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NOVEL NOR-HARMAL ALKALOID FROM *ADHATODA VASICA*

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Key Word Index—*Adhatodavasica*; Acanthaceae; alkaloids; 9-acetamido-3,4-dihydropyrido-(3,4-b)-indole; 1,2,3,9-tetrahydropyrrolo-(2,1-b)-quinazolin-9(1 H)-one(deoxyvasicinone); sitosterol β-D-glucoside; *O*-ethyl-α-D-galacto-pyranoside and D-galactose.

Abstract—A novel alkaloid and a galactoside isolated from the roots of *Adhatoda vasica* have been characterized as 9-acetamido-3,4-dihydropyrido-(3,4-b)-indole and *O*-ethyl-α-D-galactoside respectively by chemical and spectroscopic methods. In addition sitosterol β-D-glucoside, D-galactose and deoxyvasicinone have also been isolated from the roots of this plant.

Adhatoda vasica Nees, one of two Indian species of the N. O. Acanthaceae known for its pharmacological activity [1–5], has been examined for alkaloid content. From the leaves and roots of this plant vasicine and related compounds have been isolated and characterized [6–8], but no harmal-type alkaloids have been reported. In this paper, we report from the roots of the plant, apart from the alkaloids isolated by earlier workers [9], deoxyvasicinone and the isolation and characterization of a new alkaloid which appears to have a nor-harmal nucleus. The basic chloroform fraction of the EtOH extract on column chromatography resulted in the isolation of two alkaloids AV_1 and AV_2.

The compound AV_1, mp 165–166°, showed a M^+ *m/e* 212.0950 (calculated for $C_{13}H_{12}N_2O$, 212.0950), $[\alpha]_D^{33}$ −54.2° (0.35 *c*, MeOH) and AV_1 HCl, mp 187–188°. The IR showed prominent bands at 1660 (amide) and 1630 (C=N–), 1601 and 1490 cm^{-1} (aromatic). ^1H NMR (CCl_4) showed a three-proton singlet at δ 2.76 attributed to N–CO–Me protons and two multiplets (2 H each) at 3.13 and 4.1 attributed to C-4 and C-3 methylene protons. respectively. A multiplet centred at 7.5 integrating for five protons is attributed to a C-1 methine proton and four aromatic protons. The downfield shift of the C-1 proton is due to its olefinic nature and α, β to N-atoms besides being allylic to an indole nucleus.

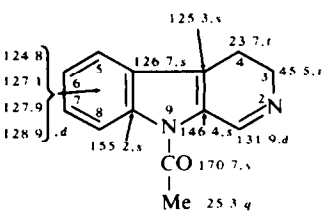
The MS of AV_1 gave the molecular formula $C_{13}H_{12}N_2O$ and the characteristic peak at *m/e* 169.075 (100%) was attributed to a dihydropyrido indole nucleus indicating the presence of –COMe, *m/e* 43 (97%) in AV_1 which is lost in the MS.

Further proof for the dihydropyrido-indole nucleus was obtained by alkaline hydrolysis of AV_1 which yielded the hydrolysed product AV_1-H, mp 205–206°, analysed for $C_{11}H_{10}N_2$, M^+ 170.1280 (calculated for $C_{11}H_{10}N_2$, 170.08439). IR of the hydrolysed product showed the disappearance of the N-COMe band at 1660 cm^{-1} and the appearance of a –NH band at 3140–3110 cm^{-1} (as expected due to the removal of –COMe, function) apart from the bands at 1625 (C=N–), 1602 and 1490 cm^{-1} (aromatic). In the ^1H NMR ($CDCl_3$) the disappearance of the N COMe signal at δ 2.76 (at the N-9 position) was observed. The position and multiplicity of rest of the signals remained unchanged. Thus, IR and ^1H NMR of the hydrolysed product clearly indicate that AV_1 has a reduced pyrido-indole nucleus.

To ascertain the olefinic nature of the C-1 proton, reduction of AV_1 with $NaBH_4$ was carried out. The ^1H NMR of the reduced product ($CDCl_3$) showed a multiplet at δ 3.26 (2 H) attributed to C-4 methylene protons and a multiplet centred at 3.8, integrating for four protons, attributed to C-1 and C-3 methylene protons respectively. A multiplet centred at 7.36 integrating for only four protons is attributed to aromatic C-5, C-6, C-7 and C-8 protons. D_2O exchange showed the presence of two exchangeable protons at 5.13 attributed to the protons at N-2 and N-9. $NaBH_4$ reduction resulted in the deacetylation of 9-N COMe as well as saturating the olefinic bond.

The structure of A_1 was further confirmed by acetylation (Ac_2O C_5H_5N) of AV_1-H and isolating the original compound AV_1 (co-TLC, mmp undepressed and superimposable IR and ^1H NMR).

In view of the above data and biogenetic considerations, the structure of AV_1 may be, therefore, represented as 9-acetamido-3,4-dihydropyrido-(3,4-b)-indole (**1**), which is also supported by ^{13}C NMR as shown in the figure.



AV_2, mp 104°, M^+ *m/e* 186 analysed for $C_{11}H_{10}N_2O$ and showed prominent IR bands at 1675 (N–C(=O)–R), 1624 (C=N–), 1603 and 1492 cm^{-1} (aromatic). ^1H NMR ($CDCl_3$) showed three multiplets (2 H each) at δ 2.3, 3.2 and 4.2 attributed to C-2, C-3 and C-1 methylene protons respectively, a multiplet centred at 7.5, integrating for 3 protons, attrbuted to C-5, C-6 and C-7 aromatic protons, while the doublet (J = 8 Hz) at 8.23 (1 H) is attributed to the C-8 proton.

From IR and ^1H NMR, AV_2 was identified as deoxyvasicinone (ref. [10], deoxyvasicinone, mp 110°), and the assignment was confirmed by comparison with an authentic sample (co-TLC, mmp undepressed, superimposable IR and ^1H NMR). Thus, the structure for AV_2 may be represented as 1,2,3,9-tetrahydropyrrolo-(2,1-b)-quinazolin-9(1 H)-one.

The dried aqueous extract of the roots on column chromatography over silica gel yielded three compounds, viz. AV_3, AV_4 and AV_5. AV_3, mp 278–280°, M^+ *m/e* 576, $[\alpha]_D^{35}$ −42.1° (0.4 *c*, MeOH), analysed for $C_{35}H_{60}O_6$, gave a positive Liebermann–Burchard test and responded to Molisch's test (after hydrolysis). Hydrolysis of AV_3 (acid and emulsin) yielded sitosterol as the aglycone (confirmed by ^1H NMR, co-TLC and mmp with an authentic sample) and glucose as the sugar moiety (confirmed by PC), thereby establishing AV_3 and sitosterol β-D-glucoside.

AV_4, mp 135–136°, M^+ *m/e* 208, $[\alpha]_D^{33}$ +170.4° (0.5 *c*, H_2O), analysed for $C_8H_{16}O_6$ and gave a positive Molisch's test (after hydrolysis). On hydrolysis of AV_4 (acid and amylase), galactose was identified as the sugar moiety by PC. The IR of AV_4 showed a prominent band due to –OH at 3390–3200 cm^{-1} and ^1H NMR (DMSO-d_6) showed a complex overlapped multiplet from δ 3.1 to 4.2 and a triplet at δ 1.36. The compound on acetylation yielded the acetate, mp 78–79°, analysed for $C_{16}H_{24}O_{10}$, $[\alpha]_D^{31}$ +113.6° (0.25 *c*, MeOH). ^1H NMR (CCl_4) of the acetylated product showed a triplet centred at δ 1.23 (3 H) which could be attributed to a methyl function adjacent to $-OCH_2-$, a group of four singlets (3 H each) from δ 1.9 to 2.13 attributed to four acetoxyl functions of the sugar, a multiplet centred at 3.6 (2 H) and a quartet at 4.03 (2 H) which could be attributed to ($-CH_2$) and ($-OCH_2$) respectively. A multiplet integrating for four protons centred at 5.3 could be attributed to methine protons of the sugar moiety. From mp, $[\alpha]_D$ and ^1H NMR of AV_4 and its acetylated product, AV_4 was identified as *O*-ethyl-α-D-galactopyranoside (ref. [11], mp 142° and confirmed by comparison with a synthetic sample, (see Experimental) (co-TLC, mmp undepressed, ^1H NMR). This appears to be the first confirmed report of its occurrence from a natural source, the only other report indicates its presence in lupin [12].

AV_5, mp 163°, M^+ *m/e* 180 $[\alpha]_D$ +141.57° (0.3 *c*, H_2O) gave a positive Molisch's test and was identified as D-galactose on PC.

EXPERIMENTAL

Mps are uncorr. ^1H NMR were recorded at 60 MHz using TMS as int. reference. ^{13}C NMR values given in the figure are also with reference to TMS. R_f values are for Si gel G.

Extraction of alkaloids. Finely cut roots (20 kg) were defatted with *n*-hexane and subsequently extracted with 95% EtOH. The EtOH extract was acidified with 3% HCl and extracted with $CHCl_3$ to remove non-alkaloidal components. The acidic extract was made alkaline with NH_3 (pH 9–10) and extracted with $CHCl_3$. Concn (*in vacuo*) of the $CHCl_3$ extract yielded total alkaloids (40 g).

Separation. Total alkaloids on TLC ($CHCl_3$–MeOH, 99:1) showed 2 spots with R_f 0.82 and 0.64. The two alkaloids, AV_1 and AV_2, were obtained in pure form by column chromatography over Si gel using C_6H_6–$CHCl_3$ (1:4). AV_1, mp 165–166° (0.103 g, 0.005%).

Alkaline hydrolysis of AV_1. AV_1 (20 mg) was treated with KOH (1%) in 50% EtOH (8 ml) and heated at 100° for 15 min. Extraction with $CHCl_3$ gave AV_1–H, mp 205–206°.

Reduction of AV_1. AV_1 (22 mg) was treated with $NaBH_4$ (35 mg) in EtOH and the mixture stirred for 16 hr. Dilution of the mixture and extraction with $CHCl_3$ yielded a crystalline compound, mp 194–196°. Found: C, 76.96; H, 7.01; N, 16.33. $C_{11}H_{12}N_2$ requires: C, 76.74; H, 6.97; N 16.27%. AV_2 (2 g, 0.01%) mp 104°. Found: C, 71.03; H, 5.62; N, 15.31. Calc. for $C_{11}H_{10}N_2O$: C, 70.95; H, 5.37; H, 15.05%.

Extraction of glycosides. The residual EtOH extract left after alkaloidal extraction was extracted with H_2O and the aq. extract vacuum-dried on a thin film evaporator to give a mixture of glycosides (24 g).

Separation. The aq. extract yielded 3 compounds viz. AV_3, AV_4 and AV_5, in pure form by column chromatography over Si gel using $CHCl_3$–MeOH mixtures of increasing polarity. AV_3 (4 g, 0.02%) mp 278–280°. Found: C, 73.13; H, 10.53. Calc. for $C_{35}H_{60}O_6$: C, 72.93; H, 10.41%. *Hydrolysis.* AV_3 (50 mg) dissolved in 5% HCl–MeOH (60 ml) was refluxed for 4 hr. On usual work-up fine needles of sitosterol crystallized from Et_2O, mp 136–137°, confirmed by co-TLC and mmp with authentic sample. PC of the aq. portion established the sugar moiety as glucose (Py–EtOAc–H_2O, 5:12:4 and *n*-BuOH–HOAc–H_2O, 4:1:5). On enzyme hydrolysis, a β-linkage was established. AV_4 (2 g, 0.01%), mp 135–136°. Found: C, 47.09; H, 7.86. Calc. for $C_8H_{16}O_6$: C, 46.15; H, 7.72%.

Hydrolysis. AV_4 (60 mg) was dissolved in 2% aq. HCl and refluxed for 2.5 hr. PC of the aq. soln established the sugar moiety as galactose (PC solvent system same as for AV_3). *Acetylation.* A mixture of AV_4 (30 mg), Ac_2O (2 ml) with a drop of cone H_2SO_4 was heated for 35 min at 100°. Usual work-up yielded colourless needles which crystallized from Et_2O–MeOH (19:1), mp 78–79°. Found: C, 52.13; H, 6.57. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06; H, 6.42%.

Partial synthesis of O-ethyl-α-D-galactopyranoside. D-(+)-Galactose (2 g) treated with 3% dry HCl–EtOH soln was reacted in a sealed tube at 100° for 52 hr. On usual work-up, column chromatography over Si gel and elution with $CHCl_3$–MeOH (7:3) yielded a few fractions which on concn yielded a crystalline compound, mp 139–140°. Found: C, 46.93, H 7.88. Calc. for $C_8H_{16}O_6$: C, 46.15; H, 7.72%. $[\alpha]_D^{30}$ +190.7° (0.35 *c*, H_2O). *Acetylation.* Using the method for AV_4 yielded a crystalline compound, mp 80–81°, $[\alpha]_D^{30}$ +117.3° (0.21 *c*, MeOH). AV_5 (1.5 g, 0.0075%) mp 163°. PC established it as D(+)-galactose (PC solvent system same as for AV_3).

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ISOLATION OF FUNIFERINE DIMETHIODIDE AND OBLONGINE FROM *TILIACORA FUNIFERA*

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Key Word Index—*Tiliacora funifera*; Menispermaceae; benzylisoquinoline; alkaloids; funiferine dimethiodide; oblongine.

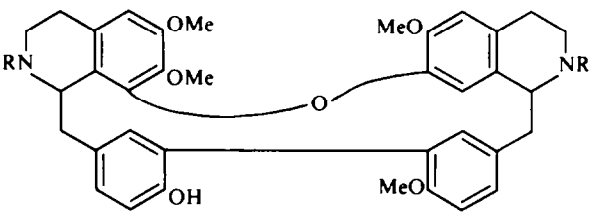
In previous papers [1–5] we have reported the isolation of various dimeric benzylisoquinoline alkaloids from the roots and leaves of *Tilicora funifera* Engl. ex Diels (Menispermaceae). In this paper we wish to present the isolation and identification of funiferine dimethiodide (**2**) and the novel quaternary benzylisoquinoline monomer oblongine (**3**) from the water-soluble alkaloid fraction of an extract of the roots of *T. funifera*.

The identity of funiferine dimethiodide (**2**) was established by a comparison of its physical and spectral properties with those of funiferine (**1**). The identity was confirmed by direct comparison of the properties of the isolated **2** with those of a specimen prepared by treating funiferine (**1**) with methyl iodide in acetone.

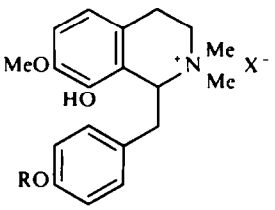
The ^{1}H NMR and MS of oblongine (**3**) suggested that it was a quaternary benzylisoquinoline alkaloid of the petaline type (**4**) [6]. That the isolated compound was oblongine (**3**) was indicated by a comparison of its spectral data with those published for oblongine (**3**) [7, 8]. The identity was confirmed by a direct comparison of the properties of the isolated compound with a synthetic racemic sample prepared by an unambiguous route [8].

Funiferine dimethiodide (**2**) is a new natural product that, to our knowledge, has not been reported previously. This is also the first report of a naturally-occurring quaternary bisbenzylisoquinoline biphenyl alkaloid. Funiferine dimethiodide (**2**) has previously been shown to be a slightly more potent muscle-relaxing agent than (+)-tubocurarine chloride [9].

Oblongine (**3**) has been found previously in *Berberis oblonga* [7] and another *Tiliacora* species, *T. dinklagei* [7].



1 R = Me
2 R = diMe; I^-



3 R = H; X = I
4 R = Me

Part XXIV in the series "Constituents of West African Medicinal Plants". For Part XXIII see (1979) *J. Nat. Prod.* **42**, 116.