CHEMICAL COMPOSITION OF ADHATODA VASICA

M. Ikram, M. Ehsanul Huq and S.A. Warsi

North Regional Laboratories, Pakistan Council of Scientific and IndustrialResearch, Peshawar

AND

Viqar-uddin Ahmad

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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A new alkaloid ($C_{11}H_{12}N_2O_2$, m.p. 246–48° dec.) provisionally named Vasicinine, has been isolated from the flowering tops of Adhatoda vasica. No vasicinone or betaine could be detected in the extracts. Vasicine and betaine have been isolated from the fresh leaves, but no vasicinone or vasicinine could be isolated.

Adhatoda vasica.—(Hindi, Arusa; Bengali, Vasaca) grows abundantly in the northern plains and lower hilly regions of West Pakistan and is used in the indigenous system of medicine as a remedy for various chest ailments.

The alkaloid vasicine was first isolated from the leaves of the plant by Hooper[1] and characterised in detail by Sen and Ghose.[2] Mithal and Schroff[3] isolated a new quaternary ammonium base, later found to be identical with betaine. Sen and Ghose[2] reported a yield of 0.2—0.4 percent of vasicine while Spath and Kesztler[4] obtained over two percent of the alkaloid by using a different and improved method of isolation.

Subsequently Mehta *et al.*[5] were able to obtain vasicinone earlier reported by Koretskaya,[6] and established it to be the oxidation product of vasicine. It was also reported that vasicine has broncho-constrictor effect[5] whereas vasicinone was broncho-dilator.

The plant was taken up for re-investigation, to assess the effect of climatic and other environmental factors on the relative proportions of the alkaloidal constituents and to determine if vasicinone is originally present in the plant or subsequently appears as a result of aerial oxidation during drying and storage. From fresh Adhatoda vasica leaves collected in different seasons and from different localities, only vasicine and betaine could be isolated. Chromatographic examination of the total alkaloidal fractions isolated from different batches of fresh leaves gave only two Dragendorff positive substances with R_f 0.15 and 0.54 corresponding to betaine and vasicine. Paper chromatography was carried out on Whatman No. 1 paper using solvent system, butanol: concentrated HCl, 98:2(V/V) saturated with water.

From fresh flowering tops (inflorescence) a new alkaloid, provisionally named vasicinine, has been isolated by employing a simpler and milder technique of isolation. The molecular formula of vasicinine was found to be $C_{11}H_{12}N_2O_2$ containing an oxygen atom in excess of the vasicine formula. Its infra-red spectra shows three characteristic peaks due to the conjugated quinazoline ring system[7] i.e. 1492, 1590 and 1627 cm^{-1} (Fig. 1). For comparison the infra-red spectra of vasicine is given which also has three characteristic peaks of quinazoline i.e. 1498, 1590, 1628 cm^{-1} (Fig. 2). Further work on the constitution of the base is in progress.

It has also been found that the amount of vasicine, minimum at the blooming stage, increases with seed formation. On the other hand, the quantity of vasicinine, maximum at the flowering stage, decreases with seed formation. Another interesting observation has been the total absence of either betaine or vasicinone in the material examined.

Melting points were taken in J.W. Tower's electrical melting point apparatus. Micro analysis was done by Dr. A. Bernhardt, 433, Mulheim (Ruhr), West Germany.

Experimental

Leaves.—Fresh leaves (1.65 kg., on moisture free basis) were extracted six times with 95% ethyl alcohol. The greenish residue, obtained on concentration of the combined extracts under reduced pressure, was chilled and repeatedly extracted with ice cold water. The aqueous extract was passed through a thick wad of cotton to remove any suspended matter. After the addition of sufficient solid potassium iodide it was allowed to stand overnight in the cold, when vasicine hydro-iodide (40 g.) crystallised out and on recrystallisation from hot water melted at 190°C.

Vasicine (3 g.) was also recovered by basifying the aqueous filtrate with ammonia, extracting with amyl alcohol and removal of the solvent at reduced pressure. The free base from the iodide, on recrystallisation from alcohol, finally melted at 208°. and was identified as vasicine by mixed melting point with an authentic sample. Paper chromatography showed it to be a uniform substance.

dissolved in methanol and purified by the addition of ethyl alcohol and some ethyl acetate and ether. The treacly precipitate thus obtained showed the presence of reducing sugars, gave very little precipitate with potassium bismuth iodide, and was rejected. The almost colourless filtrate on removal of the solvent was taken up with hot ethyl alcohol and on keeping overnight in the cold, gave a colourless crystalline deposit which on

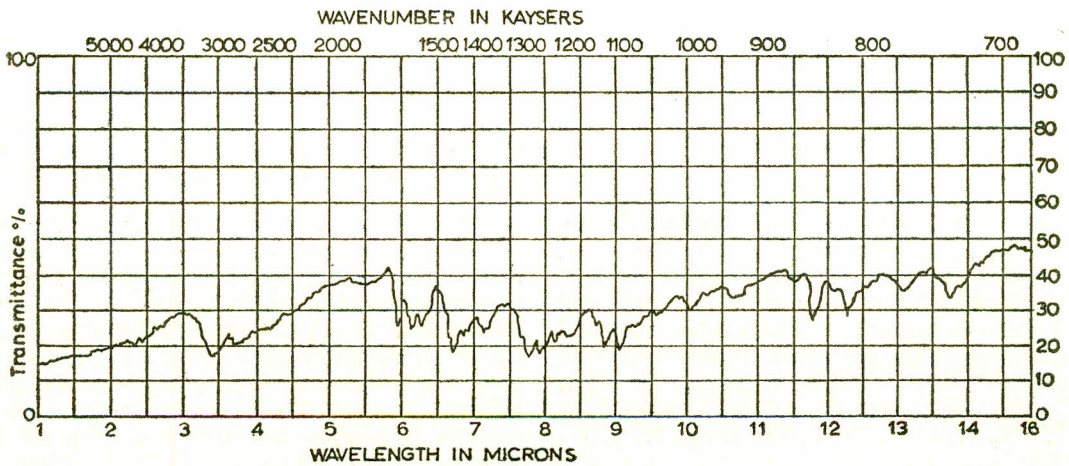

Fig. 1.—Infra-red spectra of Vasicinine (Nujol).

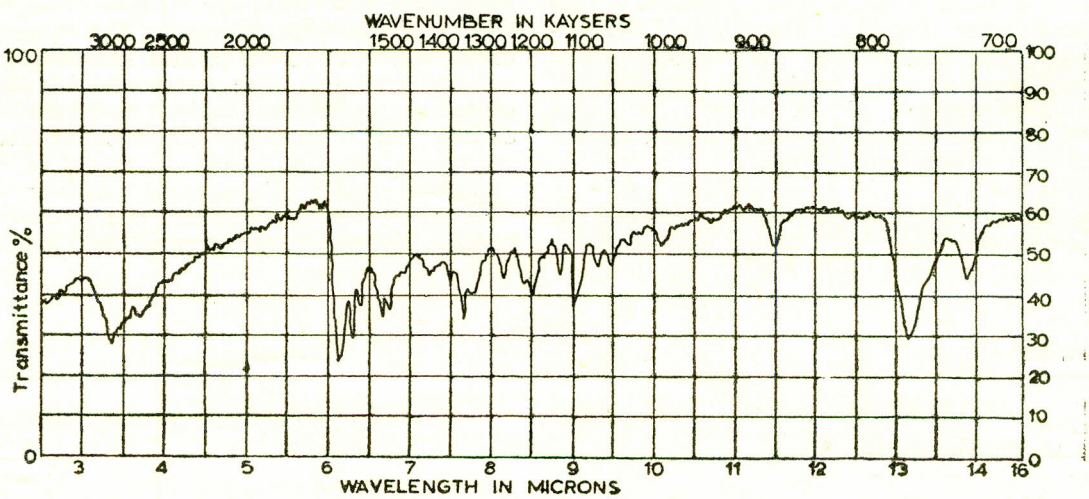

Fig. 2.—Infra-red spectra of Vasicine (Nujol).

The final aqueous mother liquor was concentrated under reduced pressure. The residue obtained on complete removal of the solvent was recrystallisation from the same solvent melted at 218-20°. It gave positive tests for K^+ and I^-. From the analysis it appears to have the structure

$(CH_3)_3.N^+.CH_2COO^-$. $(CH_3)_3.N.ICH_2COOK$. Found: C, 28.94; H, 5.53; N, 7.03; K, 9.60; I, 31.99%. Calc. for $C_{10}H_{22}N_2O_4.KI$; C, 30.00; H, 5.50; N, 7.00; O, 16.00; K, 9.75 and I, 31.75%.

The filtrate on concentration yielded a second crop of the above complex which on crystallisation from ethanol melted at 220°.

The mother liquor after the removal of the solvent was taken up in water and precipitated with Dragendorff reagent. The precipitate was centrifuged and washed thoroughly with water, and decomposed with hydrogen sulphide. The filtrate after removing the bismuth sulphide was concentrated under reduced pressure.

(*a*) The first crop of colourless prisms thus obtained, on recrystallisation from ethanol melted at 244° (dec.). It was soluble in water and methanol but sparingly soluble in ethyl alcohol. Its aqueous solution was acidic to litmus, contained iodine but no potassium. The analysis indicated it to be a complex of one mole betaine with one mole betaine iodide. Found: C, 33.92; H, 6.42; N, 7.32; O, 17.47; I, 34.22%. Calc. for $C_{10}H_{23}N_2O_4I$: C, 33.14; H, 6.35; N, 7.73; O, 17.67; I, 35.08%.

This complex was found to be stable and did not liberate any iodine on keeping. The chloride of the compound was prepared by rubbing the aqueous solution of the complex with freshly precipitated silver chloride. The filtrate after removing silver iodide was concentrated under reduced pressure. The residue on crystallisation from ethyl alcohol, melted at 226° (dec.) and was identified as betaine chloride by mixed melting point with an authentic sample.

(*b*) The second crop of crystals obtained on further concentration and cooling was obtained as straw coloured prismatic rods, m.p. 200°. (Betaine iodide—recorded m.p. 200°). It was found to liberate iodine on keeping and the chloride prepared as above on crystallisation from alcohol gave colourless crystals, m.p. 226° (dec.) identified as betaine chloride by mixed m.p. with an authentic sample.

Inflorescence.—The flowering tops (blooming stage) were collected from the Khyber hills early in April, cut into half-inch bits and repeatedly percolated with 95% ethyl alcohol until free from alkaloid. The combined extracts were first concentrated in a cyclone evaporator to a point where the chlorophyll and fatty matter began to separate out, then allowed to stand overnight in the cold and filtered through a wad of cotton. The filtrate containing mainly the alkaloid and carbohydrate fractions was finally concentrated under reduced pressure to a thick syrupy consistency. Ethyl alcohol (98%) was then added to the cooled extract when most of the sugars got precipitated. The supernatant layer was removed and the thick syrupy residue was further extracted with absolute alcohol a number of times until the residue showed a negative test for alkaloid. The alcoholic extract, after complete removal of alcohol was saturated with an excess of 10% aqueous ammonia. On keeping overnight in the cold, the bulk of the alkaloids crystallised out and were centrifuged and washed with water. The mother liquor yielded two more crops of crystals on further standing. The final mother liquor was then repeatedly extracted with chloroform until free from alkaloids. The extract after drying and filtration was freed of chloroform under reduced pressure. This was combined with the crystalline precipitates obtained earlier and divided into chloroform soluble and insoluble fractions.

Another batch of the tops (seeding stage), collected by the end of May, was similarly processed. The results are given in the Table 1.

TABLE 1.

S. No.	Materials	Flowering stage	Seeding stage
1.	Weight of the drug on moisture free basis	.. 5.5 kg.	5.9 kg.
2.	Weight of crystalline precipitate	.. 40.59 g.	25.30 g.
3.	Weight of residue from chloroform extracts	.. 32.24 g.	47.10 g.
4.	Yield of chloroform soluble fraction	.. 35.34 g.	52.1 g.
5.	Yield of chloroform insoluble fraction	.. 34.49 g.	20.3 g.

The chloroform soluble fraction was found to contain only vasicine identified from the mixed melting point of the authentic sample of the free base. The purity of the sample was also confirmed by paper chromatography which gave a single spot.

Chloroform Insoluble Fraction.—It consisted entirely of vasicinine and gave only one spot when subjected to paper chromatography. The fraction was found to be insoluble in ethyl ether, chloroform, benzene, petroleum ether and difficultly soluble in moist ethylacetate or hot dilute alcohol from which it crystallised out in colourless rectangular plates (m.p. 246-48° dec.). Found: C, 64.29; H, 5.77; N, 13.86; O, 16.08. Calc. for $C_{11}H_{12}N_2O_2$; C, 64.67; H, 5.92; N, 13.72; O, 15.68.

Vasicinine is soluble in dilute mineral acids and alkali. It reduces Tollin's reagent and decolourises alkaline potassium permanganate solution. On adding a few drops of concentrated nitric acid to a solution of the base in concentrated sulphuric acid, a deep orange colour is produced, whereas vasicine gives yellow colour. The base as well as vasicine gave positive tests for tertiary amine.

Hydrochloride.—It was prepared by adding conc. hydrochloride (36 percent) or ethereal hydrochloride to a suspension of the alkaloid in absolute alcohol until the substance went into solution. Base hydrochloride crystallised out on keeping and was recrystallised as colourless needles from absolute alcohol, m.p. 258-260°. (dec.). Found: C, 54.77, 54.74; H, 5.38, 5.51; N, 11.56, 11.47; Cl, 14.59, 14.50; O, 13.78, 13.72. Calc. for $C_{11}H_{13}N_2O_2$ Cl: C, 54.87; H, 5.44; N, 11.64; Cl, 14.73 and O, 13.72. $[\alpha]_D^{13°}$ +49.46° (C,2.98, methyl alcohol).

Paper Chromatography.—Using Whatman No. 1 chromatographic paper and n-butanol acetic acid: water (10:1:5) as the irrigating mixture (descending) the R_f for vasicinine was found to be 0.43, while vasicine showed R_f, 0.51 in the same system. Paper chromatography of the original alcohol insoluble residue showed glucose as the main component.

Vasicinine Acetate.—The acetate of the base was prepared by adding alcoholic acetic acid to a suspension of the base in absolute alcohol, until it went into solution. Crystals of the acetate appeared after some time and were recrystallised from absolute alcohol and ether in silky needles, m.p. 239-40° (dec.).

Vasicinine Sulphate.—The sulphate was prepared by dissolving the base in alcoholic sulphuric acid. Aggregates of needle shaped crystals appeared on allowing the solution to stand overnight, m.p. 190-200° (dec.).

Vasicinine Picrate.—Vasicinine picrate was prepared by adding aqueous solution of picric acid to the dilute acetic acid solution of the base. It was recrystallised with methanol (needle shaped crystals), m.p. 207-210° (dec.).

Gold Chloride Double Salt of Vasicinine.—The gold chloride double salt was prepared by adding an aqueous solution of gold chloride to the dilute acetic acid solution of the base. It charred at 280-90° without melting.

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