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CHEMICAL CONSTITUENTS OF *ALSTONIA VENENATA* R.Br.

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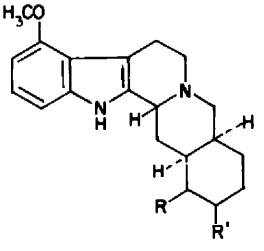
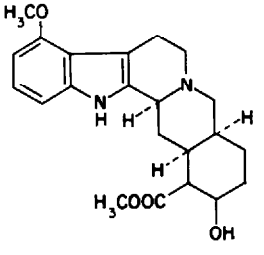
Abstract—Venenatine and isovenenatine, two new indole alkaloids from *Alstonia venenata* R.Br., have been shown to have structures I and V respectively.

IN A preliminary communication[1] we have described the chemical examination of the bark of *Alstonia venenata* R.Br. The arguments leading to the assignment of structures I and V respectively for the two alkaloids, venenatine and isovenenatine obtained from this plant have already been presented. We wish to record here experimental details of this work and the relevant spectra.

The methoxyl group in venenatine and isovenenatine have been assigned position 9 in the yohimbine skeleton on the basis of the similarity of their UV absorption spectra with that of 5-methoxy-1,2,3,4-tetrahydrocarbazole and the close resemblance of the aromatic proton region of their NMR spectra with that of mitragynine. It was observed earlier that dehydrogenation of venenatine (I) and venenatyl alcohol (III) failed to yield any identifiable product. Selenium dehydrogenation of venenatic acid (II), however, yields a methoxyyobyrine.[2] This has been identified as



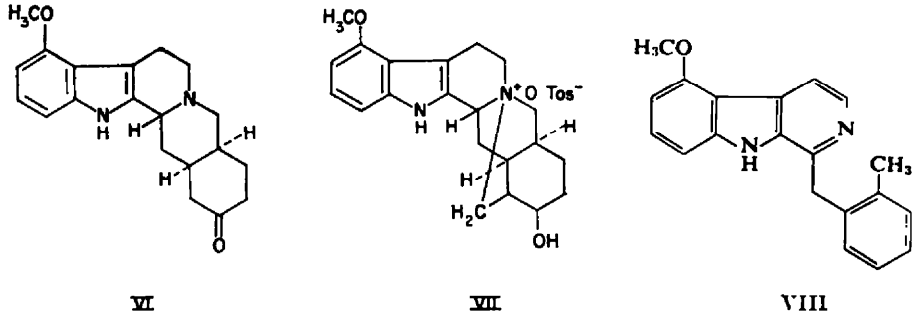


V

I: R = $COOCH_3$; R' = OH II: R = COOH, R' = OH
III: R = CH_2OH; R' = OH IV: R = $COOCH_3$; R' = O Tos

[1] T. R. Govindachari, N. Viswanathan, B. R. Pai and T. S. Savitri, *Tetrahedron Letters* No. 16, 901 (1964).
[2] A. B. Ray and A. Chatterjee, *J. Ind. Chem. Soc.* **41**, 638 (1964).

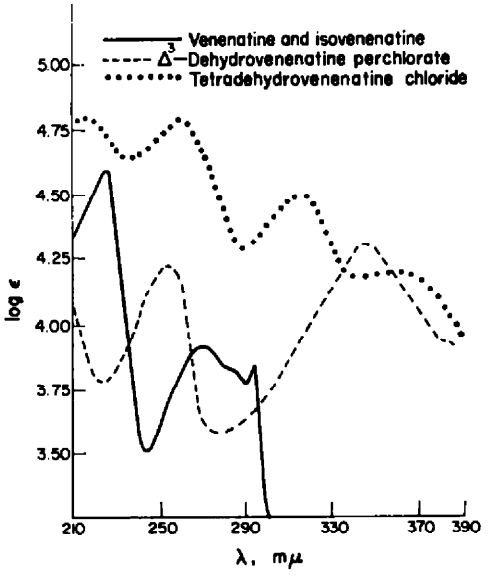
5-methoxy-yobyrine (VIII) by comparison with a synthetic sample,[3] thus confirming chemically the location of the methoxyl group.



VI VII VIII

(a)

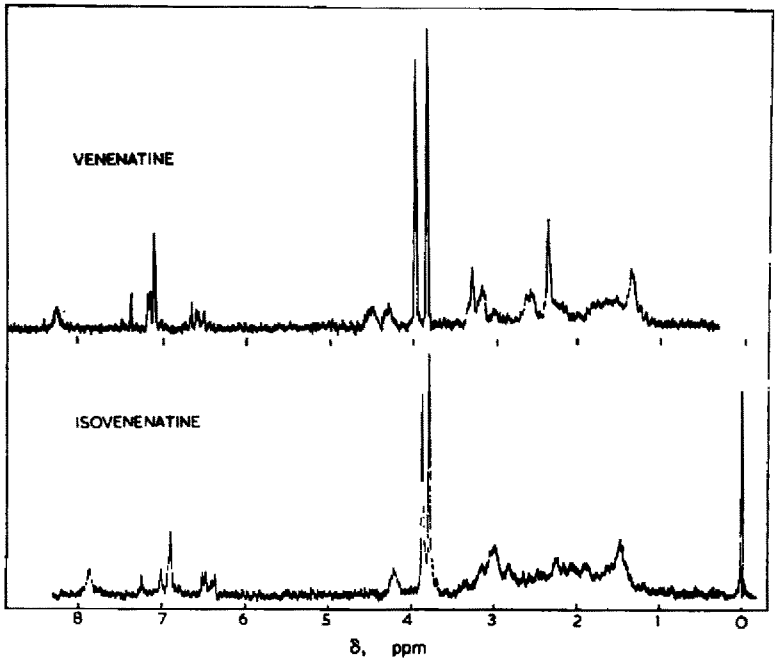
Recently, there have been some communications by Chatterjee *et al.* on the chemical investigation of *Alstonia venenata*. In their first note,[4] the authors reported the isolation of an alkaloid "alstovenine" which from the physical constants appears to be identical with isovenenatine. They suggested erroneously the presence of a 7-methoxy-β-carboline moiety in this on the basis of a colour reaction. In a later communication,[2] subsequent to ours,[1] the authors reported the isolation of venenatine and reassigned the methoxyl tentatively to position 9. Very recently they have observed the presence of venenatine-N_b-oxide (venoxidine) in the plant in addition to reserpine and kopsinine.



(b)

[3] T. R. Govindachari, P. Madhavan Pillai, K. Nagarajan and N. Viswanathan, *Tetrahedron* **21**, 2957 (1965).

[4] A. B. Ray and A. Chatterjee, *J. Ind. Chem. Soc.* **40**, 1043 (1963).



(c)

EXPERIMENTAL

All m.p.s are uncorrected. Rotations were taken in $CHCl_3$ solutions. IR and UV spectra were determined in CH_2Cl_2 and EtOH solutions respectively. NMR spectra were taken in $CDCl_3$ at 60 MC.

Extraction

1. *Isolation of stigmasterol*

Finely powdered bark (5 kg) of *Alstonia venenata* was extracted with cold hexane. Evaporation of the hexane extract gave a viscous brown gum from which by trituration with ether a colourless solid was obtained. Repeated crystallization from MeOH gave stigmasterol (0·5 g), m.p. and mixed m.p. with an authentic sample, 165–166°, $[\alpha]_D$ −53·5°. (Found: C, 84·67; H, 11·91. Calc. for $C_{29}H_{48}O$. C, 84·40; H, 11·72%.) The *acetate*, prepared in the usual way, had m.p. 140°, $[\alpha]_D$ −49·9°. (Found: C, 81·79; H, 11·35. Calc. for $C_{31}H_{50}O_2$: C, 81·88; H, 11·08%.). The *benzoate* had m.p. and mixed m.p. with an authentic sample, 158–159°, $[\alpha]_D$ −25·3°. (Found: C, 83·81; H, 10·13. Calc. for $C_{36}H_{52}O_2$: C, 83·66; H, 10·14%.)

2. *Isolation of the alkaloids*

The defatted plant material was extracted with cold acetone for 3 days. The solvent was evaporated from the extract and the residue diluted with water and left overnight. The supernatant liquid was decanted from the resinous gum, basified with solid $CaCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried (Na_2SO_4) and distilled. The residue was chromatographed over neutral alumina (600 g) in $CHCl_3$ and the eluate collected in 40 ml fractions.

(a) *Reserpine*. Fractions 1–5 in the chromatography were combined and evaporated. The material thus obtained was rechromatographed to yield reserpine (0·15 g), needles from MeOH, m.p. and mixed m.p. with an authentic sample, 260–262°. The UV and IR absorption spectra of the two samples were also identical. (Found: C, 64·87; H, 6·74. Calc. for $C_{33}H_{40}N_2O_9$: C, 65·11; H, 6·62%.) Reserpine was obtained in somewhat better yields from the plant by extraction with MeOH.

(b) *Venenatine*. The combined material from fractions 6–25 in the chromatography was crystallized several times from MeOH to yield colourless needles of venenatine (3·8 g), m.p. 123–125° (dec),

$[\alpha]_D$ −76·1°, p*Ka* 7·2 ± 0·1 (methyl cellosolve), λ_{max} 226, 271, 293 mμ (log ε 4·57, 3·92, 3·81), λ sh 281 mμ (log ε 3·84), ν_{max} 3580, 1730, 1625, 1585, 1565, 1010 cm^{-1}. (Found: C, 66·22; H, 7·51; O, 18·92; N, 6·93; OCH_3, 14·51; H^+ 0·71. $C_{23}H_{28}N_2O_4 \cdot CH_3OH$ requires: C, 66·32; H, 7·74; O, 19·21, N, 6·73; 2 OCH_3, 14·90; 3 H^+ 0·72%.) The alkaloid gave a deep green colour in the Adamkiewicz test with conc H_2SO_4 and $FeCl_3$.

Venenatine picrate, crystallized from acetic acid, had m.p. 243° (dec). (Found: C, 54·45; H, 5·31. $C_{28}H_{31}N_5O_{11}$ requires: C, 54·55; H, 5·09%.) The *hydriodide*, crystallized from acetone, had m.p. 255–257° (dec). (Found: C, 51·14; H, 5·81; O, 12·31. $C_{23}H_{29}N_2O_4$ I requires: 51·56; H, 5·71; O, 12·49%.)

The *methiodide*, prepared in $CHCl_3$-solution, was crystallized from aqueous MeOH to yield needles, m.p. 288–290° (dec). (Found: C, 52·13; H, 5·84. $C_{24}H_{31}N_2O_4I$ requires: C, 52·47; H, 5·94%.) The *acetate* prepared in the usual way (acetic anhydride—pyridine), crystallized from aqueous MeOH as needles, m.p. 100–101° (dec). (Found: 67·14; H, 7·28; O, 18·67. $C_{24}H_{30}N_2O_5$ requires: C, 67·58; H, 7·09; O, 18·76%.)

(c) *Isovenenatine*. The residue from the mother liquors of venenatine was combined with the material obtained from the later fractions in the chromatography and subjected to a 30-stage counter-current distribution between $CHCl_3$ and I N HCl. The product from tubes 11–19 gave, after chromatography over alumina in $CHCl_3$, 1·2 g more venenatine and 0·15 g *isovenenatine*. The latter crystallized from MeOH as needles, m.p. 169–170° (dec), $[\alpha]_D$ +9·42°. (Found: C, 68·48; H, 7·69; N, 7·51. $C_{23}H_{28}N_2O_4$ requires: C, 68·72; H, 7·34; N, 7·29%.) On a silica gel plate, venenatine and isovenenatine had R_f 0·31 and 0·45 respectively using 85:15 $CHCl_3$–MeOH mixture. On alumina plate, they had R_f 0·21 and 0·46 respectively using 60:40 acetone–hexane mixture.

O-*Acetylisovenenatine* crystallized from aqueous MeOH as needles, m.p. 143° (dec). (Found: C, 65·97; H, 7·66. $C_{24}H_{30}O_5N_2 \cdot CH_3OH$ requires: C, 65·48; H, 7·47%.)

(d) *Kopsinine*. Tubes 20–27 in the above counter-current distribution gave 10 g of a gum which was again subjected to a 30-transfer counter-current distribution between $CHCl_3$ and citrate-phosphate buffer of pH 5. The material from tubes 5–19 was chromatographed over alumina in acetone–hexane (1:1) mixture. The earlier fractions of the eluate gave on oily base. Its hydrochloride crystallized from MeOH–ether as needles, m.p. 244–245°. (Found: C, 61·55; H, 7·28. $C_{21}H_{27}N_2O_2Cl \cdot 2H_2O$ requires: C, 61·39; H, 7·55%.) The base regenerated from this was crystallized from ether–hexane mixture to yield prisms of kopsinine (0·15 g), m.p. 103°, $[\alpha]_D$ −74·8°, λ_{max} 204, 246, 295 mμ (log ε 4·35, 3·83, 3·45). (Found: C, 74·85; H, 7·80; N, 8·52; OCH_3, 4·47. Calc. for $C_{21}H_{26}N_2O_2$: C, 74·52; H, 7·74; N, 8·28; 1 OCH_3, 4·43%.) The methiodide crystallized from MeOH as needles, m.p. 261–262° (dec). (Found: C, 55·20; H, 6·20. Calc. for $C_{22}H_{29}N_2O_2$ I: C, 55·00; N, 6·04%.) The picrate, prepared in ether solution, crystallized from MeOH as plates, m.p. 213–214° (dec), undepressed by admixture with authentic kopsinine picrate. (Found: C, 56·85; H, 5·43. Calc. for $C_{27}H_{29}N_5O_9$: C, 57·14; H, 5·15%.) The IR spectra of the two samples were identical.

3. *Venenatic acid*

Venenatine (1 g) was refluxed with methanolic KOH (1 N; 40 ml) for 4 hr. The solution was concentrated *in vacuo* to about 10 ml diluted with water (30 ml) and concentrated again *in vacuo* to about 20 ml. The solution was cooled and acidified with 1:1 aqueous acetic acid to pH 7. The precipitated solid was filtered, washed with water, dried and crystallized from EtOH to yield venenatic acid (0·9 g), m.p. 242° (dec). (Found: C, 61·59; H, 7·28. $C_{21}H_{30}N_2O_6 \cdot 2H_2O$ requires: C, 62·05; H, 7·44%.)

A solution of venenatic acid (0·3 g) in MeOH (20 ml) was saturated with dry HCl and left overnight at room temp. Evaporation *in vacuo* and basification with NH_4OH gave venenatine (0·25 g), m.p. and mixed m.p. 123–125° (dec). The IR spectrum of the product was identical with that of venenatine.

4. *Norvenenatic acid*

Venenatine (1 g) was refluxed with 3 N HCl (60 ml) for 4 hr. The solution was evaporated *in vacuo*, the residue dried thoroughly *in vacuo* and crystallized twice from MeOH–ether mixture to yield *norvenenatic acid hydrochloride*, m.p. 306–308° (dec), λ_{max} 223, 270, 285 and 295 mμ (log ε 4·58, 3·84, 3·69, 3·66). The maxima were shifted to 275, 300 mμ on addition of a drop of alkali. (Found: C, 61·24; H, 6·59; O, 16·55; OCH_3, nil. $C_{20}H_{25}N_2O_4Cl$ requires: C, 61·13; H, 6·41; O, 16·25%). It gave a deep blue colour with $FeCl_3$ and also with Gibb's reagent.

5. *Venenatyl alcohol*

A solution of venenatine (2 g) in dry tetrahydrofuran (20 ml) was added dropwise with stirring to a suspension of LAH (1·2 g) in ether (100 ml). The mixture was stirred for 3 hr, left overnight at room temp and decomposed with water. The ether–tetrahydrofuran solution was dried (Na_2SO_4) and evaporated to yield venenatyl alcohol as an amorphous solid (1·6 g). The *methiodide* prepared in $CHCl_3$ solution, crystallized from aqueous MeOH as needles, m.p. 293–295° (dec). (Found: C, 52·77; H, 6·26; O, 9·64; OCH_3, 3·12. $C_{22}H_{31}N_2O_3I$ requires: C, 53·02; H, 6·22; O, 9·64; OCH_3, 3·13%.)

6. *Tetradehydrovenenatine chloride*

A solution of lead tetra-acetate (2·2 g) in acetic acid (100 ml) was added dropwise to a stirred solution of venenatine (0·9 g) in acetic acid (25 ml) at 60° at such a rate that only a slight excess of oxidant was present always (starch–KI test). The acetic acid was removed *in vacuo*, $CHCl_3$ (150 ml) and water (25 ml) added to the residue and the mixture made just alkaline (pH 9) by slow addition of 50% NaOH aq. The $CHCl_3$-solution was dried (Na_2SO_4), acidified with ethanolic HCl and the solution evaporated *in vacuo*. The residue (0·5 g) was a red uncrystallizable glass, λ_{max} 260, 315, 360 mμ (log ε 4·79, 4·51, 4·20).

7. *Oppenauer oxidation of venenatine*

A solution of venenatine (0·5 g) in dry benzene (10 ml) was added to anhydrous potassium t-butoxide (freshly prepared from 0·125 g K) in an atm. of N_2. Fluorenone (1·13 g) was then added and the mixture refluxed for 48 hr. The product was cooled, treated with water and extracted with ether. The ether extract was washed with 2 N HCl, the acid solution basified with ammonia and re-extracted with $CHCl_3$ to yield 50 mg of a brown gum. The products from five such batches were combined and chromatographed in $CHCl_3$ over alumina. The amorphous solid so obtained was sublimed *in vacuo* ($0·8 \times 10^{-2}$ mm) to yield the *ketone* (VI; 15 mg), m.p. 140–144°, ν_{max} 1705 cm^{-1}. (Found: C, 74·31; H, 7·41; OCH_3, 4·46. $C_{20}H_{24}N_2O_2$ requires: C, 74·04; H, 7·46; 1 OCH_3, 4·63%.)

8. *7-Chloro-7H-Venenatine*

A well-stirred solution of venenatine (0·6 g) in dry CH_2Cl_2 (25 ml) was cooled in ice and treated with triethylamine (0·3 ml). To this was added dropwise a cold 0·1 M solution (18 ml) of t-butyl hypochlorite. The addition took 20 min. After stirring for 10 min more, the solution was washed with water, dried (Na_2SO_4) and evaporated to yield the chloroindolenine.

9. *Δ^3-Dehydrovenenatine chloride*

A solution of the above chloro compound (0·6 g) in MeOH (10 ml) was saturated with dry HCl and refluxed on a water bath for 10 min. The solution was evaporated to dryness *in vacuo* and the product used as such.

10. *Isovenenatine*

A solution of the above chloride in water (10 ml) was treated with $NaBH_4$ till the red colour of the solution disappeared. The buff-coloured precipitate was extracted with CH_2Cl_2 and the product chromatographed over alumina in acetone–hexane (1 : 1) to yield isovenenatine (0·35 g), needles from MeOH, m.p. 169–170° (dec), undepressed by admixture with a sample of isovenenatine obtained from the plant. The two samples also had identical IR spectra. (Found: C, 68·61; H, 7·45. $C_{22}H_{28}N_2O_4$ requires: C, 68·72; H, 7·34%.)

11. *Mercuric acetate oxidation of isovenenatine*

A solution of isovenenatine (0·2 g) and mercuric acetate (0·7 g) in 5% aqueous acetic acid (10 ml) was heated at 80–85°. After 3 hr the precipitate was filtered, the filtrate heated to boiling and saturated with H_2S. A small amount of HCl aq was added to the cooled solution and the latter heated till the mercuric sulphide had coagulated. This was filtered and the filtrate treated with perchloric acid. The precipitated *perchlorate*, crystallized from MeOH, had m.p. 220° (dec), λ_{max} 255, 346 mμ (log ε 4·22, 4·31). (Found: C, 51·62; 51·96; H, 5·64, 5·79; N, 5·50. $C_{22}H_{27}N_2O_6Cl \cdot 1\frac{1}{2} H_2O$ requires: C, 51·98; H, 5·91; N, 5·51%.) In a parallel experiment carried out with venenatine, no mercurous acetate precipitated even after 6 hr.

12. *Quaternary tosylate* (VII) *from venenatyl alcohol*

A solution of venenatyl alcohol (0·16 g) in pyridine (0·5 ml) was cooled to 0°, treated with *p*-toluenesulphonyl chloride (95 mg) and left overnight at room temp. The pyridine was evaporated *in vacuo* below 40° and the residue treated with ice-water. The precipitated solid was crystallized from acetone–MeOH to yield the *tosylate*, m.p. 265–267° (dec). (Found: C, 63·31, 63·48; H, 6·78, 7·06. $C_{28}H_{34}N_2O_5S \cdot H_2O$ requires: C, 63·63; H, 6·82%.)

A solution of the tosylate in hot water was treated with 20% KI aq. The precipitate thus obtained was filtered, washed with water and crystallized from MeOH to yield the quaternary *iodide*, m.p. 265–266° (dec). (Found: C, 50·10, 50·01; H, 6·30, 6·46. $C_{21}H_{27}N_2O_2I \cdot 2H_2O$ requires: C, 50·20; H, 6·22%.)

13. O-*Tosylvenenatine* (IV)

A solution of venenatine (0·75 g) in pyridine (4 ml) was cooled to 5° and treated with *p*-toluenesulphonyl chloride (1·5 g). The solution was maintained at 5° and then poured on ice water. Extraction with $CHCl_3$ and chromatography of the product over alumina in acetone–hexane (2:3) gave, in one of the fractions, the tosylate (30 mg), m.p. 143–145°. (Found: C, 64·98; H, 6·75. $C_{30}H_{34}N_2O_6S$ requires: C, 64·67; H, 6·36%.)

14. *Selenium dehydrogenation of venenatic acid*

A mixture of venenatic acid (4·4 g) and Se (6 g) was ground well in a mortar and divided into 6 portions. This was heated from 270° to 300° during 10 min and maintained at 300° for 5 min more. The cooled mixture was ground with alumina (10 g) and extracted with $CHCl_3$ in a soxhlet. The product obtained was dissolved in MeOH and treated with ethereal diazomethane. The residue got by evaporation of the solvent was chromatographed over alumina in benzene. One of the fractions gave, on trituration with ether–MeOH mixture, a pale yellow solid (50 mg). Repeated crystallization from MeOH gave needles of VIII, m.p. 229°, undepressed by admixture with a synthetic sample; λ_{max} 219, 247, 289, 335, 349 mμ. (log ε 4·58, 4·74, 4·17, 3·84, 3·89.) (Found: C, 75·09; H, 5·81; $O(CH_3)$, 4·35. $C_{20}H_{18}N_2O \cdot H_2O$ requires: C, 74·97; H, 6·29; 1 $O(CH_3)$, 4·69%.)

Acknowledgements—We thank Professor C. Djerassi for determining the mass spectra and drawing our attention to the similarity between venenatine and 3-epi-α-yohimbine. Thanks are also due to Professor H. Schmid for a sample of kopsinine picrate, Dr. K. Nagarajan for the NMR spectra and Dr. S. Selvavinayakam for the microanalysis.