STRUCTURE AND STEREOCHEMISTRY OF DIVARINE: ANOTHER NEW BISINDOLE ALKALOID FROM *STRYCHNOS DIVARICANS* DUCKE

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

In course of our structural studies on divaricine, we reported[1] the isolation of a less polar alkaloid , which we name as divarine. Application of a variety of one- and two-dimensional (2D) nmr techniques established the bisindole structure (1) for divarine, composed of two 18-deoxy Wieland-Gumlich aldehyde moieties in which the *N*-1 and C-17 of one unit is linked to the C-17' and *N*-1', respectively of the second unit. Additionally, the oxygen at C-17' forms an ether bridge with C-16 while the oxygen at C-17 is part of a methoxyl group.

Divarine, a polar alkaloid, was obtained as colorless needles from MeOH-EtOAc, mp 240-242°C (decomp.), λ_{max}^{EtOH} (log ε) 233 (4.68), 244 (4.24), and 284 (4.09) nm. Its electron impact mass spectrum (AEI MS50 and KRATOS MS80) exhibited a molecular ion peak at *m/z* 600 corresponding to the formula $C_{39}H_{44}N_4O_2$.[§2] The intense ion peak at *m/z* 568 (600-32 dalton) was due to the loss of MeOH in the inlet system.

The above information along with a detailed analysis of the different nmr data (CD_3OD solution) obtained for this alkaloid established the complete structure of divarine. The proton decoupled ^{13}C nmr spectrum exhibited 38 signals, with two methylene carbons having the same chemical shift at δ 55.57, making a total of 39 carbons in the molecule. The DEPT[3] spectra revealed the presence of eight sp^3 methylene and three sp^3 quaternary carbons (Table 1), one at a low field at δ 86.23, suggesting its linkage with at least one hetero atom.[4] The presence of a methoxyl group is also confirmed by the DEPT analysis of the protonated carbons, showing that carbons at δ 55.81, 13.37, and 14.43 are methyls, of which δ 55.81 is characteristic of a methoxyl, a result consistent with the appearance of a strong three proton singlet at δ 3.07 in the proton spectrum.

[§] Our data of divarine did not agree with the values of the bisindole alkaloid of the same molecular formula, 16-methoxyisomatopensine, reported from African *Strychnos*.[2] Also because of the non availability of the reference sample of 16-methoxyisomatopensine, direct comparison with divarine was not possible.

The HMQC[5] 2D C/H chemical shift correlation plot was used to transfer the numbering system[6] from the carbon peaks to the proton peaks corresponding to the protons directly bonded to each of the carbons. The protonated carbon with the largest chemical shift is at δ 129.42. The six carbons with larger chemical shifts do not show a direct C/H correlation because they are non-protonated.

The aromatic region of the proton spectrum displays a multiplet at δ 7.07 which is shown, to represent the protons on carbons 9, 11, 9', and 11' by virtue of the correlation peaks in the HMQC spectrum.

The protons on C-10 and C-10' are shown to fall at δ 6.90 and 6.66 ppm respectively, while the multiplet at δ 6.52 is shown to represent the protons on C-12 and C-12'. The chemical shift of δ 6.52 for those protons allows assignment of 12-H and 12'-H, to the positions *meta* to the nitrogens in the indole moieties.

The HMBC 2D C/H long-range correlation[5] experiment generates peaks which appear as spots in the contour plot display and which signify that either 2-bond or 3-bond (^2JCH or ^3JCH) spin-spin coupling pathway exists for the corresponding carbon and hydrogen atoms with the chemical shift coordinates of the 2D peak The contour plot also exhibits a large enough number of correlations to overdetermine many of the bonding pathways in the molecule, thus allowing ambiguities to be resolved and a unique structure specified. The correlations C-12, 10-H and C-12', 10'-H represent 3-bond coupling pathways and confirm the presence of two indole moieties in divarine.

The chemical shifts of C-13 and C-13' establish them as the aromatic carbons directly bonded to the indole nitrogens. The long-range correlations from C-13 to 2-H, 9-H, 11-H and 17'-H; from C-13' to 2'-H, 9'-H, and 11'-H; from C-8 to 10-H, 12-H, and 2-H; and from C-8' to 10'-H, 12'-H, and 2'-H establish additional assignments, based on the fact that only 3-bond correlations are observed between aromatic carbons. Additional correlations from C-8 to 3-H and 6-H and from C-8' to 6'-H extend the indole moieties further.

The sp^3 quaternary carbons C-7 and C-7' exhibit correlations from C-7 to 2-H, 3-H, 5-H, 6-H$_2$, 9-H, and 14-H and from C-7' to 2'-H, 3'-H, 5'-H, 6'-H$_2$, 9'-H, and 14'-H. These couplings require that 2-bond and 3-bond connectivity pathways exist which are consistent with the structure of divarine.

Carbons 5 and 14 could be interchanged and still satisfy the connectivity pathways, as could 5' and 14', but additional evidence to be shown later would not be consistent with those alternatives. The 2-bond couplings C-7, 6-H$_2$ and C-7', 6'-H$_2$ are observed for both protons of the non-equivalent methylenes since the bond angle relationships are the same for both of the protons, but the 3-bond couplings C-7, 5-H and C-7', 5'-H are observed for only one of the two non-equivalent methylene protons due to the dependence of the coupling constant on the dihedral angle, ϕ , defined by four nuclei as in Figure 1. The C-7, 5-H coupling is large enough to give a good correlation peak in the 2D experiment, while the C-7-5$_a$ -H coupling is too small to give an observable correlation.

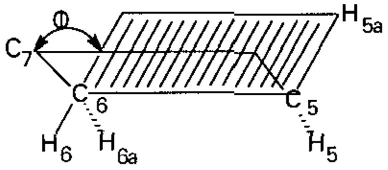


Figure 1

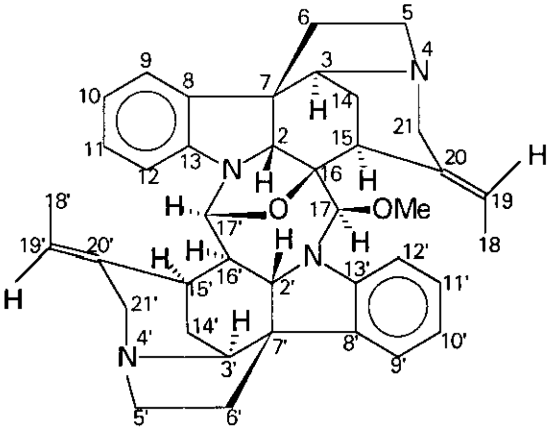
C-3 shows correlations to 2-H, 5-H, 14-H_2, 15-H, and 21-H; also C-3' correlates to 5'-H, 6'-H, 14'-H, 15'-H, and 21'-H. These are consistent with the presence of strychnine like structures in both halves of divarine.

If the correlations C-3, 5-H and C-3' and 5'-H result from 2-bond pathways, then C-3 would be directly bonded to C-5 and C-3' would be directly bonded to C-5', but the resulting 4- membered rings would leave no way for C-3 to show correlations to both 15-H and 21-H or for C-3' to correlate to both 15'-H and 21'-H. The C-3, 5-H and C-3' and 5'-H correlations must, therefore, be 3-bond correlations through an intervening atom. The chemical shifts of both protons and carbons in the positions 3, 5, and 21, 3', 5', and 21' and the lack of any additional correlations in the HMBC 2D plot are consistent with the intervening atom being the other nitrogen in a strychnine-type monomer, with C-21 and C-21' bonded to the nitrogens *N*-4 and *N*-4'. This conclusion is supported by the observation of the correlations C-21, 5-H and C-21', 5'-H arising from 3-bond connectivity pathways through the nitrogens. This leaves C-15 bonded to C-14 and C-15' bonded to C-14', which settles the ambiguities of the two indole moieties.

The correlation C-15, 2-H along with C-16, 15-H; C-16, 14-H; and C-16, 17'-H require closure of a six membered ring. Furthermore, the chemical shifts of C-16 and C-17' are best explained by joining them with a cyclic ether bridge which provides a 3-bond pathway for the observed C-16, 17'-H correlation.

Similary, correlations corresponding to C-15', 3'-H; C-15', 14'-H confirm that C-15' is bonded to C-14' as deduced previously. Additional correlations C-15', 16'-H, and C-16', 14'-H require a bonding pathway from C-16' to C-15' and thence to C-14'. The correlation C-2', 15'-H is consistent with a 3-bond coupling pathway from C-2' to 15'-H through C-16' which closes a six-membered ring of the strychnine moiety of the second half. A correlation from C-2' to 15'-H is observed which confirms the six-membered ring including C-2' and C-15'. Finally, the correlations from C-17' to 2'-H and 16'-H require C-16' to be bonded to C-2', and C-17' to be bonded to C-16' forming the seven-membered ring with the ether bridge, thus showing for the first time a common atom, i.e , C-17' appearing in both halves and representing a bridging atom in the bisindole alkaloid, divarine.

Numerous 2-bond and 3-bond long range C/H correlations (Table 1) require the placement of C-18, C-19, and C-20, and of C-18', C-19' and C-20' as are expected from the two 18-deoxy Wieland-Gumlich aldehyde moieties for the structure (1) of divarine.



(1)

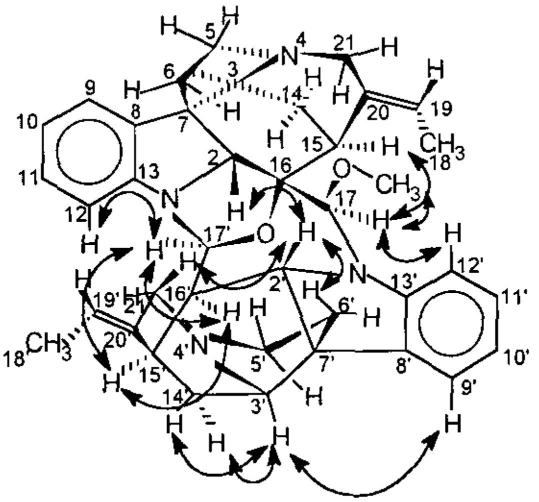
Table 1. ^{1}H (500 MHz, Varian Unity 500 spectrometer) and ^{13}C (125.8 MHz) Nmr data

carbon no.	^{13}C mult [a]	H (*J* in Hz)	long range C/H correlations from C no.[b]
2	77.35 d	4.64 s	6-H$_2$, 15-H, 17'-H
3	69.36 d	3.01 t (3)	3-H, 5-H, 14-H$_2$, 15-H, 21-H
5	55.57 t	3.06 m	3-H, 6-H, 21-H
6	45.98 t	2.41 dd (14.1, 7.5), 2.55 m	2-H
7	54.33 s		2-H, 3-H, 5-H, 6-H$_2$, 9-H, 14-H
8	139.11 s		2-H, 3-H, 6-H, 10-H, 12-H
9	123.27 d	7.09 d (7.5)	11-H
10	123.16 d	6.90 td (7.4, 0.8)	12-H
11	123.50 d	7.08 t (7.5)	9-H
12	114.00 d	6.52 d (7.7)	10-H
13	153.51 s		2-H, 9-H, 11-H, 17'-H
14	23.75 t	1.61 br m	15-H
15	37.84 d	3.09 br s	2-H, 3-H, 14-H, 19-H, 21-H
16	86.23 s		14-H, 15-H, 17'-H
17	89.23 d	4.76 s	2-H, 2'-H, 15-H and/or OCH$_3$
18	13.37 q	1.91 d (6.9)	19-H
19	121.96 d	5.64 q (7)	15-H, 18-H$_3$, 21-H$_2$
20	136.40 s		14-H, 15-H, 18-H$_3$, 21-H$_2$
21	55.57 t	3.18 d (13), 3.44 br d (13)	3-H, 5-H, 15-H, 19-H
OCH$_3$	55.81 q	3.09 s	
2'	66.34 d	4.08 d (10.6)	3'-H, 6'-H$_2$, 15'-H, 16'-H, 17'-H
3'	62.22 d	3.56 t (3)	5'-H, 6'-H, 14'-H, 15'-H, 21-H
5'	53.66 t	2.87 ddd (11.6, 9.1, 3.9), 2.97 dd (11.6, 7.6)	6'-H, 21'-H
6'	43.56 t	1.76 ddd (14.6, 10.7, 4), 2.49 m	2'-H
7'	54.74 s		2'-H, 3'-H, 5'-H, 6'-H$_2$, 9'-H, 14'-H
8'	132.86 s		2'-H, 6'-H, 10'-H, 12'-H
9'	128.63 d	7.06 d (7.4)	11'-H
10'	119.14 d	6.66 td (7.4, 0.7)	12'-H
11'	129.42 d	7.06 t (7.5)	9'-H
12'	105.92 d	6.53 d (7.9)	10'-H
13'	149.80 d		2'-H, 9'-H, 11'-H
14'	32.82 t	1.93 br m	15'-H, 16'-H
15'	32.18 d	3.09 br s	3'-H, 14'-H, 16'-H, 17'-H, 19'-H, 21'-H
16'	55.38 d	2.03 br d (10.6)	14'-H
17'	102.45 d	5.10 d (1.4)	2-H, 2'-H, 16'-H
18'	14.33 q	1.8 d (6.8)	19'-H
19'	121.58 d	5.46 q (7.1)	15'-H, 18'-H$_3$, 21'-H$_2$
20'	137.88 s		15'-H, 16'-H, 18'-H$_3$, 21'-H$_2$
21'	61.35 t	3.30 d (13); 3.41 br d (13)	3'-H, 5'-H, 15'-H, 19'-H

[a] multiplicity from DEPT spectra, [b] from HMBC spectra ; s, d, t, q, m, and br refer to singlet, doublet, triplet, quartet, multiplet, and broad

The correlations C-17, 2-H, C-17, 2'-H; and C-17, 15-H establish C-17 as bonded to C-16 and to the indole nitrogen, resulting in a tetrahydroisooxazole moiety in the bisindole alkaloid, divarine with two linkages as shown in structure (**1**).

Turning now to the data of Table 2 which summarizes the observed nOe's arising from protons which are non-bonded but in close proximity, it is possible to deduce a stereochemistry in complete agreement with the data. The easiest way to verify that the observed nOe's are consistent with the proposed stereochemistry (**2**) is to build a Dreiding model of the alkaloid. Very little force is required to distort the model into a configuration which is probably the lowest energy state of the actual molecule and which gives a measurable interproton distance in excellent agreement with the relative values of the nOe's.



(2)

Table 2. nOe data of divarine

irradiated proton (s)	observed nOe's[a]
17'-H	12-H (7%); 16'-H (4%); 15'-H (4%)
17-H	12'-H (12%); OCH_3 (2%); 15-H (6%); 18-H_3 (2%)
2-H	2'-H (7%); 6-H (4%)
2'-H	2-H (7%); 6'-H (1.5%); 21'-H (7%); OCH_3 (0.5%)
3'-H	9'-H (8%); 14'-H (2%)
16'-H	17'-H (4%); 15'-H (4%)

[a] approximate value

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6. Since data analysis had to be performed before the entire structure was known, building up the structure one carbon at a time, a temporary consecutive numbering system was used to designate the carbon atoms, begining at 1 for the carbon atom with the largest chemical shift and ending at 38 for the carbon with the smallest chemical shift. The methoxyl was not numbered. When the complete structure (**1**) had been built up, it was renumbered to conform to the accepted chemical numbering system. These numbers, used for assignment throughout this paper, are the ones designating the observed correlations which are shown to be consistent with the 2-bond and 3-bond proton-carbon couplings in (**1**).

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