AJMALINIMINE — A NEW ALKALOID FROM RAUWOLFIA SERPENTINA BENTH.

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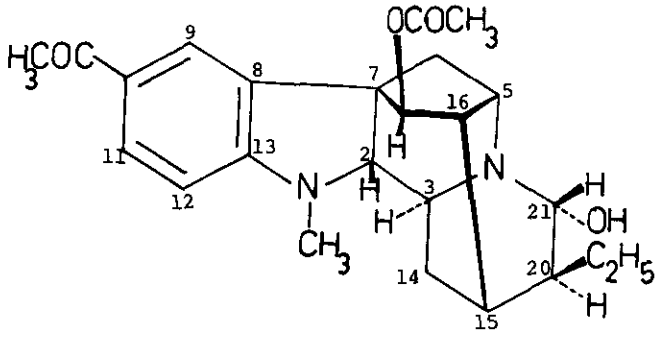
Abstract - A new dihydroindole alkaloid ajmalinimine has been isolated from the roots of Rauwolfia serpentina Benth. collected from Thailand. Its structure was determined as 10-C,17-O-diacetylajmaline on the basis of chemical and spectroscopic studies.

The present work was carried out in the alkaloidal constituents of the roots of Rauwolfia serpentina Benth., in the light of earlier results[1-5] that the alkaloidal constituents markedly differ in their yield and character on the basis of regional distribution. The novel feature of the alkaloid is the presence of C-acetyl group at indole nucleus, which has never been reported in ajmalane and yohimbane series of alkaloids.



(I)

Ajmalinimine (I) has been isolated from 2% acetic acid soluble fraction through the procedure described in the experimental. It formed elongated rods on recrystallization from moist methanol and melted at 198-199°C, $[\alpha]_D^{20}$ + 205° (c,0.3, $CHCl_3$). The UV spectrum showed[6] maxima at 212, 226 and 290 nm and the IR spectrum exhibited[6] the presence of two carbonyl groups at 1735 and 1700, a band for O-H stretching at 3410 and bands due to aromatic vibrations at 3100, 1600 and 1460 cm^{-1}.

Its molecular formula $C_{24}H_{30}N_2O_4$ was determined by elemental analysis and high resolution mass m/z 410.2201. The mass spectral fragmentation is similar to that of ajmaline with an increment of 42 mass units for all the fragments containing

the benzene ring. Apart from the molecular ion, the mass spectrum showed a peak at m/z 367.2019 $(C_{22}H_{27}N_2O_3)^+$, arising by the elimination of acetyl group, which on further loss of carbon monoxide gave a peak at m/z 339.2070 $(C_{21}H_{27}N_2O_2)^+$. The peak at m/z 352.1781 $(C_{21}H_{24}N_2O_3)^+$ is possibly due to the loss of C-21 and the ethyl group from which a further loss of acetyl group from C-17 resulted in a fragment at m/z 309.1606 $(C_{19}H_{21}N_2O_2)^+$. Another peak at m/z 279.1489 has been assigned to the fragment ion $(C_{18}H_{19}N_2O)^+$ formed by the loss of C-14 to C-17 and C-21 along with their substituents. The prominent peaks at m/z 200.0709, 199.0992 and 186.0916 attributed to the ion fragments $(C_{12}H_{10}NO_2)^+$, $(C_{13}H_{13}NO)^+$ and $(C_{12}H_{12}NO)^+$ respectively are characteristic of an indolic nucleus.[7]

The ^{1}H-NMR spectrum exhibited two doublets at δ 7.90, 6.71 and a double doublet at δ 7.83 assigned to aromatic protons H-9, H-12 and H-11 respectively. The absence of the signal for H-10 indicated a substituent at C-10 and the chemical shifts of H-9, H-11 and H-12 showed that the substituent is C-acetyl group. The H-17 also resonated 0.9 ppm downfield (δ 5.32) as compared to that of ajmaline[6,8] and showed half width of 0.5Hz whereas H-21 appeared at δ 4.26 as a broad singlet ($W_{\frac{1}{2}}$ = 0.5Hz) The three three-proton sharp singlets at δ 2.88, 2.46 and 2.21 have been attributed to N-$\underline{CH}_3$ Ar$\underline{COCH}_3$ and $\underline{COCH}_3$ respectively. The half width values of H-17 and H-21 showed that both these protons are "β" oriented.[8]

The ^{13}C-NMR spectrum of ajmalinimine afforded twenty-four signals, the assignments (Table) of which have been made through comparison of their chemical shifts with those of ajmaline series of compounds[9,10] and finally established through gated spin echo measurements. On the basis of these data and the acetylation of I, ajmalinimine has been characterized as 10-C,17-O-diacetylajmaline.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. UV spectra (CH_3OH) were recorded on Pye-Unicam SP-200G spectrometer, IR spectra ($CHCl_3$) on JASCO A-302 spectrometer, NMR spectra were recorded on Bruker AM-300 spectrometer and mass spectra were recorded on Varian MAT-112 and MAT-312 double focussing mass spectrometer connected to PDP 11/34 computer system. Thin layer chromatography was performed on silica gel (GF-254) precoated aluminium cards.

Isolation: Freshly collected undried roots of Rauwolfia serpentina Benth (6 kg) were obtained from Thailand and extracted repeatedly with ethanol after prior

overnight dip in water. The combined alcoholic extracts were freed of the solvent under reduced pressure below 40°C. The dark brownish viscous residue, was taken up in water and exhaustively shaken out with ethyl acetate, finally with the addition of 5% acetic acid. The ethyl acetate phase was defatted with petroleum ether and divided into benzene-ether and ethyl acetate soluble fractions. The ethyl acetate solution was successively shaken out with 1% and 2% acetic acid, the latter was ammoniated and extracted with ethyl acetate. After usual work, the ethyl acetate solution was freed of the solvent under reduced pressure and the resulting residue was subjected to thin layer chromatography (silica gel, 0.2 mm, chloroform, benzene-methanol (5:4.4:0.4). As a result ajmalinimine was obtained as a white crystalline solid and gave elongated on recrystallization from moist methanol mp 198-199°C, $[\alpha]_D^{20}$ + 205° (c, 0.3, $CHCl_3$), (40 mg, 6.6×10^{-4}% yield). It analyzed for $C_{24}H_{30}N_2O_4$ found: C=70.31, H=7.20, N=6.99, O=15.50%; requires: C=70.24, H=7.32, N=6.83, O=15.61%). HRMS m/z (rel.int. %), 410.2201 (M^+, calcd. for $C_{24}H_{30}N_2O_4$ 410.2205) (52), 395.1976 $(C_{23}H_{27}N_2O_4)^+$ (25), 367.2019 $(C_{22}H_{27}N_2O_3)^+$ (30), 352.1781 $(C_{21}H_{24}N_2O_3)^+$ (20), 339.2070 $(C_{21}H_{27}N_2O_2)^+$ (5), 309.1606 $(C_{19}H_{21}N_2O_2)^+$ (5), 279.1489 $(C_{18}H_{19}N_2O)^+$ (5), 242.1186 $(C_{15}H_{16}NO_2)^+$ (20), 224.1078 $(C_{15}H_{14}NO)^+$ (52), 200.0709 $(C_{12}H_{10}NO_2)^+$ (12), 199.0992 $(C_{13}H_{13}NO)^+$ (12), 186.0916 $(C_{12}H_{12}NO)^+$ (38) and 182.0973 $(C_{13}H_{12}N)^+$ (100). IR $\nu_{max}(cm^{-1})$: 3410 (O-H stretching), 3100, 1600, 1460 (aromatic vibrations) and 1735, 1700 (carbonyl stretching). UV λ_{max}(nm): 212, 226 and 290. ^{1}H-NMR ($CDCl_3$): δ 7.90 (1H, d, $J_{9,11}$=1.7Hz, H-9), 7.83 (1H, dd, $J_{9,11}$=1.7Hz, $J_{11,12}$=8.3Hz, H-11), 6.71 (1H, d, $J_{11,12}$=8.3Hz, H-12), 5.32 (1H, s, $W_{½}$ = 0.5Hz, H-17), 4.26 (1H, br s, $W_{½}$ = 0.5Hz, H-21β), 3.70 (1H, brd, $J_{3,14\alpha}$=10.2Hz, H-3α), 3.05 (2H, m, H-5), 2.98 (1H, s, $W_{½}$ = 0.5Hz, H-2β), 2.88 (3H, s, N-CH_3), 2.46 (3H, s, Ar$COCH_3$), 2.35 (1H, m, H-15), 2.21 (3H, s, $COCH_3$), 2.15 (1H, dd, $J_{5,6\alpha}$=1.0Hz, $J_{6\alpha,6\beta}$=11.5Hz, H-6α), 1.88 (1H, dd, $J_{5,6\beta}$=5.3Hz, $J_{6\alpha,6\beta}$=11.5Hz, H-6β), 1.85 (1H, m, H-14α), 1.68 (1H, ddd, $J_{3,14\beta}$=1.0Hz, $J_{14\alpha,14\beta}$=13.6Hz, $J_{14\beta,15}$=5.5Hz, H-14β), 1.41 (2H, m, H-19) and 0.95 (3H, t, J=7.0Hz, H-18).

Table

^{13}C-NMR chemical shift assignments

Carbons	Chemical shifts	Carbons	Chemical shifts
2	79.09	16	47.95
3	43.40	17	79.49
5	52.75	18	12.09
6	46.60	19	35.50
7	54.12	20	52.51
8	128.99	21	78.02
9	122.77	C=O (ester)	170.37
10	131.69	C=O (ketonic)	196.36
11	130.21	$-CH_3$	21.27
12	108.01	$-CH_3$	26.06
13	157.30	$N-CH_3$	32.86
14	31.15		
15	27.99		

All values are in ppm relative to TMS=0

Acetylation of I:

To a solution of I (10 mg) in pyridine (1 ml) acetic anhydride (1 ml) was added and the reaction mixture kept overnight at room temperature. On usual work 21-O-acetylajmalinimine was obtained as needles, mp 205-206°C, *HRMS* m/z 452.2218 (M^+, calcd. for $C_{26}H_{32}N_2O_5$, 452.2210), ^1H-NMR ($CDCl_3$) δ : 7.92-6.70 (3H, m, aromatic protons), 5.33 (1H, s, $W_{½}$ = 0.5Hz, H-17), 5.27 (1H, s, $W_{½}$ = 0.5Hz, H-21), 2.86 (3H, s, $N-CH_3$), 2.45 (3H, s, $ArCOCH_3$), 2.20 and 2.18 (6H, s, $2xCOCH_3$), 1.40 (2H, m, H-19) and 0.96 (3H, t, J=7.0Hz, H-18).

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