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# STUDIES ON RHAZYA STRICTA DCNE.

## Part I.—Isolation of a new Alkaloid "Sewarine"

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A new alkaloid, *sewarine* has been isolated from an indigenous medicinal plant Sewar (*Rhazya stricta* Dcne.). On the basis of studies in the chemical constitution of sewarine which has been found to possess marked oncolytic activity, a structure for the base has been proposed, and some of its salts and derivatives have been described.

*Rhazya stricta*[1] Decaisne (*apocynaceae*), a small glabrous erect shrub, is widely distributed in western Asia, and abundantly found in various regions of West Pakistan. It is reputed in the indigenous system of medicine for the treatment of a variety of ailments.[2–4] Since Chatterji *et al.*[5] reported the isolation of five alkaloids from *Rhazya stricta* in 1961, a considerable amount of work has been carried out in this field, but though the total alkaloidal content of the plant material has been variously reported[5a,7] to be as high as 8-10%; the yields of individual uniform crystalline bases have been extremely poor. Again, some of the alkaloids which were considered to be new were later identified with known bases.[6] The present position in regard to their physical data, structure and yields are given in Table 1.

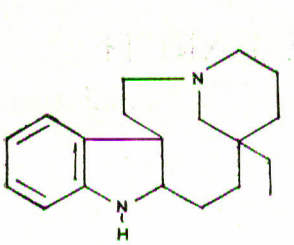
alkaloids 'Vinblastine',[9a,c] and 'Vincristine',[9b,c] have been found to possess pronounced antileukemic and also general anti-cancer activities, the present authors considered it of interest to reinvestigate the alkaloidal constituents of *Rhazya stricta*, which like *Vinca rosea*, also belongs to the alkaloid-rich *apocynaceae* family.

As a result of these investigations, which were based on the alcoholic extract of fresh undried leaves of *Rhazya stricta*, it has been possible to isolate a new base which has been named as *sewarine* after the local name *Sewar* of *Rhazya stricta* in the Sind area, from where the plant material was collected. The base, sewarine could not be separated by chromatographic techniques, which yielded small quantities of a number of known crystalline alkaloids, none of which was found

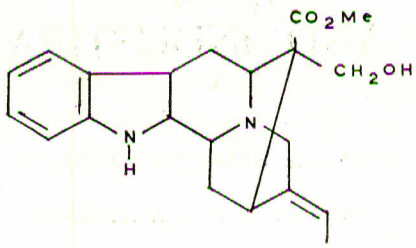
TABLE 1.

| S. No. | Name | Mol. Formula | M.P. | $[\alpha]_D$ | Yield % |
|---|---|---|---|---|---|
| 1. | (–) Quebrachamine[5a](I) | $C_{19}H_{26}N_2$ | 141° | –117° | 0.06 |
| 2. | Rhazine[5a] (II) (Akuammidine) | $C_{21}H_{24}N_2O_3$ | 234-236°d | ±0°($CHCl_3$) | 0.15 |
| 3. | Rhazinine[5a] | $C_{19}H_{24}N_2O$ | 115-116° | +4° ($CHCl_3$) | |
| 4. | Rhazidine[5d] | $C_{20}H_{26}N_2O_3.H_2O$ | 278-279°d | –20.8°(EtOH) | 0.001 |
| 5. | Alkaloid[5c] | $C_{21}H_{24}N_2O_3$ | 181-182°d | +293.8° | 0.0002 |
| 6. | 1,2-Dehydroaspido sperimidine[7](III) | $C_{19}H_{24}N_2$ | liquid | +243° | 0.21 |
| 7. | Aspidospermidine[8](IV) | $C_{19}H_{26}N_2$ | 120-121° | +17°(EtOH) | — |
| 8. | Eburnamonine[8] (V) | $C_{19}H_{24}N_2O$ | 203-204° | +89°($CHCl_3$) | — |
| 9. | Eburnamenine[8] (VI) | $C_{19}H_{22}N_2$ | (Picrate m.p 196°) | 183° ($CHCl_3$) | — |
| 10. | (±) and (+) Vincadifformine[7] (VII) | $C_{21}H_{26}N_2O_2$ | (±)m.p. 120-126° | | 0.006 |

In view of the fact that there has been an increasing interest in the evaluation of plant alkaloids for anti-cancer activity,[9] and the *Vinca rosea* to have any leucopenic activity. Sewarine was however, fairly easily obtained in relatively good yield (about 0.06%) by following the orthodox



(I)



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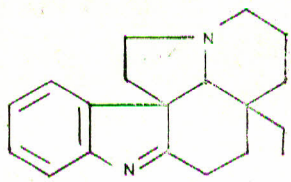

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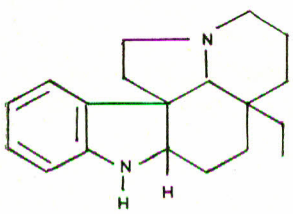

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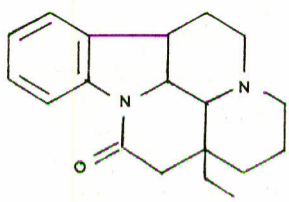

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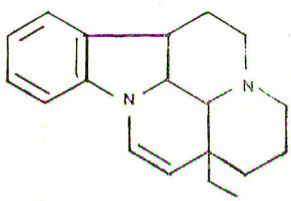
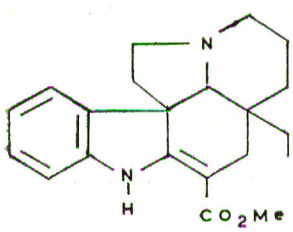
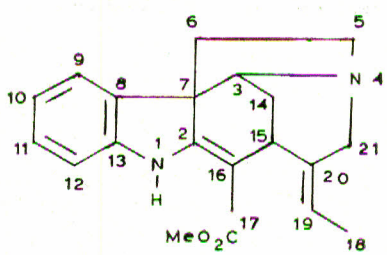


(VI)

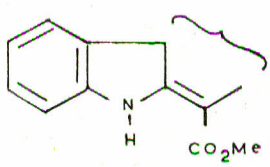


(VII)

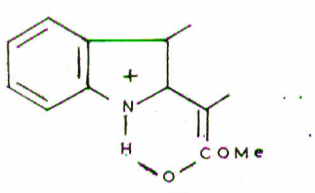


(VIII)

a, As it is
b, OH at C-14
c, OH at C-19 (19,20-Dihydro)
d, OH at C-20 and (19,20-Dihydro)
e, VIIIa with an OH in the non-aromatic portion



(IX)



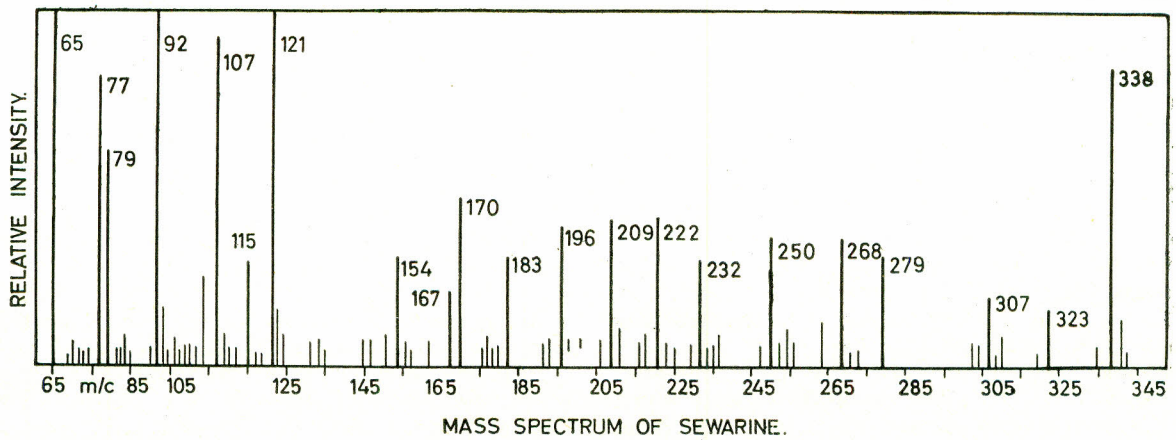
(X)

techniques of isolation, making use of the varying basic strength, and solubilities of the various alkaloids, as described in the experimental.

In its crystalline state, sewarine is almost insoluble in all the bench solvents, and this may account for its having been missed through column chromatographic techniques. However, its salts are readily soluble in alcohol, and it was possible to purify the base through them. Sewarine forms aggregates of pale yellow plates, melts with decomposition at 245°C., and yields crystalline hydrochloride, hydroiodide, picrate, chloroplatinate and methiodide. On the basis of the ultimate analysis of sewarine and its salts, it has been accorded the molecular formula,

$C_{20}H_{22}N_2O_3$. The base was further found to contain one -OMe, two active hydrogens, and one -CMe. The dubious nature of -NMe value; (found 3.04; calculated for one NMe, 4.44%) shown by sewarine has also been recorded[10] for the type of structure envisaged for this alkaloid (VIIIe). The N-methyl value in such cases probably originates[10] from the methoxycarbonyl substituent.

The mass spectrum of sewarine (Fig. 1.), shows molecular ion peak at m/e 338,




MASS SPECTRUM OF SEWARINE.

Fig. 1.

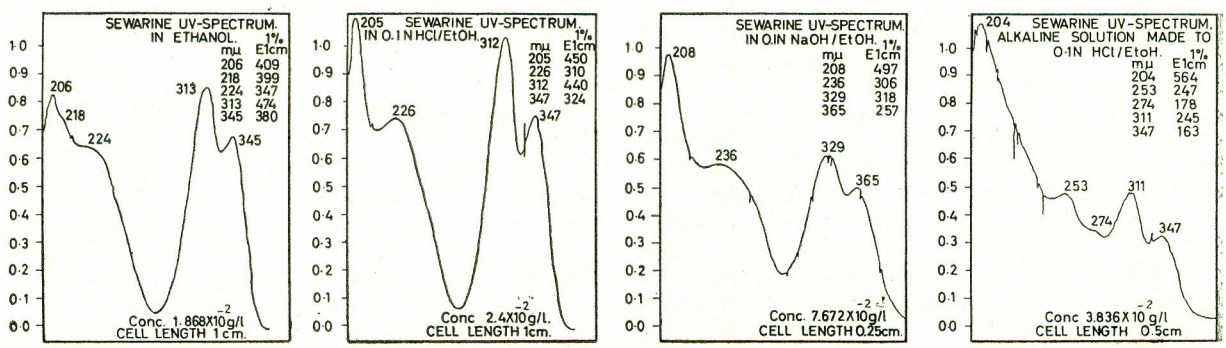



Fig. 2.

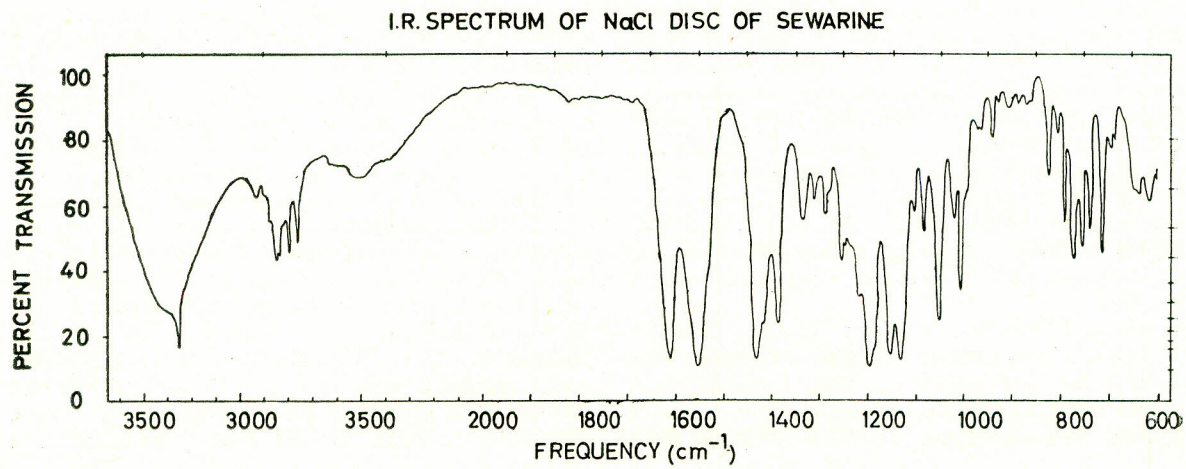



Fig. 3.

which, according to the elementary analysis of the base, fixes its molecular formula to $C_{20}H_{22}N_2O_3$. On account of the insolubility of the base in most of the organic solvents, the molecular weight of sewarine could not be obtained by the usual methods. For the same reason its NMR spectrum could not be obtained either.

The colour reactions and the UV spectra (Fig. 2) indicate that sewarine belongs to the indole group of alkaloids. In fact its UV spectrum is typical of the akuammicine group of alkaloids. From its UV spectra in 0.1N ethanolic HCl, 0.1N ethanolic NaOH and that of the latter solution reacidified to 0.1N acid concentration, as recorded above (Fig. 2), it seems probable that two essentially independent chromophoric systems are present in the molecule of sewarine; one a heterocyclic system absorbing at 205 mμ, 312 mμ and 347 mμ, which is probably reversibly dependent upon pH and a second, fairly simple system, absorbing at 224 mμ, which is not reversible to pH and which may arise from enolisation or ionisation that may indicate a breakdown of the system.

A characteristic strong band at 1661 $cm^{-1}$ shown by sewarine in its IR spectrum (Fig. 3) and an unusually high negative rotation of its hydrochloride, $[\alpha]_D^{32°}$ -656 in water (-724° in ethanol), suggest the presence of the conjugated indole group (IX) as encountered in the akuammicine and vincadifformine groups of alkaloids.[11] The absence of any band in the region 2830-2815 $cm^{-1}$ indicates[12] the absence of OMe and NMe groups and since no other active group, besides a hydroxyl and a conjugated methoxycarbonyl (which also accounts for one -OMe value) is present in the molecule ($C_{20}H_{22}N_2O_3$), therefore, sewarine, which also possesses one -CMe should have the skeletal structure of akuammicine (VIIIa) with only the position of the hydroxyl group remaining to be fixed. It may be significant that of the alkaloids represented in Eli Lilly collection,[13] only those with akuammicine (VIIIa) structure have infra-red and ultra-violet spectra similar to those of sewarine. In particular, there are marked similarities in the spectra in the 1600 $cm^{-1}$ to 1700 $cm^{-1}$ region where, presumably, the resonance stabilised chelation of the type (X) may be responsible for the rather low carbonyl frequency and for the chelation band at 2600 $cm^{-1}$.

In the light of above evidence sewarine may be provisionally represented by the structure IXe, in which the hydroxyl group may be situated in the non-aromatic portion. The physical data of sewarine, however, show that it is different from the previously reported hydroxy akuammicine alkaloids, mossambine[14] (VIIIb) echitamidine[10b] (VIIIc) and Lochneridine [15] (VIIId).

Further work on the elucidation of the structure of sewarine, and isolation of other bases from the alkaloidal fraction of *Rhazya stricta* Dcne. is in progress.

## Experimental

The infra-red spectrum was measured with a resolution of 0.8 $cm^{-1}$, on I.R. "Unicam" S.P. 100 Recording spectrophotometer with grating spectral slit width approximately 2.5 $cm^{-1}$. For U.V. Spectra "Unicam" S.P. 700 Recording spectrophotometer was used.

*Rhazya stricta* Dcne. (*apocynaceae*) was collected from the suburbs of Karachi. The alkaloidal assay of the air-dried plant materials according to the procedure of Higuchi and Brochmann-Hanssen,[16] gave the values: leaves 5.6%, roots 3.2% and stems 2.0%. The aqueous portions left after the extraction of alkaloids in these assays, still showed the presence of some unextractable water-soluble bases, with the usual alkaloidal reagents.

The initial procedure which led to the isolation of sewarine consisted in the fractionation of the total alkaloids according to their varying basic strengths. The major concentration of sewarine was found in the weakly and moderately basic fractions which crystallised out on dissolving them in a small quantity of alcohol. Later, advantage was taken of the unusual solubility character of sewarine, and the following simple procedure was evolved for its isolation.

Fresh undried leaves of *Rhazya stricta* Dcne. (7.0 Kg., which correspond to an air-dried weight of about 2.5 Kg.) were percolated four times with commercial ethanol at room temperature. The dark green extract was concentrated under reduced pressure in a cyclone evaporator below 25°C. to a small volume (until a solid began to separate). The solid, which was non-alkaloidal, was filtered off, and washed with a little ethanol. The filtrate was further concentrated in a rotary film evaporator, until a dark green viscous mass was obtained which was digested with two litres of warm distilled water, in which most of it dissolved, and the decanted solution was filtered after charcoal treatment. The clear brown solution was basified with ammonia, and the light-brown precipitate was exhaustively extracted out with ethyl acetate. The ethyl acetate extract was washed with a

little water, dried ($Na_2SO_4$), and concentrated to one third of its volume. On keeping the ethyl acetate solution overnight at room temperature sewarine separated out as cream coloured micro-needles (1.6 g.). Once it had separated out, sewarine was practically insoluble in all the bench solvents, and was purified by liberating the base from its recrystallised hydrochloride (see below). Sewarine forms pale yellow plates or aggregates of needles, m.p. 245°d [Found: C, 70.96 (71.07); H, 6.55 (6.58); N, 8.40 (8.75); O, 14.31(14.83); -OMe, 9.81; -CMe, 3.20; -NMe, 3.04; active H, 0.48%; Mol. Wt. 338 (from mass spectrum) $C_{20}H_{22}N_2O_3$ requires: C, 70.98; H, 6.55; N, 8.28; O, 14.18; -OMe (one), 9.25; -CMe (one) 4.44; -NMe (one), 4.44; active H (two), 0.59%; Mol. Wt. 338.39].

After removal of sewarine, the solvent was completely removed *in vacuo* from the ethyl acetate filtrate. The viscous residue on application of vacuum swelled into a yellow foam, which could be scraped and powdered. The *alkaloidal fraction* thus obtained (124 g.) was freely soluble in ethyl acetate, ethanol and methanol.

*Sewarine Hydrochloride.*—Sewarine (100 mg.) was taken up in 10% dry ethanolic hydrogen chloride, in which it is readily soluble. On cooling in an ice-bath the hydrochloride crystallised out, and after two crystallisations from methanol it formed further pale yellow plates, m.p. 210°d (Found: C, 65.06; H, 6.09; Cl, 9.00; N, 7.41; O, 12.53 $C_{20}H_{22}N_2O_3$. HCl requires C, 64.98; H, 6.14; Cl, 9.47; N, 7.47; O, 12.81%. Sewarine hydrochloride showed an $[\alpha]_D^{32°}$ of -724° in ethanol, (in water it was -656°).

*Sewarine Hydroiodide.*—A well cooled aqueous solution of sewarine hydrochloride was treated with a saturated solution of potassium iodide, whereupon a white silky mass separated. When recrystallised from water, the hydroiodide was obtained as colourless thin rectangular plates, m.p. 186° (Found: I,27.29;$C_{20}H_{22}N_2O_3$. HI requires: I, 27.25%).

*Sewarine Picrate.*—An aqueous solution of sewarine hydrochloride when treated with an aqueous solution of picric acid gave a yellow precipitate, which on crystallisation from methanol gave clusters of yellow prisms, m.p. 176°d (Found: N, 12.20; $C_{20}H_{22}N_2O_3.C_6H_3N_3O_7$ requires; N, 12.34%).

*Sewarine Methiodide.*—Methyl iodide (1.0 ml.) was added dropwise to a suspension of sewarine (100 mg.) in methanol, and the solution was refluxed for one hour on a water bath. The residue, obtained after removal of the solvent *in vacuo*, when crystallised from dry methanol gave the methiodide as well defined colourless needles, m.p. 258-260°d (Found: I, 25.90; N, 5.57; $C_{20}H_{22}N_2O_3.CH_3I$ requires: I, 26.45; N, 5.83%).

*Sewarine Chloroplatinate.*—A well cooled aqueous solution of sewarine hydrochloride was treated with 10% solution of chloroplatinic acid. The resulting precipitate when crystallised from methanol gave the chloroplatinate as short buff coloured needles, m.p. 225°d. (Found: Pt, 17.79; N, 5.08 $(C_{20}H_{22}N_2O_3)_2.H_2PtCl_6$ requires: Pt, 17.97; N, 5.15%.

*Chromatography of the Alkaloidal Fraction.*—The alkaloidal fraction (see above) was divided into ether-soluble and ether-insoluble portions. 20 g. of the bright yellow ether-soluble portion, was dissolved in minimum quantity of dry benzene and put on a column of neutral alumina (Woelm, activity one) prepared in dry benzene. A colourless product eluted out with benzene. On crystallisation from benzene it gave colourless needles, m.p. 142-143°, which proved to be identical with quebrachamine (IR-spectrum, and undepressed mixed melting point, same as that of an authentic sample of quebrachamine). The elution of the column was continued with a mixture of benzene and chloroform, and the proportion of chloroform was progressively increased. A viscous brown product, which failed to crystallise, was obtained, until the proportion of chloroform in the eluting mixture was 1:1, when a colurless crystalline product started coming out. It melted in the range of 115-126°. Repeated crystallisation from a mixture of chloroform and methanol gave a small amount of a product m.p. 115°, identical (undepressed mixed m.p. and superimposable IR-spectrum) with rhazinine. Further elution of the column with chloroform alone yielded akuammidine (Rhazine), m.p. 234-236°d, identical (IR-spectrum and undepressed mixed m.p.) with an authentic sample. Further investigation of the alkaloidal fraction is in progress.

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