STRUCTURE AND STEREOCHEMISTRY OF DIVARICINE, A NEW BISINDOLE ALKALOID FROM *STRYCHNOS DIVARICANS* DUCKE.

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Abstract – The complete stereostructure of divaricine, a new bisindole alkaloid from *Srychnos divaricans* Ducke., has been determined mainly by the use of one- and two-dimensional nmr techniques.

During the course of structural and pharmacological studies on a series of tertiary indole alkaloids from Brazilian *Strychnos*,[1,2] we have isolated from the roots of *Strychnos divaricans* Ducke. (Loganiaceae) a new indole alkaloid, named divaricine. Application of a variety of one- and two-dimensional (2D) nmr techniques established the 18-deoxy Wieland-Gumlich aldehyde N^4-oxide – vellosimine coupled bisindole structure (**1**) for divaricine.

Divaricine, a polar alkaloid, was obtained as colorless prisms from MeOH–EtOAc, mp 280–282 °C (decomp.), $[\alpha]_D^{22°}$ +94° (*c* 0.1, EtOH); λ_{max}^{EtOH} (log ε) 236 (4.04), 284 (3.85), and 290.5 (3.83) nm. Its electron impact mass spectrum exhibited a molecular ion peak at *m/z* 584 corresponding to the formula $C_{38}H_{40}N_4O_2$ (found *m/z* 584.3161; calcd 584.3152). The fragment ion peaks at *m/z* 130, 143, 144, 168, and 169 in the mass spectrum revealed the presence of an aromatic unsubstituted sarpagine-like framework[3,4] in divaricine. This was further supported by the presence of three other major fragment ion peaks at *m/z* 247, 248, and 249 which are comparable to those observed at *m/z* 249, 250, and 251 in the mass spectra of the bisindole alkaloids longicaudatine[5] and usambarensine,[6] characteristic of the presence of an ethylidene indoloquinolizidine moiety.

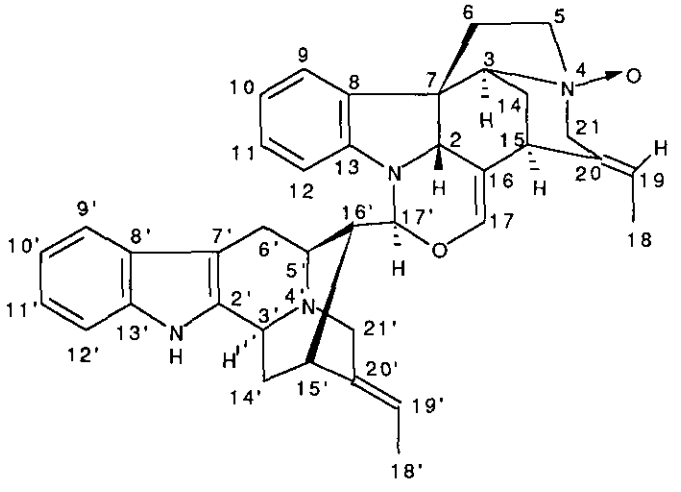
The above structural features as well as the complete structure of divaricine could be independently inferred from a detailed analysis of the different nmr data (CD_3OD solution) obtained for this alkaloid. The proton decoupled ^{13}C nmr spectrum exhibited 34 signals for the 38 carbon atoms of the molecule, four of the signals representing two carbons each. The DEPT[7] spectra revealed the presence of one sp^3 quaternary carbon, nine sp^2 quaternary carbons, eight sp^3 methine carbons, eleven sp^2 CHs, seven sp^3 methylenes and two methyl groups (Table 1). Together they accounted for

38 carbons and 39 protons thereby indicating the presence of a single exchangable hydrogen which is presumably the one linked to the indole nitrogen of the ethylidene indoloquinolizidine moiety. It could therefore be concluded that the two oxygen atoms present in the molecule of divaricine did not belong to any alcoholic group. The 500 MHz ^{1}H nmr spectrum also integrated for 39 protons and revealed the presence of two sets of four aromatic protons along with three olefenic protons, two of which belonged to two ethylidene groups (δ 0.80, 3H, dt, J = 7 and 1.8 Hz; δ 4.51, 1H, q, J = 7 Hz, and δ 1.70, 3H, dt, J = 7 and 1.8 Hz; δ 5.56, 1H, q, J = 7 Hz), leaving the third olefinic proton (δ 6.28, 1H, d, J = 1.4 Hz) to be assigned.

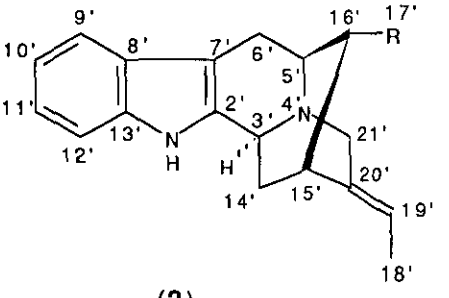
Proton-carbon chemical shift correlations for all of the carbons directly bonded to protons were established from data obtained with the conventional carbon-detected H/C 1-bond correlation 2D spectrum, and corroborated with the newer and more sensitive proton-detected (HMQC)2D experiment.[8] Furthermore, total correlation spectroscopy (TOCSY) experiments showed the presence of four aromatic CH in each indole ring. The proton-detected long range heteronuclear chemical shift correlation (HMBC) 2D spectrum[9] provided 2-bond and 3-bond ($^2J_{CH}$ and $^3J_{CH}$) long range C/H correlations which established one part as indole and the other as an indoline moiety in divaricine. This accounted for the 14 indole sp^2 carbons, leaving 6 other sp^2 carbons 4 of which could be attributed to the two ethylidene systems. By assigning proton signals at δ 0.80 and 4.51 to 18-H$_3$ and 19-H, respectively, the carbon connectivity pattern of the upper half of divaricine could be deduced in the following manner. The 19-H was correlated to C-15, C-18 and C-21, whereas the 18-H$_3$ correlated to C-20. A 2-bond correlation was also found between 18-H and C-19. Correlations of C-20 to one of the 14-H$_2$, 15-H, 21-H$_2$ and of C-19 to 21-H$_2$ were also noted, whereas C-16, a quaternary sp^2 carbon, correlated to 2-H, one of the 14-H$_2$ and 15-H. At this point, it became clear that the above mentioned third olefinic proton 17-(C)H must be linked to C-16. This attribution was fully consistent with the observed correlations of 17-H to C-2, C-15 and C-16. Strong correlations of C-2 to 3-H, 6-H$_2$, 17-H and of C-15 to 3-H, 17-H, 19-H and to one of the 21-H$_2$ established the C-3–C-14 bond. Further correlations between C-6 and 5-H$_2$, and of C-5 to 6-H$_2$ and 21-H$_2$ as well as of C-7 to one of the 5-H$_2$, 6-H$_2$ and one of the 14-H$_2$ with the insertion of N^4 completed the *Strychnos* type indoline moiety in divaricine. The low field ^{1}H chemical shift values of 3-H, 5-H$_2$ and 21-H$_2$ together with the ^{13}C data of the corresponding carbons (Table 1) indicated that they should be attached to an *N*-oxide as in **1**. A methine carbon resonance at δ 85.47 was correlated to 17-H and was assigned to a carbon which, considering its chemical shift value, must be attached to two hetero atoms as shown for C-17' in **1**. All the ^{1}H and ^{13}C chemical shifts fully supported the connectivity pattern elaborated so far.

The occurrence of two low field sp^3 methine carbons at δ 52.29 and 56.65 and a methylene carbon at δ 56.58 suggested their linkage to a nitrogen atom. These data, together with the presence of a second ethylidene group and the multplicity patterns of the remaining carbons in divaricine, led us to suspect a sarpagine-like framework, as in normacusine-B (**2**; R = CH_2OH)[10] or vellosimine (**2**; R = CHO),[11] for the indole moiety in this alkaloid. Indeed, comparison of our ^{13}C nmr spectral data with those reported for normacusine-B *O*-acetate[12] showed nearly identical values, except for the

resonance of C-17' which appeared at a lower field (δ 85.47) as would be expected due to its linkage with two heteroatoms. This structural feature was also supported by multiple bond C/H correlations. Thus, C-7' was correlated to 3'-H, 5'-H and 6'-H_2 while C-2' could be correlated to 3'-H, 6'-H_2 and 14'-H_2. The aromatic C-8' showed a correlation to one of the 6'-H_2. Furthermore, C-3' was correlated to 5'-H, one of the 14'-H_2, 15'-H and 21'-H_2. Also, the observed correlations between C-14' and 3'-H, 16'-H, between 21'-H_2 and C-3', C-5' and C-19', between C-20' and 14'-H_2, 16'-H, and 21'-H_2 with the insertion of $N^{4'}$ established the sarpagine-type connectivity. Thus, all the carbons, protons, nitrogens and oxygens of divaricine were accounted for by the structural representation (1). This structure fully accommodates all the chemical shifts along with their multiplicity patterns observed in the nmr spectra (Table 1).



(1)



(2)

The stereochemistry of divaricine (1) was supported by correlations through nOe difference experiments. From biogenetic point of view, assuming the 15α configuration as starting point, the orientation of the proton at the 2 position could be established as β because of the lack of nOe responses between these two protons. A strong nOe response between the 15-H and the 18-Me confirmed the *E* configuration of the ethylidene chain at C-20. The 19 olefinic proton also exhibited an nOe response to one of the 21 protons thereby further supporting this assignment. The 3-Hα orientation was established from its correlation with 14-Hα which exhibited nOe response to 15-Hα. Similarly, the *E* configuration of the ethylidene chain at C-20' was assigned on the basis of an nOe linking 19'-H and the protons at the 21' position. The 17'-Hα orientation was deduced from the lack of nOe response to 2-Hβ, while the expected 5'-Hα orientation was supported from its observed response to 17'-Hα. A strong nOe response to 5'-Hα established the proton resonating at δ 2.12 as due to 14'-Hα, which in turn could be correlated to 3'-Hα thereby confirming the assigned stereochemistry. Finally, the 16'*R* configuration could be assigned on the basis of the comparison of

Table 1. ^{1}H and ^{13}C Nmr data

carbon no.	^{13}C mult[a]	^{1}H (J in Hz)	long range C/H correlations from C no.[b]
2	59.50 d	3.77 br s	3-H, 6-H_2, 17'-H, 17-H
3	83.14 d	4.18 br s	2-H, 6-H_2, 15-H, 21-H
5	69.81 t	3.76 m; 4.00 m	6-H; 21-H_2
6	36.28 t	2.28 m; 2.42 m	5-H_2
7	55.91 s		6-H_2, 9-H
8	131.00 s		10-H, 12-H
9	123.75 d	7.25 d (8.5)	11-H
10	122.43 d	6.91 t (8.5)	12-H
11	130.52 d	7.25 t (8.5)	9-H
12	112.05 d	6.86 d (8.5)	10-H
13	151.27 s		9-H, 11-H, 2-H, 17'-H
14	27.24 t	1.47 dt (15, 2.5); 2.50 dt (2.5, 15)	
15	33.06 d	2.91 br s	3-H, 19-H, 21-H, 17-H
16	114.44 s		2-H, 14-H, 15-H, 17-H
17	137.78 d	6.28 d (1.4)	2-H, 15-H, 17'-H
18	13.39 q	0.80 dt (7, 1.8)	19-H
19	133.94 d	4.51 q (7)	18-H, 21-H_2
20	128.59 s		14-H_2, 18-H, 21-H_2
21	73.79 t	3.45 d (14); 3.87 d (14)	19-H
2'	139.71 s		6'-H_2, 14'-H_2
3'	52.29 d	4.28 br d (9.5)	5'-H, 14'-H, 15'-H
5'	56.65 d	3.00 br s	3'-H, 6'-H_2, 15'-H, 16'-H, 17'-H, 21'-H
6'	28.38 t	2.77 dd (15, 2); 3.04 ddd (15, 4.5, 1.6)	5'-H, 16'-H
7'	105.45 s		3'-H, 5'-H, 6'-H_2, 9'-H
8'	128.59 s		6'-H, 10'-H, 12'-H
9'	118.35 d	7.32 d (8.5)	11'-H
10'	120.02 d	7.09 t (8.5)	12'-H
11'	122.48 d	7.17 t (8.5)	9'-H
12'	112.46 d	7.39 d (8.5)	10'-H
13'	138.70 s		9'-H, 11'-H
14'	34.10 t	1.73 ddd (13.2, 4.5, 2); 2.12 ddd (13.2, 9.5, 2)	3'-H, 16'-H
15'	28.78 d	2.98 m	3'-H, 14'-H_2, 17'-H, 19'-H
16'	40.97 d	2.45 m	6'-H, 14'-H_2, 15'-H, 17-H
17'	85.47 d	5.18 d (10.2)	5'-H, 16'-H
18'	13.35 q	1.70 dt (7, 1.8)	19'-H
19'	118.95 d	5.56 q (7)	18'-H, 21'-H
20'	135.65 s		14'-H_2, 15'-H, 16'-H, 21'-H
21'	56.58 t	3.62 t (2)	3'-H, 19'-H

[a] multiplicity from DEPT spectra; [b] from HMBC spectra.

the reported[13] chemical shifts of 5'-H, 6'-Hβ and 16'-H. Also, according to molecular model construction, only the depicted 16'*R* configuration would allow the linkage beween the two moities of divaricine.

The formation of divaricine (**1**) may be rationalized by linking of the formyl group of vellosimine (**2**; R = CHO)[11] through an acetal involving the indoline NH and the OH of the enol form of 18-deoxy Wieland-Gumlich aldehyde[14,15] N^4-oxide.

EXPERIMENTAL

Melting points are uncorrected. Optical rotation was determined on a Perkin-Elmer model 141 polarimeter. Spectra were recorded with the following instruments: uv, Beckman DU-7; ir, Nicolet MX-1 IR spectrophotometer; ms, AEI MS50 and KRATOS MS80 ; nmr, ^1H (300 and 500 MHz) and ^{13}C (75.7 and 125.8 MHz) on Varian Gema and Unity spectrometers and the HMBC, 2D spectra on Unity 500 MHz machine in CD_3OD–C_6D_6 (9:1); chemical shifts are given in ppm relative to TMS (δ=0); abbreviations s, d, t, q and m in Table 1 refer to singlet, doublet, triplet, quartet and multiplet, respectively.

Plant materials. The roots of *Strychnos divaricans* Ducke. were collected in Recife, Brazil, in November 1988, by Professor Alda de A. Chippeta. Voucher specimens were deposited at LTF, UFPB.

Isolation and purification of alkaloids. Extraction of the total alkaloid mixture (470 g, dark syrup) from *S. divaricans* roots (3.05 kg) was carried out as previously described.[1] After separating the nonpolar fraction, the aqueous part was brought to pH 9.5–10 by dropwise addition of conc. NH_4OH at 0–5 °C and the liberated bases were extracted exhaustively with $CHCl_3$ (7 l) till the extract responded negative to Mayer's test. The $CHCl_3$ extract was then washed with cold water to free it from alkali, dried over anhydrous Na_2SO_4, concentrated *in vacuo* at 40 °C and the concentrated extract (3 g) was dissolved in a mixture of $CHCl_3$ (15 ml) and MeOH (2 ml), and then chromatographed over Brockmann alumina grade II-III (250 g) using EtOAc and EtOAc-MeOH (19:1, 9:1 and 4:1) as eluents. Evaporation of the fractions eluted with EtOAc furnished a brownish solid (45 mg, 0.0016%) which crystallized from MeOH in colorless needles, mp 305–306 °C, $C_{19}H_{20}N_2O$, *m/z* 292 ($M^{+\cdot}$), $[\alpha]_D^{22°}$ +35° (*c* 0.1, EtOH), λ_{max}^{EtOH} (log ε), 233.5 (3.98), 276 (sh, 3.78), 281.5 (3.79) and 289.5 (3.70) nm; ν_{max}^{KBr} (cm^{-1}), 3400 (NH) and 1713 (CO) was characterized as vellosimine. The fractions eluted with EtOAc–MeOH mixtures (19:1 and 9:1), which showed the presence of polar compounds on tlc, were combined together (0.4 g) and rechromatographed over Brockmann alumina grade II-III (50 g), using EtOAc and EtOAc–MeOH mixtures (9:1 and 4:1) as eluents. The EtOAc fractions gave, upon evaporation, a trace amount of vellosimine. The earlier fractions eluted with 9:1 EtOAc–MeOH mixture provided a solid crystallizing from MeOH–EtOAc in colorless needles (*ca.* 15 mg, 0.0005%), mp 240–242 °C, *m/z* 568 ($M^{+\cdot}$), whose structure is currently under investigation. The later 9:1 EtOAc–MeOH fractions as well as the earlier 4:1 EtOAc–MeOH fractions furnished divaricine as a slightly colored solid which

crystallized from MeOH-EtOAc in colorless prisms (107 mg, 0.0035%), mp 280–282 °C (decomp.), $C_{38}H_{40}N_4O_2$ ($M^{+\cdot}$ at *m/z* 584). All the later fractions eluted with 4:1 EtOAc–MeOH gave a mixture of polar alkaloids which were grouped together (*ca.* 150 mg) for further inverstigation.

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