ISOLATION OF DIHYDROAVICINE AND RHETSININE FROM ZANTHOXYLUM BUDRUNGA. THE REVISION OF ^1H AND ^{13}C NMR SPECTRAL ASSIGNMENTS FOR SANGUINARINE

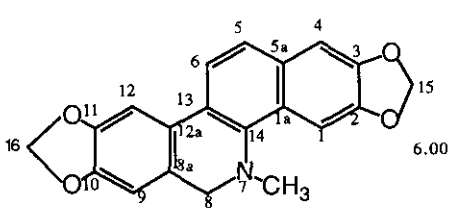
Balawant S. Joshi*[1], Mohindar S. Puar[2], Kristi M. Moore[1], and S. William Pelletier[1]
[1]Institute for Natural Products Research and School of Chemical Sciences, The University of Georgia, Athens, GA 30602, U.S.A.; [2]Schering-Plough Research, 60 Orange Street, Bloomfield, NJ 07003, U.S.A.

Abstract – Dihydroavicine (**1**) and rhetsinine (hydroxyevodiamine) (**5**) have been isolated from the bark of Zanthoxylum budrunga Wall. The ^1H and ^{13}C nmr assignments reported earlier for sanguinarine have been revised on the basis of HETCOR and selective INEPT studies.

In the course of investigating the alkaloids of Zanthoxylum budrunga Wall. we reported the isolation of the benzo[*c*]phenanthridine alkaloids dihydrochelerythrine and *N*-norchelerythrine, the quinazoline alkaloid evodiamine, and the quinolone alkaloid zanthobungeanine.[1] By extensive chromatographic separation from the same plant, we have isolated dihydroavicine (**1**), mp 212-214°C, M^+ m/z 333, as colorless needles. The identity of the alkaloid was confirmed by comparison with an authentic sample of dihydroavicine.[2] There is only one previous report of the isolation of **1** from Toddalia asiatica[3] and the alkaloid has been synthesized by Ishii et al.[2] The ^1H nmr spectrum showed four singlets at δ 6.78, 7.10, 7.28, and 7.65 assigned to H-9, H-4, H-12 and H-1, respectively.† The doublets at δ 7.48 and 7.62 were assigned to H-5 and H-6. The methylene protons at δ 4.10, 6.00 and 6.05 were assigned to *N*-(CH_3)-CH_2, and the methylenedioxy groups at H-16 and H-15, respectively. The proton assignments were confirmed by long-range 2D nOe experiments (Figure 1). The ^{13}C nmr spectrum and DEPT experiments showed 10 quaternary carbon singlets, 6 methine doublets, 3 methylene triplets and 1 quartet. The ^{13}C assignments were made by comparison with those of dihydrochelerythrine.[1] The carbon-13 nmr spectral data for most of the benzo[*c*]phenanthridine alkaloids are lacking.[4,5] However, a recent paper claimed unambiguous proton and carbon-13 nmr assignments for the benzo[*c*]phenanthridine alkaloid sanguinarine (**2**).[6] These assignments were made on the basis of APT, CSCM 1D and selective INEPT experiments. A comparison of our values for dihydrochelerythrine[1] and dihydroavicine (see Experimental) with those of sanguinarine (Table 1) indicated gross differences in the carbon-13 assignments for C-1, C-5a, C-5 and C-8a. Also, the proton assignments for H-6 (δ 8.56) and H-12 (δ 8.09) did not appear to be correct. These peri protons of the phenanthridine would be expected to be shifted downfield compared with those of H-5 and H-11.[7] The early proton assignments for sanguinarine,[8] which are very similar to

† The numbering system adopted here is based on biogenetic considerations as reported earlier.[1]

those reported by Blaskó et al.,[6] have been corrected (Table 1). In a recent paper[9] the ^{1}H nmr assignments[6] of sanguinarine have been revised, based on NOESY spectra.



1

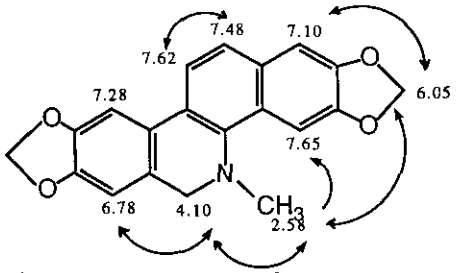
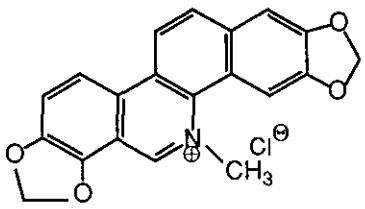
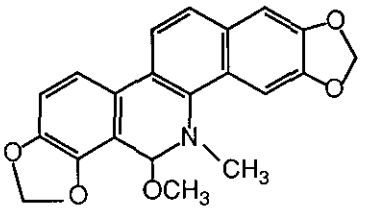


Figure 1 Long range nOe enhancements in 1



2

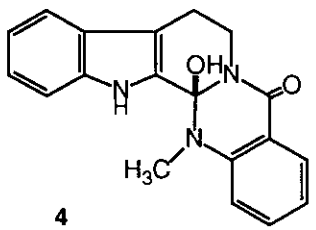


3

The carbon multiplicities and the one-bond ^{1}H-^{13}C partners were assigned, using a heteronuclear COSY (HETCOR) spectrum. This spectrum clearly indicated a correlation of the H-1 proton at δ 8.27(s) with the carbon appearing at 104.1 ppm and distinguished the proton H-5 at δ 8.29(d) which correlated with the carbon at 131.2 ppm. The H-6 proton at δ 8.77(d) showed correlation to the carbon doublet at 118.7 ppm, and the H-12 proton at δ 8.63(d) indicated a correlation with the signal at 117.2 ppm assigned to C-12. Similarly, the proton at δ 8.11(d) assigned to H-11 showed correlation with the carbon appearing at 119.8 ppm. The signal at δ 7.76(s) assigned to H-4, showed correlation with the doublet at 105.6 ppm. The remaining proton H-8 (δ 10.15) showed the expected one-bond correlation with the carbon signal at 149.8 ppm. All the methine protons were thus correlated with the corresponding carbon signals. The ^{13}C nmr spectrum of **2**, showed ten quaternary carbon signals at 109.3, 120.2, 125.5, 127.0, 131.3, 132.1, 146.1, 147.4 and 148.6 (2C) ppm which were assigned to C-8$_a$, C-1$_a$, C-13, C-12$_a$, C-14, C-5$_a$, C-9, C-10, C-2 and C-3, respectively, on the basis of selective INEPT studies (Table 2). Thus, polarisation transfer from the methyl singlet (*N*-CH$_3$, δ 4.93) to the quaternary carbon at 131.3 ppm confirmed this carbon as C-14 and the carbon signal at 149.8 as C-8 (three-bond polarisation transfer). Similar selective INEPT experiments by polarisation transfers from H-11 (δ 8.11), H-5 and H-1 (δ 8.29, 8.27 overlap), H-6 (δ 8.77) and H-8 (δ 10.15) enabled assignments of the remaining carbon signals. The earlier[6] proton and carbon-13 assignments of sanguinarine **2** and the corrected values are given in Table 1.

During the isolation of sanguinarine (**2**) from the roots of Sanguinaria canadensis L, we obtained the methanol adduct, 8-methoxydihydrosanguinarine (**3**). Although this alkaloid is reported as oc-

curring naturally[4,5,10] it is most likely an artefact.[11,12]



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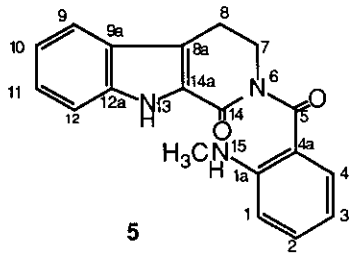

5

The indoloquinazoline alkaloid rhetsinine[13] (hydroxyevodiamine[14]) was isolated from the bark of Z. budrunga. Rhetsinine was recognized as identical with a product obtained by $KMnO_4$ oxidation of evodiamine and designated as hydroxyevodiamine (**4**).[15] Pachter and Suld showed by synthetic and uv spectral studies, that hydroxyevodiamine in fact should be formulated as the dicarbonyl compound **5**.[16] If rhetsinine (hydroxyevodiamine) has the structure **4**, the carbinolamine carbon C-14 would be expected to appear around 100 ppm. In evodiamine, C-14 appears at 69.9 ppm[1] and substitution will shift this carbon downfield by ~25-30 ppm. The carbonyls of the two amide groups at C-14 and C-5 in **5** have been assigned 150.0 ppm and 158.2 ppm, respectively, thus providing additional evidence for the formulation of rhetsinine as **5** and not as **4**. It is true that the two tautomeric structures **4** and **5** would be expected to shift their equilitrium on acid-base extractions. The alkaloid might exist in the plant as a salt of anhydrorhetsinine to afford **5** an acid-base work up.

Table 1. ^1H and ^{13}C nmr data of sanguinarine (**2**) in DMSO-d_6

Carbon No.	Present assignments	Earlier assignments[6]	Proton No.	Present assignments	Earlier assignments[6]
C-1	104.1 d	131.00	H-1	8.27 s	8.24 s
C-2	148.6 s	148.52	–	–	–
C-3	148.6 s	148.50	–	–	–
C-4	105.6 d	105.54	H-4	7.76 s	7.72 s
C-5_a	132.1 s	109.15	–	–	–
C-5	131.2 d	103.96	H-5	8.29 d	8.23 d
C-6	118.7 d	117.05	H-6	8.77 d	8.56 d
C-13	125.5 s	125.28	–	–	–
C-12_a	127.0 s	126.78	–	–	–
C-12	117.2 d	119.68	H-12	8.63 d	8.09 d
C-11	119.8 d	118.53	H-11	8.11 d	8.70 d
C-10	147.4 s	147.27	–	–	–
C-9	146.1 s	146.00	–	–	–
$C8_a$	109.3 s	131.87	–	–	–
C-8	149.8 d	149.49	H-8	10.15 s	10.11 s
N-CH_3	52.0 q	52.09	CH_3-7	4.93 s	4.91 s
C-14	131.3 s	131.00	–	–	–
C-1a	120.2 s	119.90	–	–	–
O-CH_2-O (15)	102.7 t	102.71	CH_2-15	6.35 s	6.35 s
O-CH_2-O (16)	104.8 t	104.84	CH_2-16	6.61 s	6.61 s

Table 2. Nmr data from selective INEPT experiments on **2**

Irradiation of proton assigned to	δ	Enhancement of the carbon signal assigned to* (ppm)		
		Strong	Medium	Weak
N-CH_3	4.93	149.8 (C-8) 131.3 (C-14)		
H-4	7.76	148.6 (C-2) 120.2 (C-1_a)		
H-11	8.11	146.1 (C-9) 127.0 (C-12_a)		147.4 (C-10)
H-5, H-1	8.27, 8.29	148.6 (C-3) 132.0 (C-5_a)	125.5 (C-13) 120.2 (C-1_a)	131.3 (C-14)
H-6	8.77	132.1 (C-5_a) 131.3 (C-14)	127.0 (C-12_a)	
H-8	10.15	131.3 (C-14) 127.0 (C-12_a)	146.1 (C-9) 109.3 (C-8_a)	
H_2-15	6.35	148.6 (C-2, C-3)		
H_2-16	6.61	147.4 (C-10)		

* Strong: 100%, Medium: 40-60%, Weak: < 40%

EXPERIMENTAL

Mps were determined on a Thomas-Kopfler hot stage equipped with a microscope and polarizer. Ir: Perkin-Elmer Model 1420 spectrophotometer; ^1H and ^{13}C nmr: Varian XL 300 (300 MHz for ^1H and 75.5 MHz for ^{13}C) and Varian XL 400 (400 MHz for ^1H and 100.5 MHz for ^{13}C) spectrometers; mass-spectra: Finnigan Quadrupole 4023. Abbreviations; s = singlet, d = doublet, t = triplet, br = broad. Selective INEPT experiments were carried out utilizing a Varian XL 300 nmr spectrometer. The following pulse sequence was utilized: ^1H: $90°_\chi$ - τ_1 - $180°_y$ - τ_1 - $90° \pm y$ - τ_2 - $180°_\chi$ - τ_2 - Decouple. ^{13}C: $180°_\chi$ - $90°_\chi$ - $180°_\chi$ Acquire[17]. The low-power proton decoupler power was attenuated to 16 Hz to provide a soft 90° proton pulse of 15.7 ms and the decoupler frequency was set to the proton of interest. The delays D_2 and D_3 were set equal to 30 and 40 ms, respectively, the latter corresponds to a proton-carbon coupling constant of approximately 7 Hz. For chromatographic separations on a Chromatotron, rotors were coated with 1 mm thick layer of silica gel (HF–254+366; EM 7744). Tlc was carried out on silica gel 60H (EM 7736).

Isolation of dihydroavicine (**1**) and rhetsinine (**5**): – Air dried and powdered bark of Z. budrunga (1 kg) was extracted with hexane (800 ml x 4) for 12 h and then with 95% EtOH (1 l x 4) for 12 h in a Soxhlet apparatus. The hexane and the EtOH extracts were evaporated under vacuum to give gummy residues (A) and (B) respectively. The residue (A; 9 g) was chromatographed by VLC[18] on silica gel and eluted with benzene containing increasing amounts of $CHCl_3$. The fraction eluted with benzene:5% $CHCl_3$ was purified on a silica gel rotor of a Chromatotron[19] and gradient eluted with hexane:Et_2O. The fractions collected by elution with hexane:10% Et_2O were pooled (650 mg) and

rechromatographed twice on silica gel rotors and eluted with hexane to afford dihydroavicine (**1**) 15 mg, mp 212-214°C; FAB. ms: m/z 333 (M^+); $C_{20}H_{15}NO_4$ (MW: 333); ir (nujol): ν_{max} 1460, 1385, 1315, 1295, 1270, 1240, 1228, 1160, 1110, 1080, 1040, 950, 935, 910 cm^{-1}. ^1H Nmr ($CDCl_3$): δ 2.58 (3H, s, *N*-CH_3), 4.10 (2H, s, *N*-CH_2), 6.00 (2H, s, O-CH_2-O), 6.05 (2H, s, O-CH_2-O), 6.78 (1H, s, H-9), 7.10 (1H, s, H-4), 7.28 (1H, s, H-12), 7.48 (1H, d, J=9 Hz, H-5), 7.62 (1H, d, J=9 Hz, H-6), 7.65 (1H, s, H-1). ^{13}C Nmr ($CDCl_3$): δ 100.8 (d, C-1), 148.2 (s, C-2), 147.5 (s, C-3), 104.4 (d, C-4), 125.9 (s, C-5_a), 123.9 (d, C-5), 120.2 (d, C-6), 124.5 (s, C-13), 126.3 (s, C-12_a), 107.5 (d, C-12), 147.1 (s, C-11), 147.0 (s, C-10), 103.6 (d, C-9), 130.9 (s, C-8_a), 55.2 (t, C-8), 40.8 (q, *N*-CH_3), 142.8 (s, C-14), 101.1 (2C) (t, O-CH_2-O). Tlc, ir and mixture mp of the alkaloid were identical with those of an authentic sample[2] of dihydroavicine.

The extract (B; 175 g) was stirred with $CHCl_3$ (250 ml) and 5% H_2SO_4 (200 ml). The tarry residue was separated and the acidic layer was extracted with $CHCl_3$ (150 ml x 3). The acidic solution was basified with sat. Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ extract was dried (Na_2SO_4) and evaporated to give a yellowish-brown residue (1.8 g). This was repeatedly triturated with Et_2O-MeOH to afford an insoluble residue (50 mg) which recrystallized from EtOH–Et_2O to afford **5** as yellow plates, mp 185-187°C(decomp.). FAB ms: m/z 319 (M^+); $C_{19}H_{17}N_3O_2$ (MW: 319). The free base **5** (10 mg) was dissolved in hot MeOH, a few drops of methanolic hydrogen chloride were added and kept overnight. The precipitated hydrochloride (anhydrorhetsinine hydrochloride) was collected as fine, yellow needles, mp 256-258°C (decomp.). ^1H Nmr (DMSO-d_6): δ 12.60 (1H, s, NH), 8.36 (1H, dd, J=10, 2 Hz, H-4), 8.20 (1H, br d, J=10 Hz, H-9), 8.14 (1H, dt, J=10, 2 Hz, H-11), 7.89 (1H, br d, J=10 Hz, H-12), 7.81 (1H, dt, J=10, 2 Hz, H-10), 7.71 (1H, br d, J=10 Hz, H-1), 7.53 (1H, dt, J=10, 2 Hz, H-2), 7.28 (1H, t, J=10 Hz, H-3), 4.48 (2H, t, J=6 Hz, *N*-CH_2CH_2), 4.40 (3H, brs, *N*-CH_3), 3.32 (2H, t, J=6 Hz, *N*-CH_2CH_2). ^{13}C Nmr (DMSO-d_6): δ 141.3 (s, C-1_a), 127.6[a] (d, C-1), 136.6[a] (d, C-2), 128.6[a] (d, C-3), 128.7[a] (d, C-4), 120.0 (s, C-4_a), 158.2 (s, C-5), 42.0 (t, C-7), 18.5 (t, C-8), 118.7 (s, C-8_a), 123.2 (s, C-9_a), 118.5 (d, C-9), 121.5 (d, C-10), 121.5 (d, C-11), 113.4 (d, C-12), 130.2 (s, C-12_a), 139.6 (s, C-14_a), 150.0 (s, C-14), 40.8 (q, *N*-CH_3). ([a] These assignments may be interchanged).

Isolation of sanguinarine (**2**) and 8-methoxydihydrosanguinarine (**3**); – Powdered roots of *Sanguinaria canadensis* L. (450 g) were extracted in a Soxhlet apparatus with hexane (2 l x 2) for 6 hr and 95% EtOH (2 l x 2) for 6 hr. Evaporation of the EtOH extract gave a red residue (54 g). Part of this (37 g) was stirred with 5% H_2SO_4 (1l) and washed with $CHCl_3$. The acidic layer was kept for two days to give a crystalline product (4.9 g). Part of this (500 mg) (dissolved in MeOH and adsorbed on Al_2O_3) was chromatographed over Al_2O_3 on a VLC column and fractions were collected by elution with benzene and increasing percent of EtOAc. The first 8 fractions (50 ml each) obtained by elution with benzene gave a residue (140 mg) which after two recrystallizations from CH_2Cl_2-MeOH afforded colorless cubes (**3**; 80 mg), mp 206-207°C. Ms: m/z 363 (M^+; 28%), 332 (M^+- OCH_3; 100), 317(15). $C_{21}H_{17}NO_5$ (MW: 363). ^1H Nmr ($CDCl_3$): δ 7.77 (1H, d, J=9 Hz, H-6), 7.70 (1H, s, H-1), 7.49 (1H, d, J=9 Hz, H-5), 7.42 (1H, d, J=9 Hz, H-12), 7.14 (1H, s , H-4), 6.95 (1H, d, J= 9 Hz, H-11), 6.08 (2H, s, H-16), 6.07 (2H, s, H-15), 5.40 (1H, s, H-8), 3.48 (3H, s, OCH_3), 2.80 (3H, s, *N*-CH_3).

^{13}C Nmr ($CDCl_3$): δ 100.6 (C-1), 148.1(C-2), 147.2[a] (C-3), 104.7 (C-4), 131.0 ($C-5_a$), 123.8 (C-5), 120.2 (C-6), 125.7 (C-13), 122.8 ($C-12_a$), 116.4 (C-12), 108.8 (C-11), 147.4[a](C-10), 145.6 (C-9), 113.1 ($C-8_a$), 85.9 (C-8), 138.2 (C-14), 126.8 ($C-1_a$), 54.3 (OCH_3), 40.9 (*N*-CH_3), 101.1 (C-15), 101.7 (C-16). ([a] These assignments may be interchanged).

Compound **3** (30 mg) was heated at 100°C for 5 min with 10% HCl (10 ml) and recrystallized from aq. HCl to afford sanguinarine chloride (**2**) (15 mg; 45%) as red needles, mp 274-276°C, identical (tlc, ir) with an authentic sample of sanguinarine chloride.

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