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AJMALICIDINE, AN ALKALOID FROM *RAUWOLFIA SERPENTINA*

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Key Word Index *Rauwolfia serpentina*; Apocynaceae; root; alkaloid; ajmalicidine.

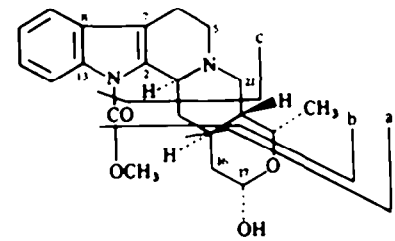
Abstract—A new heteroyohimban alkaloid, ajmalicidine, has been isolated from the roots of *Rauwolfia serpentina* of Thai origin. Its structure has been elucidated as 1-carbomethoxy-17α-hydroxy-16-decarbomethoxy 16,17-dihydro ajmalicine through chemical and spectral studies.

INTRODUCTION

Considering the importance of *Rauwolfia* alkaloids in the treatment of cardiovascular diseases and the variations recorded in the literature [1–3] with respect to the alkaloidal constituents of the roots due to varying soil and climatic conditions, studies of the alkaloids of roots collected from Nepal and Thailand were undertaken and two new alkaloids from roots originating in Nepal have been communicated earlier [4, 5]. The present paper deals with the isolation and structural elucidation of a new indole alkaloid, ajmalicidine (**1**), obtained from root material collected in Thailand.

RESULTS AND DISCUSSION

Ajmalicidine was obtained as a light yellow crystalline solid which on recrystallization from methanol–ethyl acetate formed irregular plates, mp 235–236°, $[\alpha]_D^{20°} = +190°$ ($CHCl_3$) with molecular formula $C_{21}H_{26}N_2O_4$ (elemental analysis and high resolution mass, $[M]^+$ 370.1874). The IR spectrum in chloroform showed OH stretching at 3330 cm^{-1}, in addition to a prominent band at 1720 cm^{-1} (C=O). The UV spectrum in methanol showed maxima at 208, 225 and 285 nm characteristic of an indole nucleus [6, 7]. Apart from the $[M]^+$, the mass spectrum showed peaks at *m/z* 339.1620 $[M - OCH_3]^+$, 311.1752 $[M - COOCH_3]^+$ and 170.0847, 156.0809 and 144.0813 related to ion fragments $[C_{11}H_{10}N_2]^+$, $[C_{11}H_{10}N]^+$ and $[C_{10}H_{10}N]^+$, respectively, which are characteristic of β-carbolines [8]. The spectral data of **1** showed that it belonged to the heteroyohimban series of alkaloids [9, 10]. The ^1H NMR of ajmalicidine showed a four-proton multiplet extending from δ7.38 to 6.93 for the aromatic region. The appearance of a sharp three-proton singlet at δ3.80 in the ^1H NMR and the signals at δ174.57 (C=O) and 52.36 (OCH_3) in the ^{13}C NMR suggested the presence of a carbomethoxy function in the molecule. The remaining two oxygen functions were accounted for as follows. A one-proton doublet of quartets at δ4.18 exhibited a CH_3 CH(CH) O– system, while a one-proton doublet of doublets at δ5.07 along with a signal at δ91.79 in the ^{13}C NMR suggested a hemiacetal function. These observations indicated the partial structure $-CH_2-CH(OH)-O-CH(CH)-CH_3$ which was confirmed



1

by the 2-D chemical shift correlation (COSY) spectrum. It showed that the proton at δ5.07 is coupled with two protons at δ2.29 (H-16α) and 2.64 (H-16β) while the proton at δ4.18 (H-19) is coupled with the methyl proton at δ1.19 (H-18) as well as with the methine proton at δ2.25 (H-20). Furthermore, the absence of a signal geminal to the ester function, as well as the lack of the N-H proton, led to the placement of the carbomethoxy function on the indolic nitrogen [10, 11]. The assignment of other protons was made through comparison with the published data of similar compounds [9] and was supported by COSY. The H-3 resonated at δ3.28 and suggested its α-orientation [9] while the α-disposition of the hydroxy function at C-17 could be confirmed by the coupling constants observed for H-17 ($J_{16\alpha,17}$ = 3.2 Hz, $J_{16\beta,17}$ = 4.8 Hz). Moreover, the chemical shift comparison of H-19 with that of ajmalicine and the coupling constant ($J_{19,20}$ = 3.5 Hz) indicated that the H-19 and H-20 are β-oriented. The chemical evidence for the OH group was provided through its monoacetyl derivative, H-17 being shifted to δ6.09 (*dd*, $J_{16\alpha,17}$ = 3.2 Hz, $J_{16\beta,17}$ = 4.8 Hz) in the ^{1}H NMR. In the light of these observations, structure **1** has been assigned to ajmalicidine which was also substantiated by the chemical shifts of various carbons observed in ^{13}C NMR spectrum (Table 1) [12, 13] and the fragments at *m/z* 297.1608, 267.1489 and 184.1004, corresponding to the ions $[C_{18}H_{21}N_2O]^+$, $[C_{17}H_{19}N_2O]^+$ and $[C_{12}H_{12}N_2]^+$, respectively, possibly resulting from the cleavages a, b, and c, as shown in the structure.

EXPERIMENTAL

Mps are uncorr. MS were recorded on a double focussing instrument connected to a computer system. ^{1}H and ^{13}C NMR (broad band and gated spin echo) spectra were recorded in $CDCl_3$ with TMS as int. ref., COSY expts were carried out at 300 MHz. ^{13}C NMR spectral assignments were made by comparison with published data for similar compounds [12, 13]. The purity of samples was checked by TLC on silica gel.

Isolation of alkaloid. Fresh roots (10 kg) of *R. serpentina* were cut into small pieces and repeatedly extracted with EtOH. The combined extracts were concd *in vacuo* and divided into EtOAc soluble and insoluble fractions. The EtOAc soluble fraction was successively shaken out with 2% and 5% HOAc. The latter was adjusted to pH 8 (NH_4OH) and extracted with EtOAc. After usual work up, the EtOAc soln was freed of solvent under red. press. and the resulting residue subjected to prep. TLC (silica gel; $CHCl_3$–MeOH, 9.7:0.3). As a result **1** was obtained (60 mg, 6 × 10^{-4}% yield) as a light yellow crystalline solid which on recrystallization from MeOH EtOAc formed irregular plates, mp 235 236°, $[\alpha]_D^{30}$ = +190 ($CHCl_3$). HRMS *m/z* (rel. int. %): 370.1874 (calcd. for $C_{21}H_{26}N_2O_4$ 370.1892) (100) 339.1620 $[C_{20}H_{23}N_2O_3]^+$ (14), 311.1752 $[C_{19}H_{23}N_2O_2]^+$ (8), 297.1608 $[C_{18}H_{21}N_2O_2]^+$ (28), 267.1489 $[C_{17}H_{19}N_2O]^+$ (22), 184.1004 $[C_{12}H_{12}N_2]^+$ (52) 170.0847 $[C_{11}H_{10}N_2]^+$ (38), 156.0809 $[C_{11}H_{10}N]^+$ (50) and 144.0813 $[C_{10}H_{10}N]^+$ (30). It analysed for $C_{21}H_{26}N_2O_4$ (calcd. C = 68.11, H = 7.03, N = 7.57, O = 17.29%; obsd. for $C_{21}H_{26}N_2O_4$ C = 68.20, H = 7.01 N = 7.48, O = 17.31%). IR ν_{max} (cm^{-1}) ($CHCl_3$): 3330 (OH stretching), 1380 (OH bending), 3150, 2900 and 1440 (aromatic vibrations) and 1720 (C=O). UV λ_{max} (nm) (MeOH): 208,225 and 285. ^{1}H NMR ($CDCl_3$) δ: 7.73 (1H, *dd*, $J_{9,10}$ = 7.5 Hz, $J_{9,11}$ = 1.5 Hz, H-9) 7.28 (1H, *dd*, $J_{11,12}$ = 7.5 Hz, $J_{10,12}$ = 1.5 Hz, H-12), 7.04 (1H, *ddd*, $J_{11,12}$ = 7.5 Hz, $J_{10,11}$ = 7.3 Hz, $J_{9,11}$ = 1.5 Hz, H-11), 6.69 (1H, *ddd*, $J_{9,10}$ = 7.5 Hz, $J_{10,11}$ = 7.3 Hz, $J_{10,12}$ = 1.5 Hz, H-10), 5.07 (1H, *dd*, $J_{16\alpha,17}$ = 3.2 Hz, $J_{16\beta,17}$ = 4.8 Hz, H-17β), 4.18 (1H, *dq*, $J_{19\beta,20\beta}$ = 3.5 Hz, $J_{18,19\beta}$ = 7 Hz, H-19β), 3.80 (3H, *s*, OCH_3), 3.28 (1H, *br d*, H-3α), 3.1 (1H, *m*, H-6β), 2.98 (1H, *m*, H-5β), 2.92 (1H, *dd*, $J_{20\beta,21\beta}$ = 3 Hz, $J_{21\alpha,21\beta}$ = 11 Hz, H-21β), 2.64 (1H, *dd*, $J_{15\alpha,16\beta}$ = 11 Hz, $J_{16\alpha,16\beta}$ = 12.7 Hz, $J_{16\beta,17\beta}$ = 4.8 Hz, H-16β), 2.29 (1H, *td* $J_{15\alpha,16\alpha}$ = 5.2 Hz, $J_{16\alpha,16\beta}$ = 12.7 Hz, $J_{16\alpha,17\beta}$ = 3.2 Hz, H-16α), 2.25 (1H, *td*, $J_{20\beta,21\beta}$ = 3 Hz, $J_{20\beta,21\alpha}$ = 12 Hz, $J_{19\beta,20\beta}$ = 3.5 Hz, H-20β), 2.06 (1H, *m*, H-15α) and 1.19 (3H, *d*, $J_{18,19\beta}$ = 7.0 Hz, H-18).

Acetylation of **1**. To a soln of **1** (20 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture kept overnight at room temp. On usual workup, 21 mg of acetyl ajmalicidine was obtained as needles, mp 251 252°, HRMS, *m/z* (rel. int. %): 412.1994 (calcd. for $C_{23}H_{28}N_2O_5$, 412.1998) (12). IR ν_{max} (cm^{-1}) ($CHCl_3$): 1720 and 1680. UV λ_{max} (nm) (MeOH): 208, 225 and 285. ^{1}H NMR ($CDCl_3$) δ: 7.4 7.1 (4H, *m*, aromatic protons), 6.90 (1H, *dd*, $J_{16\alpha,17}$ = 3.2 Hz, $J_{16\beta,17}$ = 4.8 Hz, H-17), 4.26 (1H, *dq*, H-19), 3.79 (3H, *s*, OCH_3), 2.08 (3H, *s*, CH_3) and 1.26 (3H, *d*, $J_{18,19}$ = 7.0 Hz, H-18).

Table 1. ^{13}C NMR data of ajmalicidine (1)

Carbon No.	Chemical shift	Carbon No.	Chemical shift
2	135.05	14	30.74
3	61.26	15	36.16
5	54.34	16	34.55
6	22.42	17	91.79
7	107.97	18	14.49
8	128.31	19	72.98
9	118.62	20	42.98
10	119.85	21	57.58
11	122.08	OCH_3	52.36
12	112.03	C=O	174.57
13	138.16		

All values are in (ppm) relative to TMS = 0 ppm.

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TOMATINE, ITS EFFECT, AND INTERACTION WITH ABSCISIC ACID ON STOMATAL OPENING IN *COMMELINA COMMUNIS*

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Key Word Index *Commelina communis*; Solanaceae; stomata; tomatine; abscisic acid.

Abstract -Tomatine, a glycoalkaloid, induces stomatal closure in epidermal peels of *Commelina communis*. It is more potent than abscisic acid (ABA) and reverses ABA-induced stomatal closure.

INTRODUCTION

Tomatine, a glycoalkaloid found mainly in the family Solanaceae, has been associated in the past with resistance to wilt caused by the fungus *Fusarium oxysporum* f. *lycopersici* [1–3]; however, its mode of action is not clear. Now we show that tomatine causes stomatal closure and is more potent than even abscisic acid, an established inhibitor of stomatal opening [4]. Thus, the tomatine associated resistance to wilt could be at the level of regulation of stomatal opening or at least partially. Recently, we demonstrated that ABA-induced stomatal closure can be reversed by a number of phenolic compounds [5]. Now we report that tomatine can also reverse ABA-induced stomatal closure. Our observations and the localization of phenolic compounds in the stomatal apparatus [6] suggest some regulatory role of secondary metabolites in stomatal mechanisms and, hence, in the process of transpiration.

RESULTS AND DISCUSSION

Both tomatine and ABA caused stomatal closure in *Commelina communis* and tomatine proved to be more potent than ABA (Fig. 1A). At 10^{-4} M, ABA caused 40% closure, while with tomatine at the same concentration the closure of stomata was total. However, a definite antagonism was noticed when ABA (10^{-4} M) and tomatine at lower concentrations (10^{-7} and 10^{-6} M) were applied together; at higher concentrations of tomatine the inhibition persisted (Fig. 1B). A similar reversal of ABA-induced stomatal closure by phenolic compounds, another group of secondary metabolites, has recently been shown by us [5]. Thus, our results show a new effect of glycoalkaloids on stomatal opening.

The mode of action of tomatine in this context is unknown. However, tomatine at neutral pH is highly membranolytic and forms a complex with cholesterol [7] as well as increasing the permeability of beet root membranes to betacyanin [8]. Thus, tomatine-induced stomatal closure is probably related to the changed permeability of guard cell membranes to K^+ or other ions. ABA also acts by directly affecting the ionic and metabolic status of guard cells [9] and binding to guard cell membranes [10].

EXPERIMENTAL

Plants of *C. communis* L. growing in their natural habitat (Shimla, W. Himalayas, *ca* 2300 m) during the rainy season were used. Abaxial epidermis peeled from fully expanded leaves closest to the apex was used. Solns of tomatine and ABA (Sigma) were prepd in 0.1 M NaPi buffer (pH 7). Epidermal strips, equilibrated in buffer soln for 1 hr, were incubated for 3 hr in buffer or buffered solns in glass Petri dishes under fluorescent light (3.4 W m^{-2}). Stomatal opening was measured using a calibrated eye piece graticule for 30 randomly located stomata as described earlier [5]. Expts were repeated twice.

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