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VASICOL, A NEW ALKALOID FROM *ADHATODA VASICA*

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Key Word Index —*Adhatoda vasica*; Acanthaceae; alkaloids; 1,2,3,4,9,11 hexahydro pyrrolo (2,1-b) quinazolin-3,11 diol; vasicinone.

Abstract—A novel alkaloid isolated from the roots of *Adhatoda vasica* has been characterized as 1,2,3,4,9,11 hexahydro pyrrolo (2,1-b) quinazolin-3,11 diol by chemical and spectroscopic methods. Vasicinone has also been isolated from the roots of this plant.

INTRODUCTION

Adhatoda vasica Nees is used extensively as a drug [1–3]. The leaves and roots of the plant are known to contain quinazoline alkaloids [4, 5]. From the roots of the plant, we report the isolation and characterization of a novel alkaloid named here as vasicol.

RESULTS AND DISCUSSION

The basic chloroform fraction of the alcoholic extract by column chromatography resulted in the isolation of two alkaloids—AV-1 and AV-2.

AV-1

Mp 210–211°, M^+ 202, analysed for $C_{11}H_{10}N_2O_2$ showed prominent IR bands at 3160–3120 cm^{-1} (—OH), 1623 ($-\overset{|}{C}=N-$), 1680 ($-\overset{O}{\overset{||}{C}}-N-$) and 1607 and 1480 cm^{-1} (aromatic). ^1H NMR (DMSO-d_6, δ) showed two multiplets (2H each) centred at 2.13 and 4.03 attributed to C-2 and C-1 methylenes, a triplet centred at 5.03 (1H) and a multiplet centred at 7.6 (3H) attributed to a C-3 methine proton and C-5, -6 and -7 protons respectively. A doublet ($J = 8$ Hz) at 8.16 (1H) can be attributed to C-8H. D_2O exchange showed the presence of one exchangeable proton at 6.06. Acetylation of AV-1 yielded a crystalline compound mp 124–25°, which analysed for $C_{13}H_{12}N_2O_3$. The spectral data of AV-1 and its acetylated product showed a similarity to vasicinone and its acetylated product (lit. [6] vasicinone mp 211–212°) which was confirmed by comparison with an authentic sample (co-TLC, mmp undepressed, superimposable IR and ^1H NMR). Thus, the structure of AV-1 may be represented as 1,2,3,9 tetrahydro pyrrolo (2, 1-b) quinazolin-3-ol-9-[1]one.

AV-2

The compound showed a M^+ at *m/e* 206.109 calculated for $C_{11}H_{14}N_2O_2$, 206.070, $[\alpha]_D^{25}$ −17.34°. AV-2-HCl mp 204–206° λ_{max}^{MeOH} 291 and 238 nm. The IR showed prominent bands at 3360–3330 ($-\overset{|}{N}-H$ and —OH) and 1603 and 1493 cm^{-1} (aromatic).

^1H NMR ($CDCl_3$, δ) showed two multiplets centred at 2.1 and 3.16 (2H each) attributed to C-2 H_2 and C-1 H_2 protons respectively. D_2O exchange simplified a complex multiplet centred at 4.2 (6H) into 2 broad singlets at 4.33 and 4.36 (1H each) attributed to C-9 H_a and H_b proton respectively and 4.5 (1H, *t*, partially obscured by the C-9 H_2 signal) attributed to the C-3 methine proton and two multiplets centred at 6.63 and 7.03 integrating for four protons are attributed to aromatic protons. The behaviour of benzylic protons (C-9 H_2) as two broad singlets instead of one observed in vasicine may probably be attributed to its slightly non-homogenous environment because of the presence of the C-11 OH.

The mass spectrum of AV-2 showed a M^+ at *m/e* 206 with the base peak at *m/e* 106 and other peaks at *m/e* 162 (14%), 161 (30%) and 133 (16%).

The molecular formula ($C_{11}H_{14}N_2O_2$) and the spectral data of AV-2 suggested that the alkaloid differs from vasicine ($C_{11}H_{12}N_2O$) by an extra H and OH functions. These functions could be best placed at N-4 and C-11 positions in order to explain the IR, ^1H NMR and mass spectrum of AV-2. Further support for the aforesaid was obtained by the methylation, acetylation and dehydration reactions of AV-2.

Methylation of AV-2 with trimethyl anilinium hydroxide [7] afforded two products AV-2-X and AV-2-Y. ^1H NMR of AV-2-X ($CDCl_3$, δ) showed two multiplets (2H each) centred at 2.1 and 3.3 for C-2 and C-1 methylenes and a broad singlet at 4.4 (2H) for C-9 methylene protons. Two multiplets centred at 6.6 and 7.13 (2H each) could be attributed to four aromatic protons (C-5 to C-8). A singlet at 3.6 (3H) could be assigned to C-3 OMe, this being also supported by the upfield shift of the C-3 methine proton observed at 4.0 compared to AV-2 in which it occurs at δ 4.5. The assignment of the signal at δ 3.6 to C-3 OMe and not to 4*N*-Me was further supported by acetylating AV-2-X. The ^1H NMR ($CDCl_3$, δ) of the acetylated product did not show any downfield shift of the C-3 methine proton as would have been the case if a *gem*-OAc had been present. The signal at 2.23 (3H, *s*) thus, could be assigned to 4*N*-OAc. The multiplicity of the rest of the signals remained unchanged.

AV-2-Y showed in its ^1H NMR ($CDCl_3$, δ) two multiplets (2H each) centred at 2.2 and 3.3 for C-2 and C-1 methylene protons and two multiplets at 6.6 and 7.13 (2H each) for 4 aromatic protons. A triplet centred at 4.36 included under a doublet integrated for 3 protons which

could be attributed to C-3 methine and C-9 methylene protons. A singlet at 2.93 (3H) could be attributed to 4 *N*-Me, this being fully supported by its acetylated product. ^{1}H NMR ($CDCl_3$, δ) of the acetylated product showed two singlets (3H each) at 2.1 and 3.03 attributed to C-3 OAc and 4 *N*-Me. The presence of C-3 OAc was also supported by the downfield shift of the C-3 methine proton observed at 5.33 ($\Delta\delta = 0.97$) as compared to AV-2-Y, in which it is observed at 4.36. The multiplicity of the rest of the signals remained unchanged.

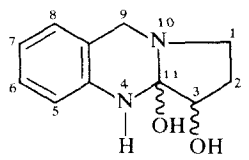
Acetylation of AV-2 yielded a crystalline compound mp 133–134°, M^+ 290, analysed for $C_{15}H_{18}N_2O_4$. IR showed prominent bands at 3320 (OH), 1748 and 1220 (OCOMe), 1680 (−N−C(=O)−Me) and 1608 and 1480 cm^{-1} (aromatic). ^{1}H NMR ($CDCl_3$, δ) showed a multiplet centred at 2.1 (2H) attributed to C-2 methylene, two singlets at 2.13 and 2.23 (3H each) attributed to C-3 OAc and 4 *N*-OAc respectively and a multiplet centred at 3.38 (2H) attributed to C-1 H_2. Two broad singlets at 4.39 and 4.4 (1H each) attributed to C-9 H_2 and a multiplet centred at 7.23 (4H) was attributed to aromatic protons. The signal for the C-3 methine proton is observed as a triplet at 5.33. D_2O exchange showed the presence of one exchangeable proton at 8.26, which was also supported by IR and mass spectrometry of the reaction product. Further acetylation failed to yield a triacetylated product. MS of the diacetate showed *m/e* at 290 (71%) with base peak at *m/e* 230 ($M^+ - 60$ due to loss of HOAc) and other peaks at *m/e* 247 (55.5%) ($M^+ - COCH_2$); *m/e* 188 (60%) ($M^+ -$ MeCOO; $-CH_2CO-$) and *m/e* 170 (40%) ($M^+ - 2 \times$ HOAc).

Passing HCl gas into the dry methanolic solution of AV-2 resulted in the formation of a product mp 204–206° characterised as vasicine hydrochloride (lit. [9] vasicine HCl mp 208°) by co-TLC, mmp, IR and ^{1}H NMR. The formation of a hydrochloride indicated the possible dehydration of the tertiary hydroxyl (C-11 OH) followed by the salt formation.

In order to support the existence of a C-11 OH, reaction of AV-2 with $POCl_3/C_5H_5N$ was carried out to effect dehydration and in turn the formation of vasicine. The reaction, however, yielded a compound mp 133–134°, M^+ 206, which analysed for $C_{11}H_{11}N_2Cl$ characterised as chlorodesoxy vasicine (lit. [4] chlorodesoxy vasicine mp 136–137°) by co-TLC, mmp, IR, ^{1}H NMR and mass spectrometry with an authentic sample of chlorodesoxy vasicine prepared by the reaction of $POCl_3/C_5H_5N$ with vasicine. The formation of chlorodesoxy vasicine from AV-2 may be caused by dehydration followed by chlorination.

Therefore, on the basis of chemical and spectral studies the alkaloid AV-2 may, therefore, be represented as 1,2,3,4,9,11 hexahydro pyrrolo (2, 1-b) quinazolin 3,11-diol.

This structure was eventually confirmed by partial synthesis from vasicine. Oxymercuration–demercuration and acid catalysed hydration of vasicine failed to yield the alkaloid AV-2. However, on heating a mixture of vasicine and water in a sealed tube at 140–150° for 16 hr AV-2 was obtained (60%) which analysed for $C_{11}H_{14}N_2O_2$ diacetate mp 133–135°. The synthetic compound and its diacetate was found to be identical by co-TLC, superimposable IR, ^{1}H NMR and mass spectrometry with AV-2 and its diacetate.



1,2,3,4,9,11 Hexahydro pyrrolo(2,1-b) quinazolin-3,11 diol

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded at 60 MHz using TMS as int. reference.

Extraction and separation of alkaloids. Finely cut roots (20 kg) were defatted with *n*-hexane and subsequently extracted with 95% EtOH. The EtOH extract was acidified with 5% HCl and extracted with $CHCl_3$ to remove non-alkaloidal components. The acidic extract was made alkaline with NH_4OH (pH 9–10) and extracted with $CHCl_3$. Concn (under vacuum) of the $CHCl_3$ extract yielded total alkaloids (40 g). The total alkaloids on Si gel TLC ($CHCl_3$–MeOH, 93:7) showed 2 spots. R_f 0.6 and 0.5. The two alkaloids AV-1 and AV-2 were obtained in pure form by column chromatography on Si gel using $CHCl_3$–MeOH (49:1).

AV-1. (1.89 g; 0.009%), mp 210–11°. (Found: C, 65.2; H, 5.02; N, 13.79, calc. for $C_{11}H_{10}N_2O_2$, C, 65.3; H, 4.98; N, 13.85%.)

Acetylation. To AV-1 (200 mg) in pyridine (10 ml) was added Ac_2O (13 ml) and the mixture refluxed for 2 hr. The reaction mixture on usual work up yielded a compound crystallized from Et_2O–Me_2CO, mp 124–125°. (Found: C, 64.10; H, 4.80; N, 11.55, calc. for $C_{13}H_{12}N_2O_3$, C 63.94; H, 4.94; N, 11.48%.)

AV-2. Gummy mass (0.8 g, 0.004%). *Methylaton.* To AV-2 (200 mg) dissolved in toluene (150 ml) was added a MeOH soln of trimethyl anilinium hydroxide (20 ml) and the mixture refluxed for 72 hr. The cooled mixture (0.5 g) after removal of solvent was chromatographed over Si gel. Column chromatography yielded two products AV-2-X (47 mg, 24% R_f 0.7 in $CHCl_3$–MeOH, 19:1) and AV-2 Y (21 mg, 10%, R_f 0.53 in $CHCl_3$–MeOH, 19:1) with $CHCl_3$ as eluant. The latter fraction on elution with $CHCl_3$–MeOH (99:1) yielded unreacted AV-2. AV-2 X crystallized from Me_2CO, mp 254–256°, M^+ 220. (Found: C, 65.25; H, 7.20, N, 12.80, calc. for $C_{12}H_{16}N_2O_2$, C, 65.43; H, 7.30, N, 12.72%). IR showed bands at 3210–3200 (−OH, −N(H)−) and 1602 and 1492 cm^{-1} (aromatic). AV-2 X acetate prepared as AV-1 acetate, mp 172°. (Found: C, 64.32; H, 7.02; N, 11.00, calc. for $C_{14}H_{18}N_2O_3$, C, 64.09; H, 6.92; N, 10.68%). IR showed bands at 3380 (−OH), 1682 (−N−C(=O)−Me) and 1605 and 1490 cm^{-1} (aromatic). AV-2 Y crystallized from Me_2CO–petrol, mp 224–226°. (Found: C, 65.32; H, 7.19; N, 12.83, calc. for $C_{12}H_{16}N_2O_2$, C, 65.43; H, 7.30; N, 12.72%). IR showed bands at 3370 (−OH) and 1603 and 1497 cm^{-1} aromatic). AV-2-Y acetate prepared as AV-2 X acetate mp 156°. (Found: C, 64.29; H, 6.998; N, 11.03, calc. for $C_{14}H_{18}N_2O_3$, C, 64.09; H, 6.92; N, 10.681%). IR showed bands at 3450 (−OH), 1740 and 1230 (−OCOMe) and 1600 and 1491 cm^{-1} (aromatic).

Acetylation of AV-2. AV-2 (100 mg) acetylated in the same manner as AV-1, yielded a reaction product crystallized from petrol–Et_2O, mp 133–134°, M^+ 290. (Found: C, 62.13; H, 6.32; N, 9.52, calc. for $C_{15}H_{18}N_2O_4$, C, 62.05; H, 6.23; N, 9.65%.)

Hydrochloride formation. Dry HCl gas was passed through a MeOH soln of AV-2 (80 mg, 25 ml) and the mixture kept at 5° for 30 min when a solid separated out, mp 204–206°. IR of the base HCl showed bands at 1685 (−C=N−), 1612 and 1493 (aromatic) 3270 and 3450 (−N−H and −OH). ^{1}H NMR (TFA, δ) of the compound showed two multiplets (2H each) centred at

2.33 and 3.5 for C-2 and C-1 methylenes and a singlet at 4.6 (2H) for C-9 methylene. A triplet at 5.16 (1H) was obtained for C-3 methine proton and a multiplet at 6.9 (4H) for C-5 to C-8 aromatic protons.

Dehydration of AV-2. To AV-2 (60 mg) dissolved in pyridine (8 ml) was added $POCl_3$ (1 ml) and the contents warmed (5 min). Usual work up yielded a reaction product crystallized from Et_2O–petrol (2:3), mp 133–134°, M^+ 206. (Found: C, 63.67; H, 5.25; N, 13.45, calc. for $C_{11}H_{11}N_2Cl$, C, 63.85; H, 5.33; N, 13.55%). IR showed bands at 1626 ($-\underset{|}{C}=N-$) and 1603 and 1493 cm^{-1} (aromatic). ^1H NMR ($CDCl_3$, δ) showed two multiplets (2H each), centred at 2.38 and 3.33 for C-2 and C-1 methylene and a singlet at 4.6 (2H) for C-9 methylene protons. A triplet (1H) at 4.66 and a multiplet (4H) centred at 7.06 was assigned to C-3 methine and C-5 to C-8 aromatic protons respectively.

Action of $POCl_3$ on vasicine. Vasicine (100 mg) was treated in the same way as that used for the dehydration of AV-2. The reaction product crystallized from petrol–Et_2O (3:2), mp 134°, M^+ 206. (Found: C, 63.65; H, 5.27; N, 13.42, calc. for $C_{11}H_{11}N_2Cl$, C, 63.85; H, 5.33; N, 13.55%)

Partial synthesis. Vasicine (0.5 g) in dist. H_2O (20 ml) was heated at 140–150° in a sealed tube for 16 hr. The contents were cooled, extracted with $CHCl_3$, the organic layer dried over Na_2SO_4 and the solvent removed by distillation. The crude reaction mixture thus obtained (0.5 g) was chromatographed over Si gel and eluted with $CHCl_3$–MeOH (99:1). The early fractions yielded a gummy mass (0.11 g) which failed to crystallize. (Found: C, 64.21; H, 6.89; N, 14.03, calc. for $C_{11}H_{14}N_2O_2$, C, 64.05; H, 6.83; N, 13.58%.) The compound (0.07 g) on acetylation and usual work up yielded from Me_2CO–petrol, a crystalline solid mp 133–133.5°. (Found: C, 62.29; H, 6.29; N, 9.83, calc. for $C_{15}H_{18}N_2O_4$, C, 62.05; H, 6.23; N, 9.65%.)

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