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THE DITERPENE ALKALOIDS: THE STRUCTURE AND STEREOCHEMISTRY OF HETERATISINE

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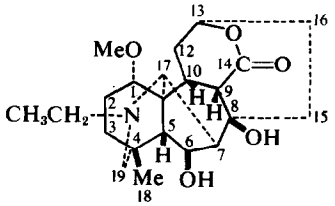
Abstract—Chemical and spectroscopic studies have shown that heteratisine ($C_{22}H_{33}NO_5$), a diterpene lactone alkaloid occurring as a monobenzoyl ester in the roots of *Aconitum heterophyllum* Wall, has stereostructure **1**. Heteratisine thus bears a closer structural relationship to the highly toxic and polyoxygenated alkaloids of the lycoctonine–aconitine type than to its companion bases of *A. heterophyllum*, atisine, atidine and hetisine. An unusual cleavage reaction occurring when 19-oxodehydroheteratisine (**28**) is treated with strong base is described.

Several structurally and biogenetically related alkaloids have been isolated from plants belonging to the *Aconitum*, *Delphinium*, and *Garrya*[3,4] genera. Among these "Aconite" or "diterpene" alkaloids, heteratisine ($C_{22}H_{33}NO_5$) has been known for almost thirty years and until recently occupied a unique position since its basic skeleton incorporates a lactone ring. Very recently, other lactone-type diterpene alkaloids have been isolated and studied.[5] In this paper, we present the details of our studies which led us to propose structure (**1**) for heteratisine.[6,7]



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Isolation and characterization

Heteratisine was first isolated 30 years ago from the dried roots of *Aconitum heterophyllum* Wall.[8] It has not been detected in any other plant. Its monobenzoyl ester has been isolated also and heteratisine may be an artefact formed during isolation. In the original isolation procedure the major alkaloid, atisine, was first removed by crystallization of the total alkaloids (as hydrochlorides), and heteratisine was obtained from the mother liquors by fractional crystallization (as the free bases). However, it is preferable to divide the total alkaloids into the strong and weak base fractions and to remove the lactonic material from the weak base fraction as an aqueous alkali solution. Details of the improved isolation procedure have been presented elsewhere.[9] A yield of 0·03% heteratisine was obtained from one batch of dried *A. heterophyllum* roots. The analytical sample was obtained from ethanol solution as colorless crystals, which were monoclinic with space group $P2_1$ (a = 13·62 Å, b = 10·65 Å, c = 8·89 Å, β = 133° 18′), m.p. 267–269° (d), $[\alpha]_D = +40°$ (c 1·0, MeOH).[10] The m.p. of heteratisine and several of its derivatives vary considerably with slight differences in the manner of determination and indeed for several derivatives mesomorphic transitions may be observed before final melting. The specific rotations also show a relatively large variation with solvent and concentration.

The functional groups

Some of the functional groups of heteratisine were recognized by Jacobs *et al.*[8] by conventional analytical determinations. These were a lactone ring (behaviour towards alkali), two OH groups (Zerewitinoff), one OMe (Ziesel) and one N-Et group (Herzig–Meyer). Unsaturation susceptible to catalytic hydrogenation was shown to be absent. These results were confirmed and corroborative evidence was provided in the present study by the spectral data (Tables 1 and 2) of heteratisine. The support necessary for some of the assignments made in Tables 1 and 2 was obtained from studies on several derivatives of heteratisine, presented later in the text.

Heteratisine is insoluble in sodium bicarbonate solution, but dissolves slowly in hot aqueous-alcoholic sodium hydroxide. Acidification of this alkali solution regenerates heteratisine; evaporation of the alkali solution to dryness yields a solid residue with IR absorption in the carbonyl stretching region at 1567 cm^{-1}, corresponding to the carboxylate ion of the opened lactone ring.

Treatment of heteratisine with acetic anhydride–pyridine yields a crystalline mono-*O*-acetyl derivative (ν 1714 cm^{-1}; δ = 2·06, 3H singlet);

Table 1. IR absorption bands of heteratisine and its derivatives

	Heteratisine	Heteratisine monoacetate	19-Oxo-heteratisine acetate	Oxo-heteratisine	19-Oxo-dehydro-heteratisine	N-desethyl-dehydro-heteratisine	N-desethyl-dehydro-heteratisine · HCl	Dehydro-heteratisine	γ-lactone carboxylic acid methyl ester
OH	3460, 3401	3395	3436	3460, 3205	3534	3195	3571, 3311	3472	
δ-Lactone	1738	1746	1739	1730	1733	1739	1742	1732	
γ-Lactone									1787
—$OCOCH_3$		1714	1739			1739	1742		
δ-Lactam			1625	1609	1626				1648
Cyclopentanone					1748			1750	1748
>C=N—						1642			
$>C=\overset{+}{N}H$							2558, 2123, 1923, 1644		
—CO_2Me									1728
—NH						3195			

Table 2. NMR absorption bands of heteratisine and its derivatives

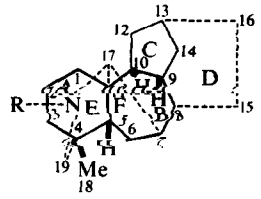
	No. of H	Heteratisine	Heteratisine monoacetate	19-Oxo-heteratisine acetate	19-Oxodehydro-heteratisine	Dehydro-heteratisine	N-Desethyl-dehydro-heteratisine	γ-Lactone carboxylic acid methyl ester	19-Oxo-heteratisine
$C\underline{H}_3C(4)$	3	0·97 s	0·85 s	1·22 s	1·22 s	0·94 s	1·20 s	1·17 s	1·18 s
$C\underline{H}_3CH_2N$	3	1·02 t (7·5)	1·03 t (7)	1·12 t (7)	1·17 t (7)	1·10 t (7·5)		1·22 t (7)	1·12 t
$C\underline{H}_3O$—C(1)	3	3·25 s	3·26 s	3·23 s	3·32 s	3·33 s	3·23 s	3·33 s	3·18 s
H—C(17)N	1	3·49 d (2)	3·58 d (2)	3·83 d (2·5)	4·09 d (2·5)	3·85 d (1·5)	4·4 m		
H—C(9)—CO—O	1	4·03							
H—C(13)—OCO	1	4·74 m	4·72 m	4·78 m	4·75 m	4·78 m	4·9 m		
H—C(6)OH	1	4·5 m							
H—C(6)OAc	1		5·29 q	5·23 q					
C(6)—$OCOC\underline{H}_3$	3		2·06 s	2·06 s			2·08 s		
H—C(17)=N	1						7·38 m		
H—C—OMe	1				3·37–3·98 m				
H_3COCO	3							6·27 s	

Note: Chemical shift values are in p.p.m. with TMS reference taken as 0·0. s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. J values are given in the brackets.

a secondary OH group is acetylated since a one-proton multiplet at $\delta = 4{\cdot}5$ (H—C—OH) in heteratisine emerges downfield as a quartet at $\delta = 5{\cdot}29$ (H—C—OAc). A second OH escapes acetylation (ν 3395 cm^{-1}) and is presumably tertiary. Parallel results are obtained on benzoylation and identical conclusions are reached from oxidation of heteratisine to dehydroheteratisine. These are discussed in the sequel. Furthermore, since heteratisine mono-O-acetate is basic, the N atom which carries only one alkyl (Et) group (see above), is tertiary and part of a ring.

The skeleton

To recapitulate, the preliminary data show that the nitrogen is cyclic, three C atoms are extra-skeletal and there is no unsaturation other than that in the lactone CO group. Since the molecular formula $C_{22}H_{33}NO_5$ permits 7 double-bond equivalents, heteratisine must possess a hexacyclic $C_{19}N$ skeleton. This fact immediately suggests a closer structural relationship between heteratisine and the group of highly toxic and polyoxygenated alkaloids of the lycoctonine-aconitine type[3b] than with its non-lactonic companion bases of *Aconitum heterophyllum*, viz. atisine, atidine and hetisine.[3a] All alkaloids of the former group contain the intact $C_{19}N$ *ent*-Aconane based skeleton (**2**).* The $C_{20}N$ *ent*-Atisane skeleton (**3**),* with occasional modification such as an altered or additional ring junction, is common to the second group. A practical decision in favour of the Atisane-type skeleton is normally possible since this type alone yields substituted naphthalenes, phenanthrenes and azaphenanthrenes on selenium dehydrogenation. Hence heteratisine was subjected to selenium dehydrogenation; however, examination showed the absence of aromatic hydrocarbon or nitrogen bases among the reaction products. The result appears to emphasize the possibility mentioned above that heteratisine has a modified *ent*-Aconane based lycoctonine-aconitine type skeleton.† This tentative conclusion was further reinforced by studies on the Sarett oxidation products of the alkaloid described in the following section. These studies provide a clear view of a portion of the molecule which is recognised as typical of the *ent*-Aconane group. This, in turn, introduces the necessary element of credibility into the subsequent analyses which, in parts, rest heavily on analogies with the structural features and physico-chemical properties of diterpene alkaloids with established structures. Nevertheless, for the sake of clarity, complete structural formulas are used to represent several derivatives of heteratisine before the structure of heteratisine is fully established.



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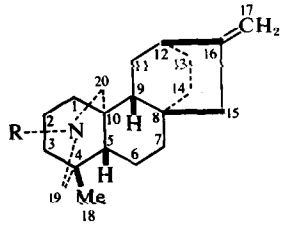


3

The environment of the nitrogen atom

Ring A/E. The oxidation of heteratisine acetate with an excess of Sarett reagent[11] gives a mixture from which three crystalline products, two neutral and one basic, can be isolated. The major neutral product, oxoheteratisine acetate, is obtained in about 70–75% yield. The second neutral product is formed in extremely small yield and no more than 10 mg could be isolated pure from *ca* 1g of heteratisine acetate. This fraction will be referred to as the *minor lactam* in the sequel.

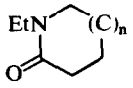
Oxoheteratisine acetate has an analysis corresponding to $C_{24}H_{33}NO_7$ and, therefore, is formed by loss of 2H and introduction of an O atom. It retains all the oxygen functions of heteratisine acetate. The IR spectrum shows a sharp peak at 3436 cm^{-1} due to an OH group. This must be tertiary since oxoheteratisine acetate could not be acetylated further. The IR absorption at 1739 cm^{-1} is indicative of a δ-lactone (or larger) ring. The O-acetyl group ($\delta = 2{\cdot}06$, 3H singlet) can be saponified to give a dihydroxy lactam, oxoheteratisine, which furnishes oxoheteratisine acetate again on treatment with acetic anhydride and pyridine. Zeisel determination shows the presence of one OMe, further confirmed by a three proton singlet at $\delta = 3{\cdot}23$ in the NMR spectrum. The NMR spectrum also shows that the two extra-skeletal alkyl groups, a quaternary Me (3 proton singlet at $\delta = 1{\cdot}22$) and N—CH_2—CH_3 (3 proton triplet at $\delta = 1{\cdot}12$ ($J = 7$ Hz) due to the Me proton of the N-Et) are present.

The nature of the new oxygen function introduced by this oxidation is revealed as follows. The compound is neutral, so it may be an amide or a lactam. The amide could only be an N-Ac derived

*For systematic nomenclature of the skeleta found in diterpene alkaloids, see reference 4, pp. xv–xvii.

†The *ent*-Atisane type skeleton present in atisine or hypognavine is not ruled out completely since skeletal modification, e.g. by location of the lactone in ring B, could conceivably preclude aromatisation.

from N-Et, the only alkyl substituent on N. Since N-Et is present intact (NMR), the compound is a lactam. The strong, sharp IR absorption band at 1625 cm^{-1} (Nujol mull) supports this assignment and further shows that the ring is 6-membered or larger. By analogy with the other Aconite alkaloids, a 6-membered N-ring may be accepted and partial structure (**4**) may be written for the oxo-derivative.



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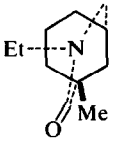

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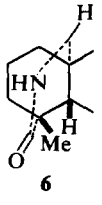
The NMR spectrum has another notable feature. The singlet due to the three protons of the quaternary Me occurs at $\delta = 1{\cdot}22$. This is at 0·33 ppm lower field than the position of this signal in heteratisine acetate. The magnitude of this deshielding suggests that (i) the CO group has been introduced in a position β- to the quaternary Me and (ii) the Me group is held rigidly in the trigonal plane of the CO group. This structural and conformational requirement is met admirably by the usual A/E ring system of Aconitum and Delphinium alkaloids.[12] Structure **4** may be extended, therefore, to **5**. The hydrolysis of oxoheteratisine acetate to oxoheteratisine was mentioned above and further transformations of the latter are discussed in the sequel. It may be mentioned here, however, that the quaternary Me protons in all compounds of the oxo series resonate at 0·25 to 0·3 ppm lower field than in the corresponding parent bases. (Table 2).

The minor lactam ($C_{20}H_{27}NO_6$) mentioned above is formed by oxidation and concomittant elimination of the N-Et group. The material isolated and characterised had also lost the O-Ac group, but the deacetylation presumably occurred during isolation procedures, which involved a protracted chromatography on alumina. Analytical and spectral data (IR, NMR, Experimental) indicate the N-desethyl-19-oxo-heteratisine containing the secondary lactam structure (**6**) for this compound. Since it does not form a vital link in our derivation of the structure of heteratisine it will not be discussed further.



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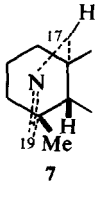

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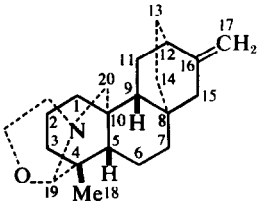
The azomethine base. The basic oxidation product is a desethyl-dehydro derivative, $C_{22}H_{29}NO_5$. The loss of the N-Et group is obvious, since the characteristic triplet absorption of its Me component is absent in its NMR spectrum (in CD_3OD). The bulk of the NMR spectrum is closely similar to that of oxoheteratisine acetate; the C—CH_3 singlet at $\delta = 1{\cdot}2$ is noteworthy.[12] In addition, there is a one-proton broad signal at $\delta = 7{\cdot}32$. There is no conjugated unsaturation (UV) and the IR spectrum has an absorption band at 1642 cm^{-1} assignable to >C=N—; additional bands due to >C=NH— appear at 2558, 2123 and 1923 cm^{-1} in the hydrochloride salt of this base. The $\delta = 7{\cdot}32$ signal thus arises from an HC=N— group. The location of this double bond in a position β- to the C—Me presumably accounts for the observed deshielding of the latter. The basic oxidation product is therefore represented ty the azomethine partial structure **7**.

Isomeric lactam or azomethine products involving C-17 (numbering sequence as in Structure 2) instead of C-19 were not isolated and there is evidence that such lactams are not formed. The quaternary Me resonance in the C-17 isomers should occur at a normal field value (*ca* $\delta = 0{\cdot}95$). However, no such peak was found in the NMR spectrum of the mother liquors obtained on crystallizing out the 19-oxoheteratisine acetate (**5**) from the total neutral fraction. This is consistent with other NMR evidence which suggests that C-17 is a methine rather than a methylene group. The protons attached to this C atom are expected to experience a deshielding effect[13] because of oxidation of the amino to the 19-oxo amide group. A comparison of the NMR spectra shows that a one-proton signal at $\delta = 3{\cdot}58$ in heteratisine acetate is shifted downfield to $\delta = 3{\cdot}83$ in 19-oxoheteratisine acetate. Similar shifts accompany the conversion of dehydroheteratisine (**26**) to 19-oxodehydroheteratisine (**28**) discussed in the sequel (17-CH at $\delta = 3{\cdot}85$ shifts to $\delta = 4{\cdot}09$). An analogous effect is observed in the azomethine (**7**) as well. In this compound, the shift of 17-CH to δ 4·4 is, as may be anticipated,[12] even more dramatic than in the 19-oxo series. These data are clearly incompatible with a 17-CH_2 or with an oxygenated 17-CH—O for which CH resonance would occur at a much lower field value. The atisine (**8**) or isoatisine (**9**) type ring E is thus firmly ruled out, leaving the hypognavine-type (**10**) and the aconitine-lycoctonine-type (**2**) skeletons for further consideration.

Further consideration of the NMR signal assigned to 17-CH is useful. In the azomethine derivative (**7**), this signal is a multiplet as would be expected on account of the additional allylic coupling to the azomethine HC=N proton. But in heteratisine and heteratisine acetate (and also in the dehydro derivatives discussed in the sequel) this absorption peak appears as a doublet, indicating coupling to only one other proton. This would be 7-CH in **2**. In the alternative skeleton (**10**) under consideration this signal would be assignable to 20-CH coupled to 14-CH. Now, in a Drieding model of



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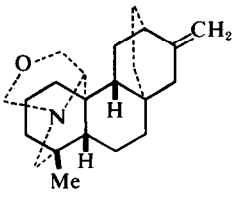

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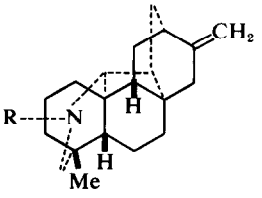

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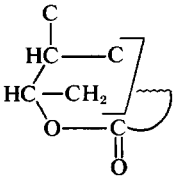

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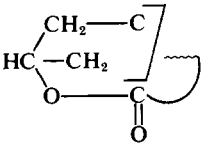

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2, the dihedral angle 17-CH–7-CH is *ca* 70°, for which the Karplus relationship[14] gives a calculated coupling constant of 1–1·5 Hz. This value is in good agreement with the values of 1·5 to 2·5 Hz actually observed for this signal. The calculated coupling constant for 20-CH–14-CH in **10** (dihedral angle *ca* 120°) is *ca* 4 Hz. A tentative decision in favour of skeleton **2** may thus be made.

The NMR evidence cited above against the hypognavine skeleton (**10**) taken by itself, is slender. Yet for any further serious consideration of a structure for heteratisine based on **10** it is necessary to explain the complete absence of aromatic hydrocarbons or bases from the products of selenium dehydrogenation of heteratisine (see above). A reasonable hypothesis is that the interruption of the carbocyclic skeleton (**10**) by the lactone ring (and the loss of one C atom) is primarily responsible for this. Because naphthalene derivatives could be formed if the lactone ring was located in rings A, C or D, location in ring B only would be consistent with this hypothesis. However, it is impossible to visualize a reasonable modification which will accommodate in ring B of the hypognavine skeleton (**10**), either of the two partial structures **11** and **12**, considered in a later section; therefore, this skeleton was eliminated from further consideration.

Location of the secondary hydroxyl group

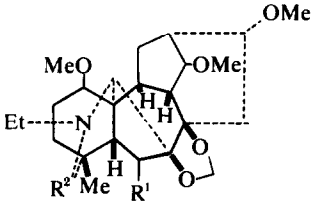
Ring B. Treatment of heteratisine with chromium trioxide in acetic acid causes oxidation of the secondary OH group to afford a keto-base, dehydroheteratisine. Similarly, oxoheteratisine yields oxodehydroheteratisine which is also formed as one of the neutral products in the oxidation of heteratisine with a chromium trioxide-pyridine reagent. Both the basic and the neutral ketones are cyclopentanone derivatives, since their IR spectra show strong absorption bands at ν_{max} 1750 cm^{-1} (CS_2) and 1748 cm^{-1} ($CHCl_3$) respectively.

However, in the UV spectrum, the $n \rightarrow \pi^*$ absorption band of the ketonic CO is at λ max 270 nm (ϵ 69), while that for the ketolactam occurs at λ max 313 nm (ϵ 30). This modest departure from normal, viz on enhancement of the intensity of the former and the red shift of the latter, has been observed earlier as a peculiarity of the pair dehydrodelpheline (**14**), λ max 269 nm (ϵ 160) and dehydrooxodelpheline (**16**), λ max 313 nm (ϵ 44).[15]

A more dramatic correspondence between the properties of these two pairs of compounds is noticed in the molar rotation changes accompanying their formation from the respective parent hydroxy compounds. Thus, $\Delta[M]_D$ is $-375°$ for the oxidation of heteratisine to dehydroheteratisine and is $-325°$ for the oxo-derivatives. The value for the oxidation of delpheline (**13**) to dehydrodelpheline (**14**) is $-202°$,* and for the oxidation of

*The value ($-120°$) quoted in reference 17 is a miscalculation.

oxodelpheline (**15**) to dehydrooxodelpheline (**16**)[15,16] the value is $-470°$. This agreement between the sign and the order of magnitude of $\delta[M]_D$ suggests that heteratisine has a β-OH structure analogous to the structure of delpheline (**13**).

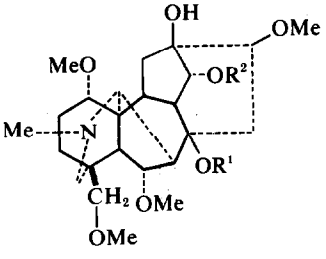


13: $R^1 = OH, R^2 = H_2$
14: $R^1 = O, R^2 = H_2$
15: $R^1 = OH, R^2 = O$
16: $R^1 = O, R^2 = O$

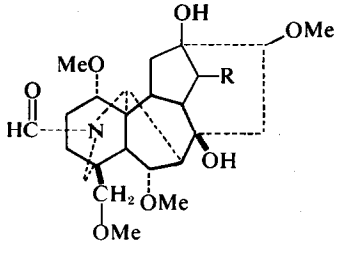
In the basic skeleton (**2**) only two sites, namely carbons 6 and 14, are available for the location of a cyclopentanone CO group in the ketonic oxidation products and hence for the secondary OH group in heteratisine. Delpheline (**13**), the alkaloid discussed above, is the only known example of a 6-OH structure based on skeleton (**2**). The 14-OH derivatives are more common and normally occur in plants as 14-benzoate esters; among these delphinine (**17**)[17a] and its non-saponifiable parent base delphonine (**18**) have been studied in great detail.[3a] Useful reference compounds are available among the oxidation products of delphonine (**18**). Thus $\Delta[M]_D$ for the oxidation of α-oxodelphonine (**19**) to α-oxodelphonone (**20**) is $+186°$ and for dihydro-α-oxopyrodelphonine (**21**) to the corresponding 14-keto compound (**22**) is $+148°$.[17b,18] Clearly, there is no correspondence between these figures and those observed in the heteratisine series. However, the delphonones are α-acyloins and are known to undergo rearrangement to the 13-keto isoseries.[17b,18] This isomerization results in a large negative change in molar rotation. The possibility was considered, therefore, that the oxidation of the secondary OH group in the heteratisine series was accompanied by a similar rearrangement—perhaps during isolation of the products by chromatography over alumina; this possibility was soon discarded, for the following reasons.

Firstly, heteratisine is not a vicinal glycol because when treated with periodate or lead tetraacetate, oxidation did not occur when after 24 hr. Further, oxodehydroheteratisine is not an α-OH ketone since it was recovered completely unchanged after being left for 60 hr with periodic acid. Nevertheless, attempts were made to reduce dehydroheteratisine back to heteratisine; no reduction was observed with $NaBH_4$ in methanol at room temperature, while in boiling methanol an intractable mixture of products was formed. Oxodehydroheteratisine behaved in a similar way; with this compound hydrogenation in acetic acid over Adams catalyst at room temperature and atmospheric pressure was also unsuccessful.

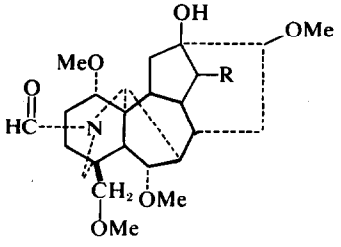
To summarize, the foregoing discussion suggests that the structures of heteratisine and dehydroheteratisine may be derived from **23** and **24** respectively.



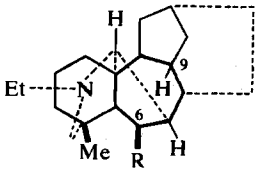
17: $R^1 = Ac, R^2 = Bz$
18; $R^1 = H, R^2 = H$



19: R = --OH
20: R = O



21: R = --OH
22: R = O



23: R = OH
24: R = O

Environment of the lactone carboxylic group

A comparison of the NMR spectra of dehydroheteratisine and heteratisine showed the expected absence of a signal due to CHOH in the former. Surprisingly, another one-proton signal at $\delta = 4{\cdot}03$ in the heteratisine spectrum is also missing from the corresponding region of dehydroheteratisine. Initially, this observation was an additional cause for the fears expressed above that the oxidation of the secondary alcoholic group was accompanied by skeletal rearrangement. However, if a skeletal rearrangement has not occurred, then presumably the signal has suffered a large change in its chemical shift. Search for this signal in the spectrum of dehydroheteratisine has been unsuccessful, but conceivably it is hidden under the OCH_3 ($\delta = 3{\cdot}37$) or the general methine–methylene signals. The chemical shift increment on introduction of the carbonyl group is thus at least 0·7 ppm.

A plausible rationale for this observation emerged from a comparison of molecular (Dreiding[19]) models of **23** and **24**. In the 6-keto structure(**24**), the 9-CH(β) is located on an axis perpendicular to the trigonal plane of the CO group, approximately 2·7 Å from the centre of the group. In other words, this proton is situated in the diamagnetic shielding region of the anisotropic CO group[20] and is expected to resonate at a (considerably) higher field value than 9-CH in the 6-OH analogue (**23**). No other proton in **24** falls in the strong shielding regions of the field of the 6-keto CO. Therefore the signal at $\delta = 4{\cdot}03$ in the heteratisine spectrum may be assigned to 9-CH(β).

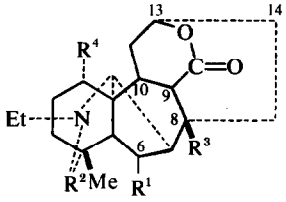
The low chemical shift ($\delta = 4{\cdot}03$) for the signal requires an electron withdrawing substituent on 9-C. This is unlikely to be the OMe group because in the *ent*-Aconane skeleton (**2**), 9-C is a tertiary ring junction. The lactone ring oxygen cannot be accommodated here either (see later). Hence, the lactone CO group must be attached to 9-C.

The environment of the lactone ring oxygen atom

To put degradation studies (next section) on the dehydro-derivatives in proper perspective, it is necessary to define the environment of the lactone ring O atom and the relative locations of the other oxygen functions in heteratisine.

A one-H signal at δ 4·74 in heteratisine, and at δ 4·72 to 4·9 in its derivatives, is assigned to H—C—OCO of the lactone because (i) the chemical shift is appropriate and (ii) the signal is absent from the spectrum of tetrahydroheteratisine (see below). In all spectra the δ 4·7–4·9 signal appears as a broad multiplet indicating spin–spin coupling of H—C—OCO with 3 or 4 adjacent protons as e.g. in **11** and **12**, respectively. These data, together with the 6- or larger membered lactone ring indicated by IR absorption can be accommodated if the lactone oxygen is linked to either 14-C or 13-C in rings C-D leading to an extension of structure **23** to **25**.*

Reduction of heteratisine with LAH in THF yielded a tetrahydro derivative lacking IR absorption for lactone >C=O and an NMR signal near 4·8 for H—C—OCO. On oxidation with Sarett reagent, the reduction product was converted into 19-oxodehydroheteratisine (**28**) showing the skeletal rearrangement accompanying LAH reduction was improbable. Heteratisine itself did not react with periodic acid and therefore contains no vicinal glycol group. Hence, the two OH groups, the lactone >C=O or ring oxygen cannot be located on adjacent C atoms in heteratisine.



25: $R^1 = OH$, $R^2 = H_2$, $R^3 = OH$, $R^4 = OMe$
26: $R^1 = O$, $R^2 = H_2$, $R^3 = OH$, $R^4 = OMe$
27: $R^1 = OH$, $R^2 = O$, $R^3 = OH$, $R^4 = OMe$
28: $R^1 = O$, $R^2 = O$, $R^3 = OH$, $R^4 = OMe$

The location of the tertiary hydroxyl group

Useful deductions may be made from the splitting pattern of the 9-CH(β) signal in the NMR spectrum of heteratisine. This occurs as a nearly symmetrical doublet $J = 7$ Hz and is, therefore, spin coupled to only one proton on an adjacent carbon atom, i.e., 10-C; presumably the other adjacent C atom, i.e., 8-C is tertiary and carries the OMe or the tertiary OH substituent. The latter assignment is more attractive by analogy with the structures of other C_{19}-aconite alkaloids and further provides an understanding of the results of alkaline degradation reactions of dehydroheteratisine and 19-oxodehydroheteratisine, described in the sequel.

An incentive for studying the reaction of the keto derivatives with strong alkalies was provided by some apparently conflicting observations in the following experiments. Dehydroheteratisine and oxodehydroheteratisine gave a positive Zimmermann color reaction,[21] which would appear to indicate that both these molecules contained CH_2—CO residues. Yet oxodehydroheteratisine did not yield a formyl derivative when treated with ethyl formate and sodium methoxide. These experiments, together with unsuccessful attempts (i) to deuterate the CH_2 group located α to >C=O, and (ii) to reduce the ketonic CO group with sodium bobohydride, appeared to indicate that the molecules may

*For easier comprehension and economy of space, **25** and subsequent structures display complete rather than partial structures.

be undergoing degradation under the influence of strong bases.

The treatment of heteratisine with hot aqueous alkali causes only hydrolysis of the lactone ring. This hydrolysis and consequent dissolution in aqueous alkali occurs with dehydro derivatives **26** and **28** as well, but continued heating with aqueous alkali leads to decarboxylated products lacking the lactone group. The neutral derivative 19-oxodehydroheteratisine (**28**) is a better substrate for the study of this degradation than the basic dehydroheteratisine (**26**), but even with the former a mixture of products was formed from which pure compounds could not be isolated. Thus the action of aqueous potassium hydroxide (0·2–2·0%) under reflux (1 to 30 min) on (**28**) gave an alkali-insoluble mixture of products in 50 to 70% yield. The IR spectrum showed lactam (1631 cm^{-1}) and OH (3571 cm^{-1}) bands; the lactone CO absorption (*ca* 1740 cm^{-1}) was absent, and a new strong band appeared at 1648 cm^{-1}. In the NMR spectrum, the broad one-H signal (*ca* $\delta = 4{\cdot}8$) characteristic of H—C—OCO of the lactone ring was absent, but signals for the OMe, N—Et and C—Me appeared to be intact.

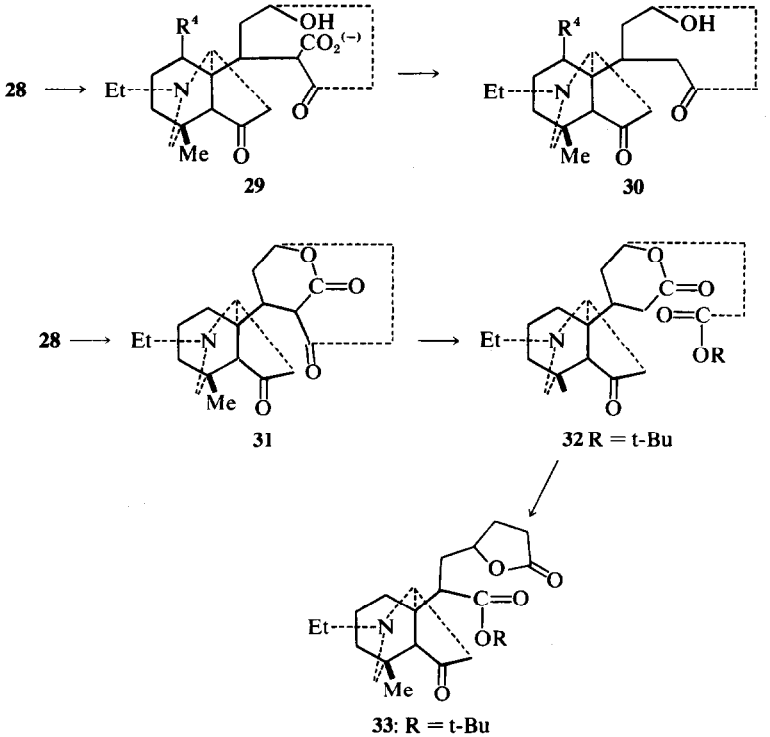
If location of the t-OH at C-8 suggested by NMR is accepted, a rational path for the observed decarboxylation reaction can be written as in **28–30**. The alkali causes a retro-aldol cleavage of the β-OH-ketone (**28**) to give the β-keto-carboxylate (**29**) which is susceptible to ready decarboxylation to **30**. The exact cause of the breakdown of **30** is uncertain.

Presumably the α-methylene ketone (**30**) is formed in the Zimmerman test also and is responsible for the positive color reaction given by the dehydroheteratisines.

An analogous cleavage was affected under non-hydrolytic conditions using potassium t-butoxide in t-butanol as the base. The final product actually isolated was a γ-lactone carboxylic acid (**34**) which was characterized as its methyl ester (**35**). The intermediate steps leading to this product would comprise an initial retro-aldol reaction to the β-keto ester (**31**) followed by a retro-Claisen reaction to a δ-lactone carboxylic acid derivative (**32**), and finally isomerization of the latter to the γ-lactone (**33**). These steps are plausible and show that the tertiary OH group is located on a C atom which is situated β- to both the cyclopentanone and the δ-lactone ether oxygen as in **28**.

Location of the secondary methoxyl group

The discussion so far has left little doubt that heteratisine contains an *ent*-Aconane skeleton in which the normal cyclopentane C ring has been modified into a δ-lactone and that the skeleton carries a secondary OH and a t-OH at C-6 and C-8,



respectively. The skeleton has no primary or tertiary carbon available as the location for an OMe group because protons have been assigned to these already. Therefore, the OMe group in the alkaloid must be present on a secondary C atom. Now, in all other known alkaloids based on this skeleton,[3,4] secondary OMe groups are present at C-1 and/or C-16. In the present case, the latter is ruled out since such a OMe would suffer ready β-elimination during treatment of 19-oxodehydroheteratisine with potassium t-butoxide. Some support for a OMe at C-1 is available from analysis of the NMR spectral data. The 60 Mz NMR spectra of heteratisine and heteratisine acetate do not contain a signal ascribable to H—C—OMe in the field region lower than the OMe singlet (δ 3·25). Presumably, this signal must be located at a field value higher than normal. The one proton multiplet between δ 3·37–3·98 in the spectrum of 19-oxodehydroheteratisine is assignable to H—C—OMe. Whatever the cause* for the additional shielding experienced by H—C—OMe in heteratisine, it would appear that the combined influence of the lactam >C=O and the cyclopentanone >C=O at positions 19 and 6 respectively, brings this resonance back to a more normal field value. Models (Dreiding[19]) show that only the 1-C β-proton in **28** lies in the trigonal planes of both these >C=O groups and is *ca* 5 Å distant from each. It is thus suitably placed to experience the deshielding influence of the lactam and the cyclopentanone C=O groups.

The evidence and arguments given in this paper thus lead to the complete structure of heteratisine which can be represented by **25≡1**. Edwards and Ferrari have recorded some chemistry of heteratisine which is consistent with this structure.[22] An identical structure has been derived by Przylylska[23] by X-ray crystallographic analysis of heteratisine hydrobromide monohydrate. Several other hydrogen halide salts of heteratisine was found to give crystals unsuitable for X-ray studies.[10,23]

The absolute stereochemistry shown in structure **1** was originally suggested by analogy with that established for other diterpene alkaloids, but has been confirmed by studies on the pyrolysis products of heteratisine monoacetate.[7]

EXPERIMENTAL

General experimental procedures. M.ps are corrected and were taken on a hot stage equipped with a microscope and polarizer. Finely powdered samples were placed on the stage 15° below the m.p. and the temp was raised at a rate of about 2°/min. Rotations were taken in chloroform unless otherwise noted. UV spectra were determined in 95% EtOH on a Beckman Model DU or a Perkin-Elmer Model 203 spectrophotometer and IR spectra on Perkin-Elmer Model 221 and Infracord spectrophotometers. NMR spectra were taken on a Varian A-60 spectrometer in $CDCl_3$ with TMS as an internal standard and are reported as δ values in ppm.

Light petroleum refers to a fraction of b.p. 60–70°. The removal of solvents *in vacuo* was accomplished with a Craig-type rotating flash evaporator at 15–20 mm Hg and with the water bath usually at 35–50°. Organic solutions were usually dried over $NaSO_4$, prior to evaporation.

Thin layer chromatography. TLC was carried out on silica gel G and alumina plates. The solvent mixtures utilised are indicated in each experiment. Visualization of spots was effected by exposing the developed plate to iodine vapor until spots became visible, removal of the plate from the iodine chamber, and exposing the surface of the plate briefly to a steam jet. The plate was then re-exposed to iodine vapor. The steam treatment and re-exposure to iodine vapor gave much more intense spots than a single exposure to iodine.

Heteratisine. The alkaloid was isolated from dried roots of *Aconitum heterophyllum* Wall as described earlier.[9] The material used for most of the degradation work had m.p. 261–265° (dec), with the m.p. rather dependent on the rate of heating and the temp of which the sample was placed on the block. An analytical sample showed m.p. 267–269°; $[\alpha]_D^{28}$ +40° (c = 1·0, MeOH); ν max (Nujol, CaF_2 prism) 3460, 3401 cm^{-1} (OH), 1738 cm^{-1} (δ-lactone); ν max ($CHCl_3$, CaF_2 prism), 1724 cm^{-1}; ν max (KBr, NaCl prism); 1382 cm^{-1} (C—CH_3); NMR, see Table 1. (Found: C, 67·57, 67·55, 67·57; H, 8·60, 8·53, 8·31; N, 3·74, 3·69; OMe, 7·62; C—Me, 3·64, 3·71. $C_{22}H_{33}NO_5$ requires: C, 67·49; H, 8·50; N, 3·58; OMe, 7·93; C—Me, 3·85).

Preparation of the sodium salt of heteratisine. To heteratisine (39·1 mg) dissolved in 1 ml abs EtOH was added 1 ml of a 0·1 N ethanolic NaOH. The soln was refluxed for 1 hr on the steam bath, and then was evaporated to dryness *in vacuo*. The crude material was taken up in water and washed 3 times with benzene to remove any unreacted lactone (or ethyl ester). The aqueous layer was then evaporated to dryness *in vacuo* and flashed repeatedly with benzene to prepare a dry powder, ν max (Nujol) 1567 cm^{+1} (broad max) in the CO stretching region.

Benzoylation of heteratisine. Heteratisine (250 mg) in 2·0 ml dry pyridine was treated with 0·75 ml freshly distilled benzoyl chloride. The red-colored mixture was allowed to stand at room temp for 3 hr and then stored in the refrigerator overnight. The mixture was poured into ice water and after being made basic with Na_2CO_3 aq was extracted 3 times with chloroform. Evaporation of the chloroform extract gave a residue which was dissolved in benzene and extracted with 2% H_2SO_4 (5x, 3 ml). The acidic extract was made basic with dil Na_2CO_3 aq and extracted (3x) with chloroform. The extract was taken to dryness and flashed several times with benzene. After seeding with natural heteratisine benzoate, 188 mg., m.p. 212–215° and 35 mg, 214–216° were collected. Recrystallization of the combined fractions from hot benzene gave 122 mg of large trapezoidal crystals, m.p. 212–214°, undepressed when mixed with a sample of naturally derived heteratisine benzoate. (Found: C, 70·22; H, 7·56. $C_{29}H_{37}NO_6$ requires: C, 70·28; H, 7·53%).

Selenium dehydrogenation of heteratisine. Heteratisine (2·0 g) was ground well with 6·5 g powdered selenium and transferred to a 50-ml flask equipped with a side arm. The

*The shielding effect probably arises from the anisotropy of the nitrogen or the lactone ring oxygen, or conceivably is associated with conformational changes in ring A.

mixture was heated gradually in a Wood's metal bath up to 340° under N_2 and then held at 340–345° for 3½ hr. After cooling, the hard selenium cake was pulverized and extracted with ether in a Soxhlet apparatus for 6 hr. The extract was evaporated to dryness and the residue taken up in benzene and decanted from the small amount of precipitated selenium. The benzene soln was extracted with 10% HCl (3 ×) and with 5% NaOH (3 ×). The benzene soln containing neutral components was evaporated to dryness *in vacuo*, taken up in 75 ml benzene and chromatographed over 20 g of Woelm neutral alumina. Elution with benzene gave only 7 mg of ill-defined noncrystalline material. Only traces of material were present in the HCl and NaOH extracts.

Heteratisine acetate. Heteratisine (200 mg), pyridine (2 ml) and Ac_2O (2 ml) were set aside, in a stoppered flask, at room temp for 18 hr. MeOH (5 ml) was added and after 1 hr, the mixture was evaporated to dryness under reduced pressure. This evaporation was repeated after addition of MeOH (2 ml) and benzene (4 ml) to the residue. The residue was stored overnight in a vacuum desiccator over P_2O_5 to remove the last traces of pyridine. Crystallization from EtOAc-light petroleum (b.p. 60–70°) gave a first crop (94 mg), m.p. 172–174°. The material in the mother liquors, on recrystallization from ether was obtained as colorless prismatic needles (98 mg), m.p. 173–175°; $[\alpha]_D^{27}$ 22·3° ($CHCl_3$); ν max 3395 (OH), 1746 (δ-lactone), 1714 cm^{-1} (OAc); δ = 0·85 (3H s, C—C$\underline{H}_3$), 1·03 (3H t, J = 7 cs, C$\underline{H}_3$—CH$_2$—N). 3·26 (3H s, C$\underline{H}_3$O), 3·58 (1H d, J = 2 Hz, N—C$\underline{H}$), *ca* 4·72 (1H m, $\underline{H}$—C—OCO of lactone), 5·29 (1H q, $\underline{H}$—C—OAc), 2·06 (3H s, C$\underline{H}_3$COO). (Found: C, 66·42; H, 8·24; Acetyl, 9·55. $C_{24}H_{35}NO_6$ requires: C, 66·49; H, 8·14; Acetyl, 9·93%).

Sarret oxidation of heteratisine acetate to 19-oxoheteratisine acetate (**5**). Heteratisine acetate obtained by the treatment of heteratisine (1·021 g) with Ac_2O-pyridine (excess, overnight, room temp) and removal of the volatile material under reduced pressure was used without further purification. The adduct[11] of CrO_3 (1·206 g) and pyridine (10 ml) was added to it with cooling (5°) and stirring. The mixture was allowed to warm, and after 24 hr at 25°, pyridine removed by evaporation under reduced pressure. The residue was suspended in water (100 ml) containing $NaHSO_3$ (1 g) and acidified with 10% H_2SO_4. Extraction with chloroform (6 × 25 ml), washing the extract with water, drying (Na_2SO_4) and evaporation to dryness gave a crude neutral and acidic (lactam) fraction (710 mg).

The aqueous layer was neutralized with Na_2CO_3 and extracted with chloroform (10 × 20 ml) and with ether (5 × 20 ml). The combined organic layer was evaporated to dryness, but was redissolved in chloroform (75 ml) and extracted into 2% H_2SO_4 (50 ml). The acidic soln was neutralized with cold 5% Na_2CO_3 aq (35 ml) and extracted with chloroform (3 × 20 ml). The chloroform soln was washed with water (2 × 15 ml), dried briefly over Na_2SO_4, filtered and evaporated to dryness. The residue (250 mg) was the crude basic fraction.

The crude lactam fraction described above was dissolved in chloroform (15 ml) and chromatographed on activity-1 neutral alumina (15 g). Elution with chloroform (15 ml) afforded 620 mg of material, which on crystallization from EtOAc yielded 19-oxoheteratisine acetate (590 mg); m.p. 273–278°. TLC (5% MeOH in chloroform) showed the presence of a trace of impurity but otherwise the product was identical with analogous material obtained in an earlier smaller scale experiment on the Sarret oxidation of heteratisine acetate. This material was crystallized from a mixture of EtOAc and chloroform and after drying at 100°/0·1 mm Hg for 3 hr, had m.p. 280–282°; ν 3436 (OH), 1739 (δ-lactone and O—Ac), 1625 cm^{-1} (δ-lactam); δ1·22 (3H s, C—C$\underline{H}_3$), 1·12 (3H t, J = 7 Hz, C$\underline{H}_3$CH$_2$N), 2·06 (3H s, C$\underline{H}_3$CO, 3·23 (3H s, C$\underline{H}_3$O), 3·83 (1H d, J = 2·5 Hz, N—C$\underline{H}$), *ca* 4·78 (1H m, $\underline{H}$—C—OCO of lactone), 5·23 (1H q, $\underline{H}$—C—OAc). (Found: C, 64·61; H, 7·55; N, 3·06; OMe, 6·92. $C_{24}H_{33}NO_7$ requires: C, 64·41; H, 7·43; N, 3·13; OMe, 6·93%).

The minor lactam (N-*desethyl*-19-*oxoheteratisine*) **6**. After recovering the bulk of the oxoheteratisine acetate from the chromatographic column in the preceding experiment, further elution of the column with chloroform (8 × 15 ml) yielded 75 mg of a mixed fraction which was combined with the mother liquors from the crystallization of the 620 mg of **5** in the preceding experiments. The mixture was again applied (in chloroform-EtOAc, 3:1) to a column of alumina (neutral, activity 1, 10 g). Elution with EtOAc initially yielded a small amount of impure oxoheteratisine acetate but later fractions contained a new compound (20 mg). Three crystallizations from EtOAc-chloroform, afforded colorless crystals (10 mg); after drying at 100°/0·1 mm Hg for 4 hr, the product showed m.p. 264–266°; ν max 3195 (OH and NH), 1736 (δ-lactone and OAc), 1637 cm^{-1} (δ-lactam); the material was insufficient for a good NMR spectrum but the characteristic 3H triplet (*ca* δ = 1) and 3H singlet (*ca* δ = 2) of the N—CH$_2$C$\underline{H}_3$ and OCOC$\underline{H}_3$ respectively, were clearly absent, while the OC$\underline{H}_3$ absorption was intact and the quaternary C—C$\underline{H}_3$ absorption was shifted to a low field value of δ = 1·15. (Found: C, 63·75; H, 7·22. $C_{20}H_{27}NO_6$ requires: C, 63·64; H, 7·21%).

The azomethine base (**7**). A soln of the crude basic fraction (250 mg) obtained from the Sarret oxidation of heteratisine acetate in chloroform (7 ml) and benzene (3 ml) was chromatographed over a column of 15 g of neutral alumina (activity 2). Elution with chloroform–benzene mixtures (1:4, 1:2, 1:1) brought a fore-run (rejected). Most of the material (140 mg) was eluted with chloroform. The product was crystallized twice from EtOAc-chloroform and then twice more from acetone. The colorless crystalline product, which appeared as a single spot in TLC (5% MeOH in benzene) was dried at 110°/1 mm Hg for 1 hr; m.p. 294–298; $[\alpha]_D^{27}$ +125° (*c* 0·5, MeOH); ν max 3195 (OH), 1739 (δ-lactone and OAc), 1642 cm^{-1} (>C=N); δ = 1·20 (3H s, C—C$\underline{H}_3$), 2·08 (3H s, C$\underline{H}_3$COO), 3·23 (3H s, OC$\underline{H}_3$), 4·4 (1H m, N—C$\underline{H}$), 4·9 (1H m, $\underline{H}$—C—O—CO of lactone), 7·38 (1H m, $\underline{H}$C=N). There is no signal for NCH$_2$—CH$_3$. (Found: C, 65·81; H, 7·37; N, 3·45. $C_{22}H_{29}NO_6$ requires: C, 65·89; H, 6·78; N, 3·49%).

The azomethine base hydrochloride. A slightly impure sample (18 mg) of **7** in MeOH was treated with ether saturated with conc HCl. Evaporation to dryness under reduced pressure was repeated after adding ether (3×) and acetone (1×). After three crystallizations from MeOH-acetone, colorless crystals (10·3 mg) of the azomethine hydrochloride were obtained. After drying at 110°/0·1 mm Hg for 12 hr the product showed m.p. 325–330°; ν max (Nujol) 3571, 3311 (OH); 2558, 2123, 1923, 1644 (>C=N$^+$H), 1742 cm^{-1} (δ-lactone and OAc). (Found: C, 59·83; H, 6·91. $C_{22}H_{29}NO_6$HCl: requires: C, 60·09; H, 6·88%).

Attempted acetylation of 19-oxoheteratisine acetate.

Pure, crystalline 19-oxoheteratisine acetate (9 mg) was treated with anhyd pyridine (1 drop) and Ac_2O (4 drops). After 4 days at room temp, MeOH (2 ml) was added and the mixture evaporated to dryness under reduced pressure. Crystallization of the residue from EtOAc-light petroleum gave colorless crystals (6·8 mg), m.p. and m.p. of mixture with starting material, 280–283°; the IR spectra (Nujol) of the two materials were also identical.

Oxoheteratisine (**27**) *by saponification of 19-oxoheteratisine acetate.* A soln of **5** (32·5 mg) in EtOH (3 ml) was treated with $NaCO_3$ (42 mg) in water (2 ml). The mixture was heated under reflux under N_2 for 1·25 hr. The alcohol was removed by evaporation under reduced pressure, the residue diluted with water to 10 ml and acidified with dil H_2SO_4. After warming for 5 min the mixture was extracted with chloroform (4×5 ml). The extract was washed with Na_2HCO_3 aq, dried over Na_2SO_4 and evaporated to dryness. The residue (30 mg) was crystallized twice from MeOH. The first crop (13·5 mg) from the second crystallization dried at 100°/0·2 mm Hg for 3 hr, gave **27**, m.p. 345–347°; $[\alpha]_D^{22}$ +50° (*c* 0·5, MeOH); νmax 3460, 3205 (OH), 1730 (δ-lactone), 1609 cm^{-1} (δ-lactam); for NMR data, see Table 2. (Found: C, 65·02; H, 7·73; N, 3·57. $C_{22}H_{31}NO_6$ requires: C, 65·1; H, 7·71; N, 3·45%).

Work up of the mother liquors yielded more material (8·5 mg), m.p. 342–347°.

Reduction of heteratisine with lithium aluminum hydride to "Tetrahydroheteratisine". A 100 mg sample of heteratisine was reduced by refluxing with 50 mg LAH THF for 3 hr. After the soln had been cooled to room temp several ml of EtOAc were added, followed by moist ether and several ml water. Filtration from the coagulated salts and evaporation to dryness *in vacuo* of the organic layer gave 106 mg of an oil which on trituration with light petroleum gave crystals, m.p. 126–128°. Recrystallization from a large excess of chloroform furnished 53 mg of flat trowel-shaped blades, m.p. 128–132°; ν max (Nujol) *ca* 3300 cm^{-1} (OH), with no absorption in the CO stretching region. Drying for 6 hr at 100° *in vacuo* failed to remove one mole of solvent of crystallization. (Found: C, 53·73, H, 7·28. $C_{22}H_{37}NO_5$. $CHCl_3$ requires: C, 53·75; H, 7·44%). Repeated recrystallization from acetone/ligroin gave crystals with m.p. 119–123°. (Found: C, 66·44; H, 9·62. $C_{22}H_{37}NO_5$ requires: C, 66·80; H, 9·43%).

A slightly impure sample of tetrahydroheteratisine which was contaminated with very small amounts of three other compounds (TLC) showed no NMR signal at δ = 4·9–4·8. This sample was subjected to oxidation with CrO_3-pyridine reagent,[11] and worked up according to the procedure described in the sequel for the oxidation of heteratisine. The neutral fraction, on crystallization from EtOAc yielded a crystalline product with an IR spectrum in Nujol identical with that of **28**.

"Tetrahydroheteratisine triacetate". To a soln of 43·4 mg of tetrahydroheteratisine-chloroform adduct in 1 ml of pyridine was added 1 ml of Ac_2O. After standing for 2 days, the soln was concentrated and flashed repeatedly with EtOH and benzene. The crude product was taken up in benzene, washed with Na_2CO_3 and with water and evaporated to dryness *in vacuo*. The resulting 43·9 mg of resin was recrystallized from ligroin to give 40 mg of crystalline product. Several recrystallizations from ligroin gave an analytical sample, m.p. 152–154°. (Found: C, 64·15; H, 8·15; acetyl, 24·78. $C_{28}H_{43}NO_8$ requires: C, 64·47; H, 8·31; acetyl, 24·76%).

Dehydroheteratisine (**26**). A soln of heteratisine (157 mg) in glacial AcOH (2 ml) was mixed with a soln of CrO_3 (53 mg) in glacial AcOH (1 ml), water (0·25 ml) and conc H_2SO_4 (0·01 ml). A brown gum separated out but redissolved on the addition of more water (2 ml). After 1 hr at room temp, the excess CrO_3 was destroyed by the addition of EtOH (4 drops) and the mixture was evaporated to dryness under reduced pressure. The residue was redissolved in 15 ml of water and the soln (pH2) extracted with chloroform (4×5 ml) to remove acidic and neutral products (discarded). The aqueous soln was made alkaline (pH8) with $NaHCO_3$ and then extracted with chloroform (5×10 ml). Evaporation of the extracts to dryness yielded a residue (143 mg) which yielded prismatic needless from light petroleum, m.p. 122–124°. Recrystallization from light petroleum gave a colorless crystalline product which showed only one spot on a thin layer chromatogram developed with 15% MeOH in benzene, m.p. 124–125°; $[\alpha]_D$ −56° (*c* 1·87 in $CHCl_3$); λ max 270 nm (ε 69); ν max (CS_2) 3742 (OH), 1750 (cyclopentanone), 1732 cm^{-1} (δ-lactone); δ = 0·94 (3H s, quat. C—CH_3), 1·10 (3H t, *J* = 7·5 Hz, CH_3CH_2—), 3·33 (3H s, CH_3O), 3·85 (1H d, *J* = 1·5 Hz, N—CH), 4·78 (1H m, H—COCO of lactone). (Found: C, 67·98; H, 8·07, N, 3·72; OMe, 7·84. $C_{22}H_{31}NO_5$ requires: C, 67·84, H, 8·02; N, 3·60; OMe, 7·96%).

19-Oxodehydroheteratisine (**28**). The complex[11] prepared by dissolving CrO_3 (600 mg) in anhyd pyridine (8 ml) was added to a cold (5°) soln of heteratisine (250 mg) in anhyd pyridine (2 ml). The mixture was allowed to warm to room temp (22°) and set aside for one day. Pyridine was then removed by evaporation under reduced pressure below 30°. The dark brown residue was quickly mascerated with cold Na_2CO_3 (2·5%, 10 ml) and then acidified with 10% HSO_4 aq. To this dark brown soln, $NaHSO_3$ was added till the soln turned deep green. The soln was made up to 50 ml with water and repeatedly extracted with chloroform (8×20 ml). The combined chloroform extracts, after washing with dil $NaHCO_3$ aq followed by water, drying over Na_2SO_4 and evaporation to dryness, yielded a colorless solid (215 mg). The crude neutral product was crystallized (Norit) from MeOH–EtOAc. The first crop, on recrystallization, yield pure **28**, which melted at 310–315° after a change of crystal form at 280–290°; $[\alpha]_D^{20}$ −30·2° (*c* 1·0, MeOH); λ max 310 nm (ε 30); ν max 3534 and (OH), 1733 (δ-lactone), 1748 (cyclopentanone), 1626 cm^{-1} (δ-lactone); δ = 1·17 (3H t, *J* = 7 Hz, CH_3—CH_2N); 1·22 (3H s, CH_3—C); 3·32 (3H s, CH_3O); 3·42 (1H s, OH); 4·09 (1H d, *J* = 2·5 Hz, CH—N); 4·75 (1H m, H—COCO, lactone). (Found: C, 65·35; H, 7·26; N,3·27. $C_{22}H_{29}NO_6$ requires: C, 65·49; H, 7·25; N, 3·47%).

The mother liquors from crystallization contained several components (by TLC) which after rechromatography on neutral alumina, (activity grade 1) in EtOAc and over silica in chloroform–MeOH, yielded a further quantity of 19-oxodehydroheteratisine.

Also, 19-oxodehydroheteratisine was obtained if 19-oxoheteratisine was used in place of heteratisine as the substrate in the above CrO_3-pyridine oxidation.

19-Oxodehydroheteratisine by chromic acid oxidation of 19-oxoheteratisine. To a soln of 19-oxoheteratisine (81 mg) in glacial AcOH (8 ml), was added, with stirring at room temp (27°), a soln of CrO_3 (23 mg) in glacial AcOH (1 ml). After stirring for 2 hr, the soln was saturated with SO_2 and evaporated to dryness *in vacuo* below 40°. Water (4 ml) was added and the product was extracted with chloroform (4×15 ml). The chloroform extract was cooled to 0–5° and

washed with dil $NaHCO_3$ aq and with water, dried briefly over $NaSO_4$ and evaporated to dryness. The residue was crystallized twice from EtOAc–MeOH to give colorless crystals, m.p. 313–317°; $[\alpha]_D^{22}$ −31° (*c* 0·5, MeOH); its IR and NMR spectra were identical with those of the sample described in the preceeding experiment. (Found: C, 65·72; H, 7·54, N, 3·19. $C_{22}H_{29}NO_6$ requires: C, 65·49; H, 7·25; N, 3·27%).

Action of potassium t-butoxide in butanol on 19-*oxodehydroheteratisine* (**28**). A soln of t-BuOK was prepared by adding K metal (14 mg) to anhyd t-BuOH (15 ml). To the resulting soln, 19-oxodehydroheteratisine (300 mg) was added and the mixture stirred at 30–35° for 3 hr. All these operations were carried out under dry N_2. After cooling to ice-bath temp, the mixture was acidified with cold dil 5% H_2SO_4, reheated at 70–80° for ½ hr, diluted with water to 50 ml and extracted with chloroform (4×15 ml). The chloroform soln was evaporated to dryness under reduced pressure (to remove t-BuOH), the residue macerated with sat $NaHCO_3$ aq (30 ml), and re-extracted with chloroform (3×15 ml). Work up of the chloroform extract gave a solid (108 mg) identified as unchanged starting material. The sodium bicarbonate soln was acidified with dil H_2SO_4 and extracted successively with (i) benzene (5×15 ml), (ii) chloroform (2×15 ml), (iii) chloroform (3×5 ml), and (iv) chloroform again (3×15 ml). All chloroform extracts showed absorption near 1785 cm^{-1} (γ-lactone), 1750 cm^{-1} (cyclopentanone), 1648 cm^{-1} (δ-lactone), but were treated separately. Extract (iii) was evaporated to dryness and dissolved in ether-MeOH and treated with an excess of diazomethane in the same solvent mixture. After 2 hr, the mixture was evaporated to dryness. The residue showed two peaks for $COOCH_3$ and two peaks for *quat*-C—CH_3 in the NMR spectrum. A portion of this crude methyl ester (34·0 mg) was chromatographed on a column of 7 g of Woelm neutral alumina (activity 2). After elution with benzene (150 ml) and light petroleum-benzene (1:1, 150 ml), the first 15 ml of chloroform eluate yielded 19·1 mg of material which was rechromatographed over 2 g of neutral alumina (activity 1). Elution with benzene–ether (1:1, 45 ml) furnished a forerun (3·3 mg). Elution with 15 ml of chloroform yielded a colorless solid (14·3 mg) which showed only one spot (R_f 0·25) in TLC on silica gel G plates developed with MeOH–benzene (1:99); λ_{max} 255 nm (ε 200), shoulder 300 nm (ε 60); ν max (CCl_4) 1787 cm^{-1} (γ-lactone), 1748 cm^{-1} (cyclopentanone), 1728 cm^{-1} (COOMe), 1648 cm^{-1} (δ-lactam), -no OH or NH absorption; δ = 1·17 (3H s, C—CH_3); 1·22 (3H t, *J* = 7·5 Hz, CH_3—CH_2N); 3·33 (3H s, OMe); 3·73 (3H s, COOMe). For analysis, the sample was "crystallized" from EtOAC. The first crop (9·3 mg) showed m.p. 90–92°. (Found: C, 63·15; H, 7·73. $C_{22}H_{33}NO_7$ requires: C, 65·43; H, 7·64%).

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