

Isolation and Structure of Ajmalinol - a New Alkaloid From *Rauwolfia vomitoria* Afzuelia

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Summary: A new phenolic dihydroindole alkaloid has been isolated from *Rauwolfia vomitoria* Afzuelia and provisionally named as ajmalinol. It could be identified as 11-hydroxy ajmaline through chemical and spectral evidence.

Taking into account the importance of *Rauwolfia vomitoria* as the main source for the industrial production of *Rauwolfia* alkaloids for therapeutic use, systematic studies were undertaken on its chemical constituents with particular reference to their isolation in optimum yield. In this context it may be noted that the techniques employed primarily follow the classical procedures in preference to adsorption chromatography which were noted in the present study to entail considerable losses of the known bases, and failed to yield any new subsidiary alkaloids. As a result of these studies, a dihydroindole alkaloid, provisionally named as ajmalinol has been isolated from the water soluble fraction of the methanolic extract of *Rauwolfia vomitoria*. The methanolic extract was worked up by carefully avoiding contact with strong alkali and acids or excessive heat, according to the procedure described in the experimental.

Ajmalinol forms colourless rectangular plates, and analyzed for $C_{20}H_{26}N_2O_3$, m.p. 209-10°C, $(\alpha)_D^{30} + 132^\circ$ (chloroform). The molecular formula of ajmalinol $C_{20}H_{26}N_2O_3$ was confirmed by mass spectrometry (M^+ 342). It contains 3 hydroxyls, one N-methyl and one C-methyl group. The u.v. spectrum showed maxima at 254 nm. and 294 nm. which is characteristic of dihydroindole alkaloids. The i.r. spectrum of ajmalinol shows OH stretching at 3400 cm^{-1} . In addition there are other peaks at $2900\text{-}3000\text{ cm}^{-1}$ (C-H stretching), 1610, 1470, 685-690 (C=C vibrations of the benzene ring) and C-OH stretching of alcoholic groups in five and six membered rings at 1050 cm^{-1} . The peaks at 738 cm^{-1} and 875 cm^{-1} represent C-H bending of 1,2,4, trisubstituted benzene. There is no strong peak between $1630\text{-}1800\text{ cm}^{-1}$ showing the absence of carbonyl group in the alkaloid.

The calculation of ring equivalents by molecular formula, and close similarity in i.r. spectrum with ajmaline, provide strong evidence in favour of ajmaline-like skeleton for ajmalinol. This fact finds further support by various colour reactions which are common to ajmaline¹ and ajmalinol.

Ajmalinol also reduces ammoniacal silver nitrate

showing carbinol amine - aldehyde imine tautomerism in analogy to ajmaline. However, it contains one hydroxyl group more than ajmaline and the characteristic bathochromic shift in u.v. maxima at pH 13, indicated the phenolic nature of this group. Although ajmalinol is only sparingly soluble in dilute alkali when pure and gives no colour with alcoholic ferric chloride, a solution of the base in dilute hydrochloric acid produces a brownish red colour with an aqueous solution of the reagent. The presence of a phenolic group was further confirmed through the formation of triacetyl derivative on heating with acetic anhydride on the water-bath. It crystallised out from methanol as colourless slender needles, melted at $250\text{-}52^\circ\text{C}$, and gave molecular ion peak in the mass spectrum at 468 in agreement with the molecular formula $C_{20}H_{23}N_2O_3$ $(\text{COCH}_3)_3$. It is more basic in nature than the parent base and shows a sharp peak at 1750 cm^{-1} in the i.r. spectrum due to the O-acetyl group, which cannot be attributed to the absorption of a secondary or tertiary amide carbonyl group as they absorb in the range $1630\text{-}1680\text{ cm}^{-1}$. Moreover, in contrast to ajmalinol, triacetyl ajmalinol does not give any colour with aqueous ferric chloride in the acidic medium, confirming the acylation of the phenolic group. The pattern of ferric chloride colour reaction with ajmalinol as described above, has been noted in the case of phenolic alkaloids marckine and sarpagine isolated from *Alanguim lamarckii*² and *Rauwolfia vomitoria*³ respectively.

The presence of ajmaline skeleton and the phenolic group was confirmed by the high resolution mass spectrum of ajmalinol, which is similar to that of ajmaline⁴⁻⁸, showing an increment of 16 for all the fragments containing the benzene ring. Aside of molecular ion peak at 342 it showed peaks at m/e 327 and 324 due to loss of methyl group and water respectively. The peak at m/e 314 represents the loss of CO from C-17 and also from the aldehyde imine tautomeric form, while the intense peak at m/e 313 is a doublet arising from the elimination of ethyl group from C-18 as well as aldehyde group from C-17 and the aldehyde imine tautomeric form respectively⁴. The elimination of C-14 to C-17 along with the C-21 substituent (OH)

gives the ion $C_{16}H_{17}N_2O$ at mass 253 which further loses C-18 to C-21, with or without rearrangement of a hydrogen atom to N-4, to form the β -carboline ion $C_{12}H_{10}N_2O$ at m/e 189 and β -carbolinium ion $C_{12}H_{11}N_2O$ at m/e 199 respectively.

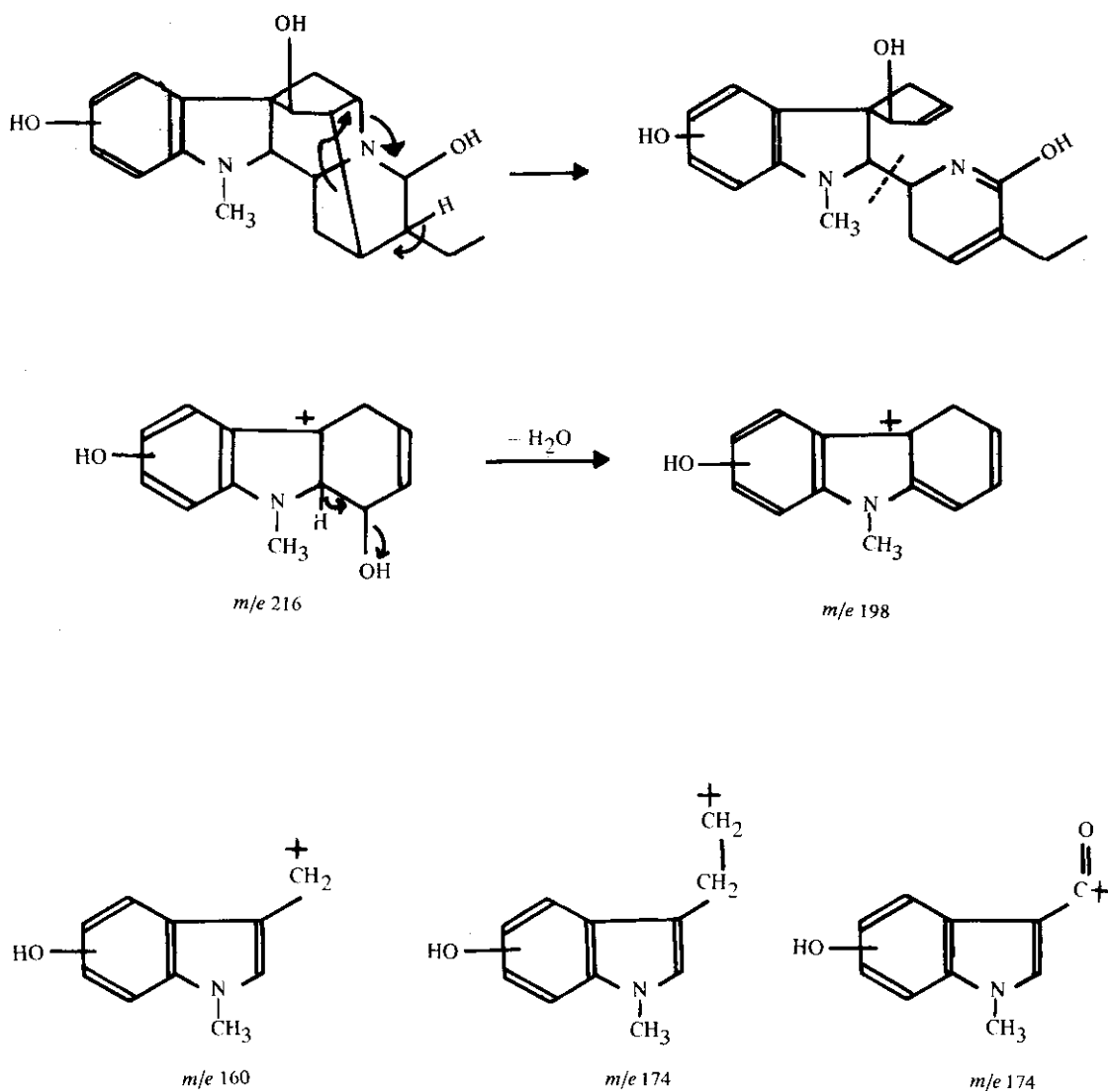
The weak peak at m/e 216 is due to ion $C_{13}H_{14}NO_2$ which is decomposed further by elimination of the C-17 hydroxyl as H_2O which leads to the more stable carbazolinium ion $C_{13}H_{12}NO$ at mass 198. The formation of these ions can be explained as⁴⁻⁸:

The third of the 198 triplet has a high H:C ratio indicating a nonaromatic species which is formed by cleavage of the 2,3- and 7,17 bond with simultaneous aromatization of ring B and formation of a 3,17 bond,

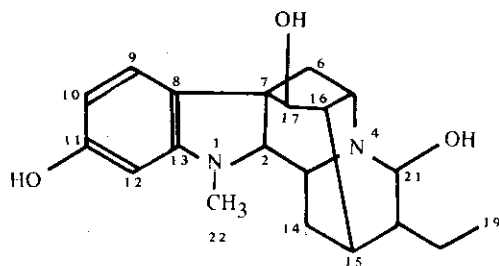
followed by rupture of the 5,6 bond⁴.

The base peak at m/e 160 ($C_{10}H_{10}NO$) and intense peak at 173 ($C_{11}H_{11}NO$) correspond to the N-methyl indolic system with one or two additional carbon atoms and are typical fragments of N-methyl substituted indole alkaloids^{5,6}. The peak at m/e 174 represents the ions which require oxygen at C-17 and is characteristic of dihydro N-methyl indolic skeleton with C-17 to C-7 bond and an oxygen atom at C-17⁴. The appreciable peak at m/e 176, attributed to $C_{10}H_{10}NO_2$, is present in all N-methyl dihydro indolic bases of the ajmaline family of alkaloids carrying a hydroxyl function at C-17⁴⁻⁶.

The position 11 has been assigned to the aromatic



hydroxyl group in ajmalinol through proton NMR spectrum in deuterated trifluoro acetic acid. It shows a doublet at δ 7.00 and may be assigned to H-12 which has a higher electron density. The proton showed meta-coupling with H-10 ($J=2.5$ c/s) and a small paracoupling with H-9 ($J=0.6$ c/s) visible under high resolution only. The signal of H-10 is at δ 7.6 as a distorted quartet. It exhibited ortho coupling with H-9 ($J=9$ c/s) and meta coupling with H-12 ($J=2.5$ c/s). The H-9 signal is again a doublet at δ 7.9 showing ortho coupling with H-10 ($J=9$ c/s) and para coupling with H-12 ($J=0.6$ c/s) visible under high resolution only. The structure of ajmalinol may therefore tentatively be represented as:



This was further confirmed by comparing the ^{13}C NMR spectrum of ajmalinol with that of ajmaline. The spectrum was recorded in deuterated chloroform and showed 20 signals the chemical shifts of which are described below in ppm with reference to TMS=O. The particular significance in the context of this data is a singlet for C-11 which is shifted downfield due to oxygen substituent.

153.90	(C-11, singlet);	133.5	(C-13, singlet);
127.31	(C-8, singlet);	122.94	(C-9, doublet);
119.18	(C-10, doublet);	109.52	(C-12, doublet);
88.1	(C-21, doublet);	79.41	(C-21, doublet);
77.65	(7.16, and 75.04 (C-2, C-3 and C-5, doublets);		
56.22	(C-7, singlet);	50.53	(C-22, quartet);
48.14	(C-16 doublet)	45.35	(C-18, doublet)
43.17	(C-15 doublet);	34.91	(C-6 triplet)
34.12	(C-14, triplet);	25.38	(C-20 triplet);
12.2	(C-19, quartet);		

Experimental

Note: All melting points are recorded in glass capillary and are uncorrected.

2 kg. of the darkish brown viscous methanolic extract was partitioned between ethyl acetate and water. A small quantity of insoluble darkish material at the interface was neglected. The aqueous layer was treated with a little dilute ammonia bringing up the pH to 6 and a dilute solution of common salt then added on to it to throw out some more darkish resinous material which

was cottoned off. The turbid cottonate was heavily charcoaled and filtered after adding celite. The bright red clear filtrate was successively treated with common salt and potassium iodide to separate off the bases forming sparingly soluble hydrochloride and hydroiodide salts. The clear light yellow filtrate was then basified with dilute ammonia and the liberated bases extracted out with ethyl acetate.

The residue left on removal of solvent from the ethyl acetate extract after washing and drying, was taken in dilute acetic acid and treated with sodium chloride to eliminate the residual ajmaline hydrochloride which was filtered off. The creamish white precipitate obtained on basification of the filtrate, had a tendency to redissolve in excess of ammonia. The filtrate was just brought to basic pH, saturated with common salt and exhaustively extracted out with ethyl acetate. The ethyl acetate layer was washed, dried, concentrated in vacuo and triturated with ether and petroleum ether which threw out the darkish brown resinous impurities. The clear solution was freed of the solvent in a nitrogen atmosphere, when a cream coloured semi-crystalline residue was obtained. It was taken up in methanol and left in the ice chest for several days when a colourless crystalline settled down which was filtered and well washed with cold methanol. It showed major concentration of a base on t.l.c. along with a subsidiary component in minute quantity. The crystalline mixture was subjected to fractional crystallization through methanolic benzene when the head fractions yielded ajmalinol as colourless rectangular plates which melted at $209-10^{\circ}\text{C}$, ($\alpha_{\text{D}}^{30} + 132^{\circ}$ (chloroform); The yield was 800 mg. — about 0.4% on the weight of the methanolic extract. Ajmalinol is soluble in alcohol, chloroform and ethyl acetate, fairly soluble in benzene and ether and insoluble in petroleum ether. It analysed for $\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_3$ -C, 70.20%; H, 7.84%; N, 8.10% and mol.wt. by mass spectrum 342 ($\text{C}_{20}\text{H}_{26}\text{N}_2\text{O}_3$, requires: C, 70.17%; H, 7.6%, N, 8.18% and M.342). The presence of 1 N-methyl and 3 hydroxyl groups were respectively shown through Herzig Meyer method and acetylation of the base.

Acetylation of Ajmalinol — 11, 17, 21, -tri O-acetyl ajmalinol.

50 mg. of ajmalinol was dissolved in 2 ml. acetic anhydride and refluxed on a water bath for 10 minutes. Acetic anhydride was completely removed in vacuo and the product was crystallised from methanol when tri-acetyl ajmalinol was obtained as colourless slender needles m.p. $250-52^{\circ}\text{C}$. It gave molecular ion peak in the mass spectrum at 468 in agreement with the molecular formula $\text{C}_{20}\text{H}_{23}\text{N}_2\text{O}_3 (\text{COCH}_3)_3$. It is more

basic in nature and showed no hydroxylic absorption in i.r. spectrum but a sharp peak at 1750 cm^{-1} due to the O-acetyl group.

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