STRUCTURE AND ABSOLUTE CONFIGURATION OF SERPENTICINE, A NEW ANHYDRONIUM BASE FROM RAUWOLFIA VOMITORIA AFZUELIA

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Abstract — The structure and absolute configuration 9 of serpenticine, a new anhydronium base isolated from Rauwolfia vomitoria Afz., has been determined with the aid of UV, IR and ^{13}C-nmr spectral data of the base along with ORD, proton nmr and high resolution mass spectra of its tetrahydro product 8.

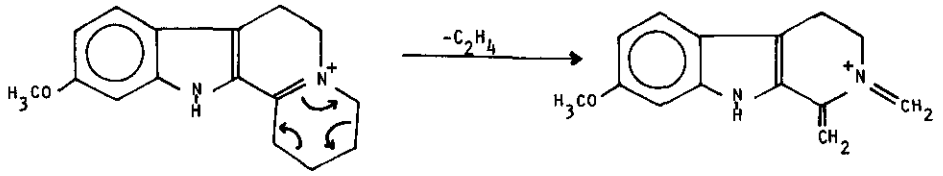
The isolation and structure of two new alkaloids from the methanolic extract of Rauwolfia vomitoria, has been communicated earlier.[1,2] Following further studies in the alkaloidal constituents, a new yellow anhydronium base has been isolated from the strongly basic fraction and provisionally named as serpenticine. The procedure of its isolation, details of which are given in the experimental, was based on the sparing solubility of its hydroiodide in water and alcohol. Serpenticine forms lemon yellow shining plates, melts at 275-276° and analysed for $C_{22}H_{22}N_2O_4.5H_2O$. It contains one C-methyl and two methoxyl groups.

The UV spectrum of serpenticine is very similar to serpentine, showing maxima at 265 nm (log ε 4.4), 310 nm (log ε 4.2) and 365 nm (log ε 3.6). The IR spectrum shows indolic NH (3400 cm^{-1}) and twin peaks of equal intensity at 1695 cm^{-1} and 1618 cm^{-1} due to carbonyl and C=C in the grouping $H_3COOC-\underset{\beta}{C}=CH-OR$.[3,4] Prominent bands at 1580, 1460 cm^{-1} and other bands at lower frequency region between 650-900 cm^{-1} provided evidence of aromaticity. The absorption at 1200 cm^{-1} represented C-O-C asymmetric stretching in the grouping C=C-O-C while that at 1260 cm^{-1} corresponded to $O-CH_3$ group. The multiple bands from 1235-1300 cm^{-1} are due to the C-C(=O)-C stretching of the ester of α,β-unsaturated acid. The completely dehydrated base in vacuo did not show the presence of NH group. This along with colour, strongly basic character and UV spectrum, strongly suggested an anhydronium base structure for serpenticine.

On catalytic reduction serpenticine yields tetrahydroserpenticine $C_{22}H_{26}N_2O_4$, m.p. 264-265°C, $(\alpha)_D^{25}$ +68° (chloroform). The presence of methoxycarbonyl group was established by alkaline hydrolysis of tetrahydroserpenticine to an amorphous amino acid - tetrahydroserpenticinic acid, from which the mother base could be regenerated by esterification with diazomethane. The UV spectrum of tetrahydroserpenticine exhibits maxima at 230 and 298 nm, with inflection at 246-250 nm. The summation of the UV spectra of 2,3-dimethyl-6-methoxyindole with 2,6-dimethyl-3-carbomethoxy-5, 6-dihydro-1,4-pyran is superimposable on the spectrum of tetrahydroserpenticine, and the latter is therefore to be considered as a derivative

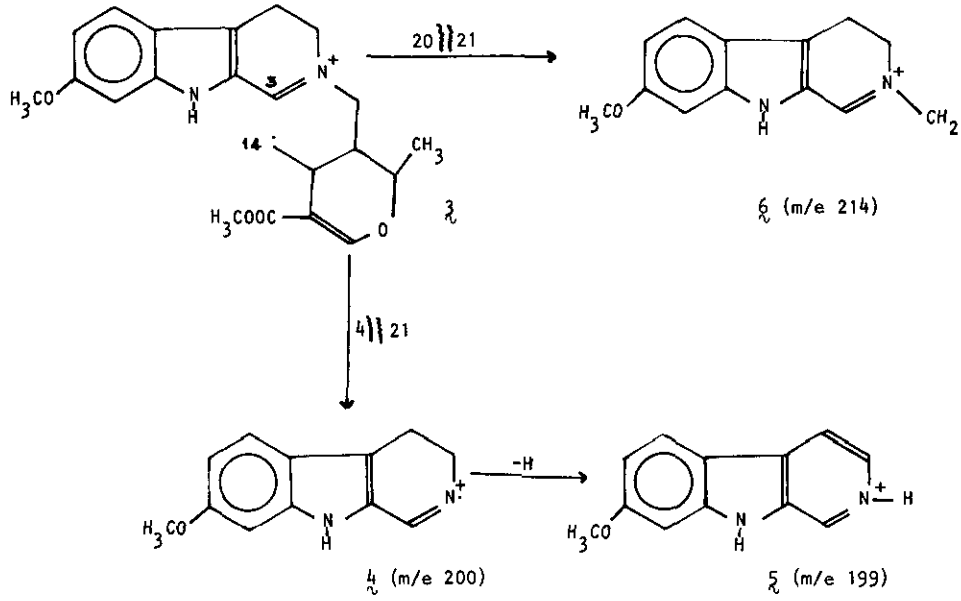
of 7-methoxytetrahydrocarboline. This part of the structure in the molecule is supported by the positive Adamkiewicz reaction, as well as close similarity in UV and IR spectra of tetrahydroserpenticine with those of *tetraphylline*[5] and reserpinine.[6]

The high resolution mass spectrum of tetrahydroserpenticine shows a fragmentation pattern similar to that of ajmalicine and yohimbine[7-9] with an increment of 30 for all the fragments containing the benzene ring. The molecular ion peak was at 382.1892 which agrees with the molecular formula $C_{22}H_{26}N_2O_4$. Aside of that, it showed intense M-1 peak at 381 resulting from the loss of a hydrogen atom from C-3, and peaks at m/e 367, 351 and 323 due to the loss of methyl group, $O-CH_3$ and $COOCH_3$ respectively. The peak at m/e 255 is due to fragment 1 of composition $C_{16}H_{19}N_2O$. It arises from the cleavage at D/E ring junction.[7] The loss of ethylene from 1 gives another prominent peak at m/e 227 for fragment 2.



1 (m/e 255) 2 (m/e 227)

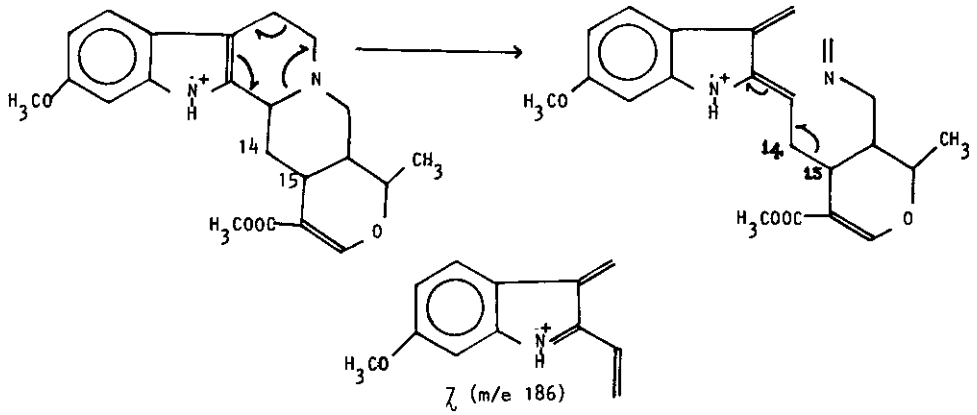
The strong peaks at m/e 214 ($C_{13}H_{14}N_2O$), 200 ($C_{12}H_{12}N_2O$) and 199 ($C_{12}H_{11}N_2O$) result from the homolysis of allylically labilized 3-14 bond of tetrahydroserpenticine to give the intermediate 3.[7] Further cleavage of the 4-21 linkage then leads to the ionized dihydro-β-carboline 4 (m/e 200), while the expulsion of an additional hydrogen atom from 4 offers a route to the β-carbolinium ion 5 (m/e 199). On the other hand, cleavage at 20-21 linkage gives ion radical 6 (m/e 214).



6 (m/e 214)

4 (m/e 200) 5 (m/e 199)

A very intense peak at m/e 186 arises from the ion $C_{12}H_{12}NO$. Its genesis can be explained through the mechanism proposed earlier by Djerassi et al.[7] which involves retro Diels - Alder fragmentation of ring C, followed by homolytic fission of the allylically activated 14-15 bond with generation of the conjugated ion 7 (m/e 186).



7 (m/e 186)

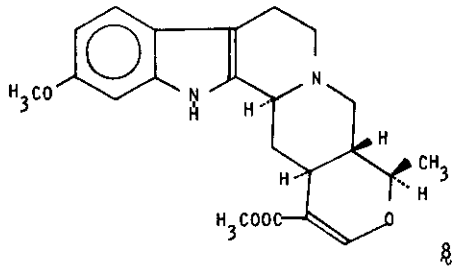
On the basis of cumulative evidence tetrahydroserpenticine appears to be a stereoisomer of reserpinine, isoreserpinine and tetraphylline. This was supported by the proton nmr spectrum in $CDCl_3$. It showed a 1H broad singlet at δ 8.20 for indolic NH, a three proton doublet at δ 1.35 for C-19 methyl (J=7.1 Hz), a sharp 3H singlet at δ 3.89 for aromatic methoxyl group and another 3H singlet at δ 3.7 due to $COOCH_3$. A one proton singlet at δ 7.5 is attributed to the ethylenic proton in the grouping ROOC-C=CH-OR. The signal for H-19 comes at δ 4.3. It is broken into a quartet by the methyl group (J=7.1 Hz) and then into an octet due to proton at C-20 (J=10.5 Hz). A doublet at δ 6.8 may be assigned to H-12 which has a higher electron density. The proton showed meta coupling with H-10 (J=2.5 Hz) and a small para coupling with H-9 (J=0.5 Hz) visible under high resolution only. The signal of H-10 is at δ 7.7 as a distorted quartet. It exhibited ortho coupling with H-9 (J=9 Hz) and meta coupling with H-12 (J=2.5 Hz). The H-9 signal is again a doublet at δ 8.00 showing ortho coupling with H-10 (J=9 Hz) and para coupling with H-12 (J=0.5 Hz) visible under high resolution only.

Tetrahydroserpenticine showed a complex pattern of Bohlmann bands in the IR spectrum between 2700-2900 cm^{-1}, which is typical of C-3 hydrogen α and axial to ring D.[10-13] The C/D ring junction is therefore trans with reference to the bond C-3 to H and the free electronic doublet of N_b. This conclusion is further supported by proton nmr spectrum of N_b-methiodide of tetrahydroserpenticine which showed N^+-CH_3 chemical shift at δ 3.36, which is typical of trans quinolizidine system.[6]

The configuration at C-20 was established through ORD studies. Tetrahydroserpenticine gave two Cotton effects, one at 270-278 mμ (shoulder, $[\phi]$=+8900 to + 8450) and the other at 238-253 mμ (peak at 253 mμ, $[\phi]$=+ 15100; trough at 238 mμ, $[\phi]$=- 3500; molecular amplitude 186). The latter arises from α,β-unsaturated ester grouping in ring E.[14] The positive sign of this effect can be explained by any one of the three possible combinations (3α,20β-; 3β,20α- and 3β,20β-).[14] In view

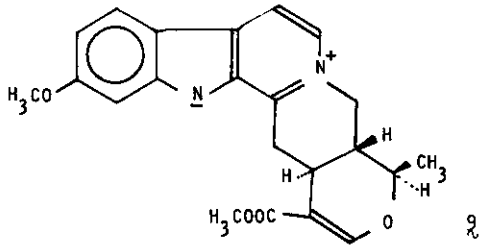
of axial hydrogen at C-3, the acceptable combination is 3α,20β. This is further confirmed by small amplitude of Cotton effect at 235-255 nm. It depends on relative positions of the two chromophores (α,β-unsaturated ester and indole nucleus). In 3α,20β combination the two chromophores are more nearly coplanar, and the ORD amplitude is small.[14] Considering α configuration at C-15 (Wenkert rule),[15] the D/E ring junction is trans and tetrahydroserpenticine, therefore, belongs to normal series of heteroyohimbine alkaloids.

The configuration at C-19 was established through proton nmr spectrum. Ajmalicine and tetraphylline, having α and axial configuration of C-19 methyl group, show methyl doublet at δ 1.16, further upfield than any other heteroyohimbine alkaloid.[14] The methyl doublet of tetrahydroserpenticine comes at δ 1.35 which compares well with those of raumitorine and rauvanine, having β and equatorial configuration of C-19 methyl group. Tetrahydroserpenticine is, therefore, 19-epimer of tetraphylline. This is further confirmed by large coupling constant (J=10.5 Hz) between H-19 and H-20 due to trans diaxial interaction. The absolute configuration of tetrahydroserpenticine can therefore be represented as 8.



8

Tetrahydroserpenticine is a reduction product of serpenticine, into which it can be converted back through dehydrogenation with lead tetraacetate. Consequently the following structure 9 can be assigned to serpenticine, keeping in view the relationship between ajmalcine and serpentine.



9

Conclusive support to the above structure is provided by ^{13}C-nmr spectrum of serpenticine. It showed the following signals which were identified through comparison with the spectrum of serpentine. Of particular significance in the context of this data is the downfield shift for the singlet of C-11 due to oxygen substituent, and also comparative downfield shifts of C-19 and C-24 due to epimerization

at C-19. Such shifts have been described by Wenkert et al.[16] in case of ajmalicine and 19-epimer of its iso base. The chemical shifts are referred to tetramethylsilane (δ 0.00).

Carbon atom	Multiplicity*	Serpenticine	Serpentine
C-2	singlet	128.48	128.50
C-3	singlet	156.12	156.10
C-5	doublet	143.51	143.52
C-6	doublet	117.72	117.77
C-7	singlet	106.33	106.24
C-8	singlet	126.98	126.92
C-9	doublet	117.47	117.41
C-10	doublet	109.2	118.99
C-11	singlet	155.6	119.1(d)
C-12	doublet	95.67	110.85
C-13	singlet	136.1	135.95
C-14	triplet	34.44	34.32
C-15	doublet	30.75	30.21
C-16	singlet	106.66	106.5
C-17	doublet	154.24	154.27
C-19	doublet	75.6	73.2
C-20	doublet	43.91	40.45
C-21	triplet	70.00	71.28
C-22	singlet	166.91	166.95
C-23	quartet	50.7	50.8
C-24	quartet	17.85	14.00
O-CH_3	quartet	55.8	-

*Refers to off resonance spectrum.

EXPERIMENTAL

Darkish viscous methanolic extract of Rauwolfia vomitoria (2 kg) was partitioned between ethyl acetate and water. The aqueous filtrate was successively treated with NaCl and KI to separate the bases forming sparingly soluble hydrochloride and hydroiodide salts. Through a repeated procedure of solvation of the hydroiodide in ethanol and gradual precipitation with the addition of water a dilute ethanol insoluble fraction of the hydroiodide was eventually obtained along with fraction which was fairly soluble in it. The insoluble fraction was dissolved in dilute acetic acid and treated with a solution of KI till turbidity and kept in cold. The light brown sticky precipitate crystallized out from methanol in prismatic rods, m.p. 286-289°C. The free base liberated from it with caustic alkali was fractionally crystallized from 1:2 methanolic benzene. From the top fractions serpenticine was obtained as lemon yellow shining plates which showed single spot on HPLC and melted at 275-276°C (yield 2 g; 0.1% on the weight of the crude methanolic extract).

The combined filtrate of the hydroiodide referred to above, was saturated with KI and the resulting hydroiodide was sucked and well washed with water and dried. After repeated solvation of the hydroiodide in alcohol and purification with the addition of small quantities of water, the fraction comparatively more soluble in ethanol-water, afforded crystalline hydroiodide (380 mg). It was worked up according to the reported procedure[17] to yield crystalline base which could be identified through mixed m.p. and superimposable spectral data as alstonine.

Characterization of serpenticine: The base is soluble in chloroform, a mixture of hot ethanol or methanol with benzene, sparingly soluble in ethanol, methanol, benzene, ether and water, and practically insoluble in pet. ether. It analyzed for $C_{22}H_{22}N_2O_4.5H_2O$ with 2-methoxyls and one C-methyl group. (Found: C, 56.37; H, 6.80; N, 6.00; O, 30.83; OCH_3, 13.1; C-CH_3, 3.3%. Calcd. for $C_{22}H_{22}N_2O_4.5H_2O$: C, 56.41; H, 6.83; N, 5.98; O, 30.76; OCH_3, 13.24 and C-CH_3, 3.20%).

Catalytic reduction of serpenticine - tetrahydroserpenticine: Serpenticine (2g) was dissolved in absolute methanol (200 ml), brought to pH 10 with methanolic alkali and treated with hydrogen over platinum oxide (400 mg) for 10 hr. The solution was warmed to dissolve the product, filtered, concentrated in vacuo and diluted with water. On cooling in the ice chest colourless prismatic plates of tetrahydroserpenticine (1.4 g) were obtained which melted at 264-265°C, $(\alpha)_D^{25}$ +68° ($CHCl_3$) and analyzed for $C_{22}H_{26}N_2O_4$ (Found: C, 69.23; H, 6.77; N, 7.19 and M^+ peak at 382. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.10; H, 6.80; N, 7.33% and mol.wt. 382).

Alkaline hydrolysis of tetrahydroserpenticine: A solution of the base (200 mg) in 2N methanolic KOH (10 ml) was refluxed for 5 hr. At the end of this period, the golden yellow solution was cooled and treated with hydrochloric acid till acidic to Congo red. The precipitated potassium chloride was filtered off and the filtrate was freed of the solvent in vacuo. The resulting hydrochloride was worked up by following the procedure reported earlier for tetraphylline[5] and aricine,[18] to afford tetrahydroserpenticinic acid as an amorphous powder which on drying at 100°C in vacuo, analysed for $C_{21}H_{24}N_2O_4$ (Found: C, 68.51; H, 6.49; N, 7.80% and M^+ peak at 368. Calcd. for $C_{21}H_{24}N_2O_4$: C, 68.47; H, 6.52; N, 7.60% and mol. wt. 368). The methanolic solution of the acid on treatment with ethereal solution of diazomethane, yielded crystalline base which could be identified through mixed m.p. and comparative Rf value as serpenticine.

Salts of serpenticine:

Hydrochloride:	bright yellow glistening rods, m.p. 289-290°C
Hydrobromide:	light yellow rectangular plates, m.p. 292°C.
Picrate:	lemon yellow needles, m.p. 196-198°C.
Methiodide:	lemon yellow slender needles, m.p. 282°C.
Chloroplatinate:	pale yellow needles, m.p. 246°C.

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