obtained from the molecular ion by the same mechanism as the fragment with m/e 180 from imperialine. α-, γ-, and δ-Cleavages of the bonds and stabilization of the ions formed by the splitting off of hydrogen from C-15 give the fragment with m/e 218. Cleavages of the bonds between C-3 and C-4 and between C-1 and C-10 and the migration of a hydrogen atom from C-2 to C-4 lead to the production of a fragment with m/e 358 $(M-57)^+$ (in edpetilidine) and an ion with m/e 358 $(M-55)^+$ (in eduardine). In the spectra of edpetilidine and eduardine, the peaks of the ions $(M-47)^+$, $(M-45)^+$, and $(M-43)^+$ have a very low intensity. In both cases the ion (M-29) is formed after the elimination of an ethyl radical from ring F. The fragment $(M-18)^+$ is obtained after the elimination of the tertiary hydroxy group in the form of water, and the fragment $(M-15)^+$ by the loss of a methyl group.

The results of a comparative study of the NMR spectrum of eduardine also confirm the structural formula proposed for it. The NMR spectrum has the following signals: a doublet at 9.02 τ (3H, C-17 methyl group), a doublet at 9.37 τ (3H, C-21 methyl group), and a singlet at 9.3 τ (3H, C-19 methyl group) [15]. The electron densities of the protons of the C-19 methyl group in eduardine and acetylimperialine are similar. In eduardine, this chemical shift is due to

the presence of a carbonyl group in position 3 and the C-5 OH group.



Experimental

Imperialine, edpetilidine, and eduardine were isolated from P. eduardi [4, 7]. Imperialone and deoxodihydroimperialone were obtained from imperialine by a published method [10].

Oxidation of edpetilidine. A mixture of 1 g of edpetilidine and 0.5 g of chromic anhydride in 28 ml of acetic acid was heated at 80° C for 30 min. The solvent was evaporated in vacuum, and the residue was dissolved in water, made alkaline with sodium carbonate solution, and extracted with chloroform. After the chloroform had been distilled off, the base was dissolved in 5% sulfuric acid, made alkaline with ammonia, and extracted with ether.

The crystals from the ethereal solution were recrystallized from ethanol, mp 247°–251° C, $[\alpha]_D$ −54.77° (c 0.603; methanol). Yield 0.7 g. UV spectrum (in ethanol): λ_{max} 290 mμ (log ε 1.7).

Found, %: C 78.7; H 10.8, 10.70; N 3.44, 3.46. Calculated for $C_{27}H_{43}O_2N$, %: C 78.39; H 10.48; N 3.33.

A mixture of the base obtained (mp 247°–251° C) with eduardine melted at 247°–251° C.

Reduction of eduardine. In 20 ml of 0.5% hydrochloric acid in the presence of 0.034 g of platinum catalyst, 0.1 g of eduardine was shaken in an atmosphere of hydrogen, 5.5 ml of hydrogen being absorbed. The acid solution was made alkaline with ammonia and the reaction product was extracted with ether. The crystals isolated had mp 227°–228° C.

The IR spectra were taken by a UR-10 double-beam spectrophotometer with the substance in the form of molded KBr tablets, and the UV spectra in ethanolic solution were taken by a SF-4 spectrophotometer.

The mass spectra were recorded in a MKh-1303 mass spectrometer with a glass inlet device at 35–50 eV and 50–70 Ma, and the NMR spectra in deuterochloroform in a JNM-4H-100 instrument. Tetramethylsilane was used as the internal standard.

Summary

1. The mass spectra of imperialine, imperialone, deoxodihydroimperialone, eduardine, edpetilidine, zygacine, and isoverticine have been studied and fragmentation schemes for them have been proposed.

2. The structural formulae of imperialine, eduardine, and edpetilidine have been established.

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