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STRUCTURE AND CONFIGURATION OF STENANZINE

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From the total alkaloids isolated by chloroform extraction of the epigeal part of *Rhinopetalum stenantherum* a new base has been isolated — stenanzine — with mp 203-205°C, $[\alpha]_D$ −44°, $C_{27}H_{43}NO_3$. On the basis of a study of the IR, NMR, and mass spectra of stenanzine and its conversion products the configuration and structure of 3β,23α-dihydroxy-5α-veratr-12-enin-6-one have been established for this alkaloid.

From the combined alkaloids obtained by the chloroform extraction of the epigeal part of *Rhinopetalum stenantherum* Rgl. [1, 2], by chromatography on a column of alumina, a new alkaloid stenanzine with mp 203-205°C $[\alpha]_D$ −44° $C_{27}H_{43}NO_3$, (I), has been isolated.

The IR spectrum of (I) shows absorption bands at (cm^{-1}) 3425-3125 (—OH, NH—), 2930-2830, 1475, 1455, 1420 (—CH_3; —CH_2—); 1713 (C=O). The mass-spectrometric fragmentation of stenanzine took place similarly to that of veratramine: peaks were observed with m/z 96, 114 (100%), 115, 141, 256, 315, $(M-1)^+$, 429 M^+ [3-5].

The acetylation of stenanzine with acetic anhydride in pyridine yielded 0,0',N-triacetylstenanzine (II), the IR spectrum of which had absorption bands at (cm^{-1}) 1740, 1245 (C=O, ester); 1717 (C=O), and 1648 (N—$COCH_3$) and no absorption bands of hydroxy groups. When 0,0',-N-triacetylstenanzine was saponified in a methanolic solution of caustic soda, N-acetylstenanzine (III) was obtained with M^+ 471. Its IR spectrum contained absorption bands at (cm^{-1}) 3450 (OH); 2970-2860, 1455, 1430 (—CH_3; —CH_2—); 1717 (C=O); and 1595 (N—$COCH_3$), and the absorption bands of the ester carbonyl group had disappeared.

The reduction of stenanzine with sodium tetrahydroborate led to a dihydro derivative $C_{27}H_{45}NO_3$ (IV), M^+ 431. Details of the NMR spectra of (I) and (II) are given in Table 1.

A comparison of the NMR and mass spectra of stenanzine and of peimisine (V) (see Table) [3] shows that stenanzine belongs to the C-nor,D-homosteroid alkaloids of the jervine group [3-5]. The NMR spectrum of (I) shows the signals from two protons geminal to hydroxy groups at 3.76 ppm (br.s, $W_{1/2}$ = 6 Hz) and 3.65 ppm ($W_{1/2}$ = 22 Hz). Consequently, both hydroxy groups have a secondary nature, as was confirmed by the production of 0,0',N-triacetylstenanzine. In the mass spectrum of (I), together with the peak of the molecular ion with m/z 429, the peak of an ion with m/z 114 (100%) is also observed, which shows the position of one of the hydroxy groups in ring F [4, 5]. The hydroxy group may occupy one of the two possible positions at C_{23} and C_{24}. Of these, from biogenetic considerations, the position at C_{23} is most suitable.

The position of the other secondary hydroxy group and of the carbonyl group was determined by comparing the chemical shifts of the 19-CH_3 group of stenanzine (I) and its acetate with those of the 19-CH_3 groups in the spectra of peimisine (V) and its acetyl derivative

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TABLE 1

Substance	Chemical shifts, δ*							
	19-CH_3 s	18-CH_3 s	21-CH_3 d	27-CH_3 d	$OCOCH_3$ s	N–$COCH_3$ s	$H_3HC-OCOCH_3$ m	$H_3H-C-OH$ m
I	0,63	1,56	0,87 J=7 Hz	0,93 J=7 Hz				3,76; 3,65
II	0,66	1,66	0,79 J=7 Hz	0,95 J=7 Hz	1,96 (6H)	2,04	5,04; 4,61	
III	0,62	1,56	0,85	0,90				
IV	0,64	1,65	0,79	0,97	1,96 (3H)	2,04	4,64	

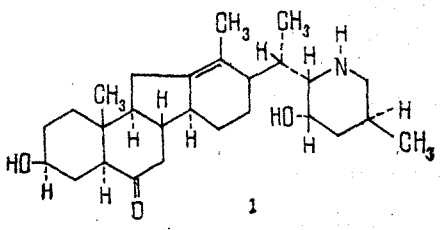
*s — singlet; d — doublet; m — multiplet.

(VI) (see Table 1). As can be seen from Table 1, the positions of the signals of the 19-CH_3 groups in the NMR spectra of (I) and (II) are close to those in (V) and (VI), which shows the position of the hydroxy group at C_3 and of the carbonyl group at C_6, and also the trans linkage of rings A/B and B/C.

From the value of its chemical shift and the half-width of the C_3—H signal, the hydroxy group at C_3 is present in the equatorial orientation (C_3—H is α-axial). This is confirmed by the existence of a multiplet at 4.61 ppm from C-3αH in the NMR spectrum of 0,0',N-triacetylstenanzine [6, 7].

The signal of a second proton geminal to an acetoxy group at 5.04 ppm in the NMR spectrum of 0,0',N-triacetylstenanzine shows the axial orientation of the C_{23}—OH group and the equatorial orientation of C_{23}—H [4, 8, 9]. The orientations of the methyl groups at C_{20} and C_{25} were determined on the basis of the following facts. The chemical shifts of the 21-CH_3 and 27-CH_3 groups of stenanzine and of peimisine are almost identical (see Table 1), which shows the identical orientations of these methyl groups [3, 9].

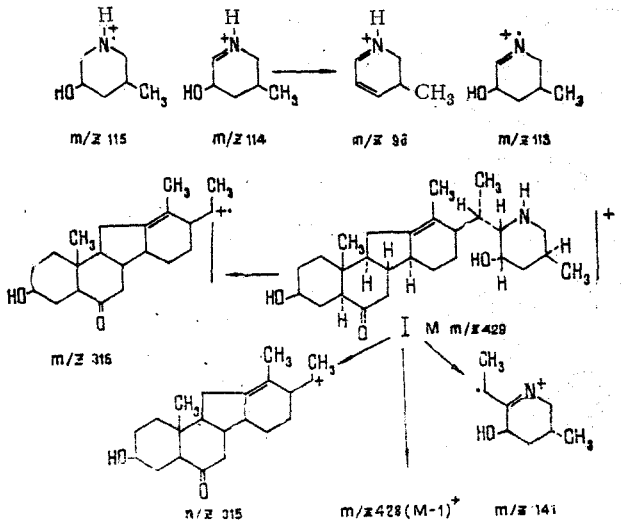
On the basis of the facts given, for stenanzine we propose as the most probable structure and configuration that of 3β,23α-dihydroxy-5α-veratr-12-enin-6-one [10].



1

The mass spectra of stenanzine and its derivatives confirmed the correctness of the proposed structure and permitted the structural features of similar compounds to be studied.

The fragmentation of stenanzine under electron impact forms the ions given in the following scheme:



The molecular peak of stenanzine, M^+ 429, is less intense than the peak of the $(M-1)^+$ ion. In agreement with literature information [4, 5, 10], it may be assumed that this is characteristic for all alkaloids of the veratramine type. As the result of the cleavage of a bond in the α,β position with respect to the nitrogen atom, ions with m/z 113, 114, 115, 315, and 316 are formed. An ion with m/z 96 can be formed from the ion with m/z 114 after the splitting out of water. An ion with m/z 141 is formed as the result of β-cleavage with the migration of hydrogen to the neutral fragment.

EXPERIMENTAL

KSK silica gel (100 μ) was used for TLC with the chloroform–methanol (10:1) system and Dragendorff's solution as the revealing agent. IR spectra (KBr tablets) were taken on a UR-20 spectrophotometer, NMR spectra on a JNM-4H-100/100 MHz instrument with $CDCl_3$ as solvent and HMDS as internal standard (the values are given in the δ scale), and mass spectra on a MKh 1310 instrument using a system for the direct introduction of the sample at an ionizing voltage of 50 V with a collector current of 60 μA, and a temperature of the separator of 110-120°C.

Separation of the Combined Alkaloids. The combined alkaloids isolated by chloroform extraction [2] (7.76 g) were dissolved in chloroform–methanol (10:0.2) and chromatographed on a column of alumina with elution by chloroform and chloroform–ethanol (10:0.2, 10:0.5, and 10:1).

From the methanol–chloroform (10:0.5) eluate, after rechromatography on a column of silica gel with elution by the same solvent system, stenanzine was obtained with mp 203-205°C (acetone), $[\alpha]_D$ −44° (c 0.5; chloroform). M^+ 429 (mass spectrometry), R_f 0.12.

0,0'-N-Triacetylstenanzine. A mixture of 90 mg of stenanzine, 1 ml of pyridine, and 1 ml of acetic anhydride was kept at room temperature for three days. After the elimination of the solvent, 5% sulfuric acid was added to the residue and the mixture was then made alkaline with ammonia and extracted with chloroform, and the solvent was distilled off. This gave amorphous 0,0',N-triacetylstenanzine with R_f 0.86.

Mass spectrum: m/z 96, 138, 139, 198 (100%), 356, 357, 496, $(M-43)^+$, $(M-42)^+$, $(M-15)^+$ 555 M^+.

N-Acetylstenanzine. A solution of 80 g of 0,0',N-triacetylstenanzine in 1 ml of methanol was treated with 5 ml of 5% methanolic caustic potash and the mixture was heated under reflux for an hour. Then the solvent was evaporated off, water was added, a chloroform extract was made, and the chloroform was distilled off. Treatment of the residue with acetone gave N-acetylstenanzine with mp 235-236°C (acetone), R_f 0.65.

Mass spectrum: m/z 96, 114, 115, 156 (100%), 157, $(M-43)^+$, $(M-42)^+$, $(M-15)^+$, 471 M^+.

Dihydrostenanzine. To a solution of 20 mg of stenanzine in 3 ml of methanol was added 30 mg of sodium tetrahydroborate over 30 min. Then the reaction mixture was kept at room temperature for 30 min. The solvent was evaporated off and the residue was diluted with water and extracted with chloroform. Acetone treatment gave dihydrostenanzine with mp 255-257°C (acetone), R_f 0.07.

Mass spectrum: m/z 96, 113, 114 (100%), 115, 121, 141, 316, 317, $(M-1)^+$, 431 M^+.

SUMMARY

From the total alkaloids obtained by chloroform extraction of the epigeal part of *Rhinopetalum stenantherum* Rgl. the new alkaloid stenanzine has been isolated. Its structure and configuration have been established as 3β,23α-dihydroxy-5α-veratr-12-enin-6-one.

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AMYLASE INHIBITORS OF *Actiniae* OF THE CARIBBEAN SEA

AMYLASE INHIBITOR OF PROTEIN NATURE FROM *Stoichactis helianthus*

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Natural amylase inhibitors have been found in actiniae of the Caribbean Sea. From the actinia *S. helianthus* an inhibitor has been isolated by gel filtration on Sephadex G-50 and ion-exchange chromatography on CM-cellulose that is highly specific in relation to the amylases of marine mollusks and is inactive in relation of the amylases from other sources.

Amylase inhibitors of protein nature have been detected in wheat and barley [1] and in potato tubers [2], and also in the bacterium *Streptomyces griseosporeus* YM-15 [3, 4]. The amylase inhibitors from wheat have been studied most fully [1, 5-9].

In a systematic search for carbohydrases in marine invertebrates [10] it has been found that extracts of some species of *Actinia* lack the amylase and β-1,3-glucanase activity that is widespread in the animal kingdom. It must be assumed that these sources contain an inhibitor of carbohydrases.

We present the result of a study of the protein amylase inhibitors which we have detected in actiniae of the Caribbean Sea.

The inhibiting capacity of an extract of one of the actinia — *Stoichactis helianthus* — was tested on 13 carbohydrases (six β-1,3-glucanases and seven amylases) from various sources (see Table 1). It can be seen from Table 1 that the amylase activities of the crystalline styles of the mollusks *Chlamys abbidus* [11], *Spisula sachalinensis* [12], and *Patinopecten* sp. [12] are effectively inhibited by an extract of *S. helianthus* (1-5 μg/ml). The α-amylase of human saliva was completely inhibited at concentrations of the extract of about 1 mg/ml. The remaining amylases, and also β-1,3-glucanases from various sources were not inhibited in the range of concentrations of the extract tested. It is possible that the inhibitor contained in *S. helianthus* is specific in relation to the amylases of marine mollusks.

These facts do not contradict the behavior of known protein inhibitors. Thus, the specificity of the inhibitors from wheat were tested on 66 amylases from various sources [6]. The amylases of plants, birds, and mammals were far less subject to their action than the amylases of marine origin and those from insects. The inhibitors from the bacterium *S. griseosporeus* YM-25 acted only on α-amylases of animal origin [3].

It can be seen from Table 2 that the inhibiting capacity of an extract of *S. helianthus* is 2-3 orders of magnitude higher than for extracts of other species of actiniae with respect to the amylase of *Ch. abbidus*. They did not act on the β-1,3-glucanase of *Ch. abbidus*.

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