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Alkaloids from the Antarctic Sponge *Kirkpatrickia varialosa*. Part 2: Variolin A and *N*(3')-methyl tetrahydrovariolin B

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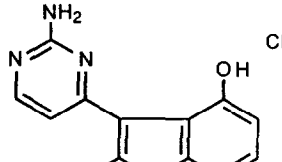
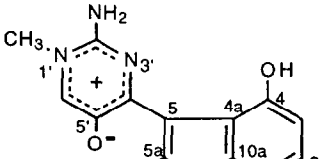
Abstract: Two pyridopyrrolopyrimidine alkaloids, variolin A (**2**) and *N*(3')-methyl tetrahydrovariolin B (**3**), have been isolated from the Antarctic sponge *Kirkpatrickia varialosa*, and their structures determined by X-ray crystallography and interpretation of spectral data respectively. *N*(3')-Methyl tetrahydrovariolin B (**3**) is moderately cytotoxic and showed antifungal activity, while variolin A (**2**) is weakly cytotoxic.

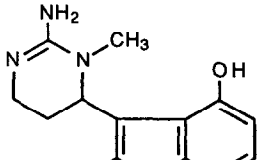
Relatively little is known of the natural products chemistry of sponges from Antarctica and other cold water environments. Studies of the ecology of Antarctic communities have suggested that, while some sponges are protected from predation by conspicuous spicules, there are other sponges that must rely on chemical defenses against predation.[1,2] Diterpenes[3] and alkaloids[4] have been reported from *Dendrilla membranacea*, while McClintock and Gauthier[5] have demonstrated that an ethanol extract of the sponge *Kirkpatrickia varialosa* Kirkpatrick (family Myxillidae, order Poecilosclerida) inhibited the Gram -ve bacteria *Klebsiella pneumoniae* and *Serratia rubidaea* and the yeast *Candida tropicalis*. In Part 1[6] we reported the isolation and identification of variolin B (**1**), while in this part we report the isolation and identification of two additional variolins, variolin A (**2**) and *N*(3')-methyl tetrahydrovariolin B (**3**) from *K. varialosa*.



variolin B (**1**)



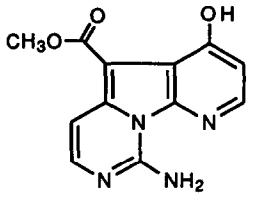
variolin A (**2**)



N(3')-methyl tetrahydrovariolin B (**3**)

For the investigation at Scripps (SIO), a specimen of *K. varialosa* collected from Cape Armitage, Antarctica, in 1989 was extracted with methanol and the red-colored butanol-soluble material from the extract was repeatedly chromatographed on Sephadex LH-20 to obtain variolin A (**2**, 0.024% dry wt.) and *N*(3')-methyl tetrahydrovariolin B (**3**, 0.24% dry wt.). As the crude extract showed no antimicrobial activity against the available micro-organisms, ^{1}H NMR spectroscopy was used to detect the compounds of interest.

For the investigation at the University of Canterbury (UCant), *K. varialosa*, collected from Cape Armitage, Antarctica, in 1988 was extracted with MeOH and MeOH/CH_2Cl_2 mixtures. The red extract was chromatographed on C18 reverse phase flash chromatography, then on Baker Bond® Diol, Sephadex LH20, and finally a Baker Bond® NH_2 column to yield variolin A (**2**), stabilised as its TFA salt (0.0234% w/w sponge). Additional processing of the C18 column fractions gave variolin B (**1**, 0.0134% w/w sponge) and variolin D (**4**) as described in the preceding paper.[6] For the UCant separations, the location of the variolins was monitored by a routine P388 bioassay. There was no suggestion at any stage in this isolation scheme for the presence of *N*(3')-methyl tetrahydrovariolin B (**3**).



variolin D (**4**)

Variolin A (**2**), a minor constituent of these specimens of *K. varialosa*, was isolated as a red-colored solid, mp. 196°C (dec). The compound was quite unstable under basic conditions and much of the compound decomposed on attempted recrystallization from pyridine. Although the molecular formula, $C_{15}H_{13}N_7O_2$, could be determined by high resolution mass spectrometry, extensive application of NMR techniques failed to yield a satisfactory structure for variolin A (**2**). Repeated attempts at UCant to obtain a crystalline sample suitable for X-ray crystallography finally yielded, from aqueous TFA, a few very fine needles, one of which was analysed at -100°C on a Siemens P4RA (rotating anode) diffractometer at Georgetown University.

Variolin A (**2**) crystallized in the monoclinic space group $P2_1/n$ with cell parameters a = 6.7548 (9), b = 10.3752 (12), c = 31.910 (3) Å, β = 91.312 (9)°. Refinements were conducted with SHELXL93 on F^2 using all data, leading to values for the weighted crystallographic index on F^2, wR2, of 0.174 for unique 3087 data and for the conventional R index on F for the 1809 data with I > 2 s(I) of 0.060.[7] All hydrogen atoms on the variolin A (**2**) moiety were located in difference Fourier maps, and several of chemical interest were allow to refine independently of the atom to which they were attached.

The asymmetric unit comprises one well-ordered variolin A (**2**) moiety (as illustrated by **2**) that is fully protonated at N8 and half-protonated at O5'. The identity of atoms as carbon or nitrogen was crystallographically confirmed. The C5'-O moiety with a bond length of 1.345(6) Å is phenolic in character, and the N1'-CH_3 bond at 1.492 (6) Å is consistent with the unusual zwitterionic character of this pyrimidine ring (see Figure 1 for crystallographically determined bond lengths). The carbon atom C5 maintains unusually long bonds of 1.391 (6), 1.447 (6) and 1.452 (6) Å with its three carbon neighbours. Balancing charge are 1.5 disordered trifluoroacetate moieties. One TFA^- site is fully occupied, although two distinct positions exist for the complete molecule, with the carboxylate moiety hydrogen bonded to hydrogen atoms at N8 and H_2N9. The other TFA^- site is half occupied with one carboxylate oxygen hydrogen bonded to HO5', and in the absence of TFA^- by an ill-defined assortment of methanol and water molecules, some of whose positions coincide with atoms from the TFA^- moiety. The disorder of the first TFA^- is correlated with the compositional disorder at the second TFA^- site. In addition to the intermolecular hydrogen bonds there are two intramolecular hydrogen bonds: one between N1 and H_2N9 (H_2N9..N1 2.732 (5) Å and N1..H-N9 133°, assuming N-H of 0.88 Å) and

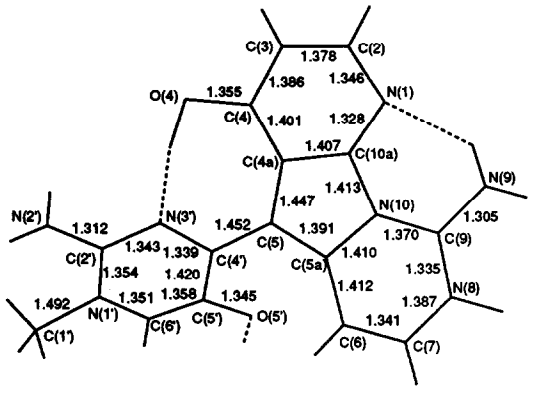


Figure 1 Bond lengths (Å) in variolin A (2)

one very strong one between HO4 and N3' (refined H-O4 1.14 (6) Å, (H)O4..N3' 2.762 (5) Å, 168 (3)°) that leads to the 2-aminopyrimidime ring being inclined 42.0 (2)° with respect to the pyridopyrrolopyrimidine ring system. The pyrido ring appears to be delocalized and isolated from the pyrrolo ring, and in both pyrimido rings the C-C bonds are close to that for an isolated C=C double bond.

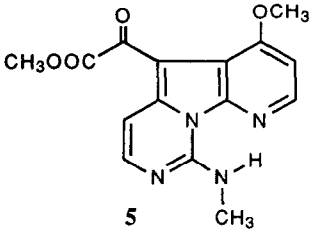
With the structure of variolin A (2) now defined, the NMR spectra could be assigned (Table 1). Signals in the ^{1}H NMR spectrum of 2 (as the free base in DMSO) at δ 8.04 (d, 1 H, J = 6.0 Hz), 6.63 (d, 1 H, J = 6.0 Hz), 7.80 (d, 1 H, J = 6.6 Hz), and 7.64 (d, 1 H, J = 6.9 Hz) were assigned to H2, H3, H6, and H7 respectively. The one additional sharp signal at δ 7.69 (s, 1 H) was assigned to H6'. Irradiation of either the δ 10.0 or 10.7 ppm broad exchangeable signals caused the disappearance of the other, indicating that they were due to hydogens on the same nitrogen atom (H_2N9 by analogy with the similar protons in variolin B (**1**)[6]). A two-proton broad exchangeable signal at δ 8.5 was assigned to H_2N2', while the remaining broad exchangeable signal at δ 17.6 ppm could be assigned to the HO4 proton. The assignment of the ^{13}C NMR spectrum followed from correlations observed in the HMQC and HMBC spectra.

Table 1. ^{1}H and ^{13}C NMR Data for Variolin A (2)

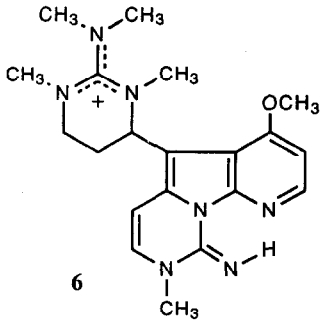
position	C[a]	mult	J (Hz)	H[b]	mult	J (Hz)	H[c]	mult	J (Hz)	HMBC correlations (J= 8.3 Hz)
2	143.9	d	183	8.36	d	5.4	8.04	d	6.0	C4, C10a, C3, C4a[d]
3	108.4	dd	163, 8	7.08	d	5.4	6.63	d	6.0	C4, C2, C4a, C5[d]
4	159.7	dd	8, 2							
4a	111.9	d	6							
5	99.5	s								
5a	136.2	br s								
6	102.3	dd	177, 4	7.11	d	6.9	7.80	d	6.6	C7, C5a, C5[d]
7	137.4	br d	181	7.7	br d	6.9	7.64	d	6.9	C9, C5a, C6
9	149.2	d	11							
10a	145.9	d	15							
2'	150.7	br s								
4'	159.1	br d	5							
5'	141.3	s								
6'	133.7	br d	189	8.08	s		7.69	s		C2', C4', C5', NCH_3, C5[d]
NCH_3	40.9	br dq	142, 5	3.82	s		3.71	s		C2', C6'
4OH							17.6	br s		
9NH_2				9.48	br s		10.0	br s		
9NH_2				10.09	br s		10.7	br s		
2'NH_2				8.37	s		8.5	br s		

[a]Recorded in $(CD_3)_2SO$ as TFA salt at 75 MHz. [b]Recorded in $(CD_3)_2SO$ as TFA salt at 300 MHz. [c]Recorded in $(CD_3)_2SO$ as free base at 300 MHz. [d]J = 2 Hz.

In the early stages of the SIO investigations on variolin A (**2**), an attempt was made to methylate **2** with methyl iodide and silver oxide in DMF. Although the major product of this reaction could not be completely characterized due to lack of material, all spectral evidence points to the α-keto ester derivative **5**. A high resolution mass measurement indicated that the α-keto ester **5** has the molecular formula $C_{15}H_{14}N_4O_4$. From a consideration of the mass spectral data and the ^1H and ^{13}C NMR spectra which required two *O*-methyl [δ 3.99 (s, 3 H), 3.84 (s, 3 H); 56.5 (q), 51.92 (q)] and one *N*-methyl [δ 3.19 (d, 3 H, J = 5 Hz); 27.8 (q)] groups, it was obvious that the variolin A skeleton had undergone extensive degradation. The ^1H NMR spectrum also contained signals at δ 7.24 (d, 1 H, J = 5.7 Hz, H-4), 7.54 (d, 1 H, J = 6.2 Hz, H-6), 8.00 (d, 1 H, J = 6.2 Hz, H-7), and 8.37 (d, 1 H, J = 5.7 Hz, H-4) that could be assigned to the pyridopyrrolopyrimidine ring system. The remaining signal in the ^1H NMR spectrum at δ 9.91 (br q, 1 H, J = 4.6 Hz) was assigned to the HN9 signal that is coupled to the CH_3N9 signal at δ 3.19. By assigning one *O*-methyl signal to the oxygen at the 4-position, the remaining *O*-methyl and the unassigned "C_2O_2" fragment from the molecular formula must be placed as a pyruvate group at C5. The ^{13}C NMR spectrum contained weak signals at δ 174 and 186 that were assigned to the ester and ketone groups and signals at 101.1, 104.2, 143.3, and 149.0 that were assigned to the aromatic protonated carbon signals, but the remaining carbon signals could not be observed. The formation of the α-keto ester **5** from variolin A (**2**) requires cleavage between C5' and C6', and hydrolysis of the bond between C4' and N3'.



5



6

N(3')-Methyl tetrahydrovariolin B (**3**), $[\alpha]_D$ = -22.4° (*c* 3.5, MeOH), was obtained as a yellow solid, mp. 226°C (dec.). The molecular formula, $C_{15}H_{17}N_7O$, was obtained from a high resolution mass measurement and required just eleven unsaturation equivalents. The UV spectrum showed a complex series of absorptions between 256 and 385 nm, which implied the presence of an aromatic system. The IR spectrum was not very helpful, but contained a series of bands in the region 2500-3600 cm^{-1} that could be due to amines or phenols and an imine band at 1633 cm^{-1}. The ^1H NMR spectrum (1:1 DMSO-d_6/acetone-d_6) contained exchangeable signals for five protons between δ 7.5 and 12.0, three sharp signals at 8.11 (d, 1 H, J = 6 Hz), 7.28 (d, 1 H, J = 6.5 Hz) and 7.09 (d, 1 H, J = 6 Hz) and a series of broad non-exchangeable signals between 6.6 and 2.2. The two signals at δ 8.11 and 7.09 correspond to ^{13}C NMR signals at δ 143.9 (d) and 106.8 (d), respectively, and were assigned to the α and β protons on a tetrasubstituted pyridine ring.[8] When recorded at -80°C, the ^1H NMR spectrum revealed two sets of aliphatic signals indicating the presence of major and minor conformers for the aliphatic ring system. Although a COSY spectrum recorded at -80°C revealed the presence of -CH=CH- and -CH_2-CH_2-CH< partial structures, we could not conveniently perform the low temperature HMQC and HMBC experiments that were required to complete the structural assignment.

N(3')-Methyl tetrahydrovariolin B (3) was exhaustively methylated with methyl iodide and potassium carbonate in dry acetone to obtain a hexamethyl derivative (6). The molecular formula, $C_{20}H_{28}N_7O$, that was obtained by HRFABMS, suggested that the derivative was a salt. One exchangeable proton had not been methylated and this was assumed to be HN9 as it is involved in hydrogen bonding with N1. The ^1H and ^{13}C NMR spectra were assigned as shown in Table 2 by interpretation of the COSY, XHCORR, COLOC (J = 10 and 6 Hz), HMBC (J = 8 Hz) and NOEDS experiments. At room temperature, the signals for the two CH_3N2' groups were broad due to rotation about the C2'-2'N bond. On heating the sample, the signals merged together at 45°C and became sharp at 65°C, while on cooling the sample to -30°C the rotation ceased and the methyl signals were as sharp as all others. The NOEDS experiment performed at -30°C showed that the signals at δ 2.91 and 3.13 were due to the CH_3N2' groups adjacent to the H_3C1' and H_3C3' groups, respectively. The calculated barrier to rotation was 14.9 kcal/mole.[9]

Table 2. NMR Data for *N*(3')-Methyl (3) and Hexamethyl (6) tetrahydrovariolin B

	N(3')-methyl tetrahydrovariolin B (3)					Hexamethyl tetrahydrovariolin B (6)					
position	C[a]	#H[b]	H[c]	mult	*J* (Hz)	C[d]	#H[b]	H[e]	mult	*J* (Hz)	HMBC correlations
2	143.9	1	8.11	d	6.0	144.4	1	8.28	d	6	C3, C4, C10a
3	106.8	1	7.09	d	5.5	102.4	1	7.05	d	6	C2, C4, C4a
4	159.4	0				159.3	0				
4a	112.6	0				111.5	0				
5	101.7	0				101.5	0				
5a	133.5 (br)	0				134.4	0				
6	99.8	1	6.58	br s		92.2	1	6.71	d	7.5	C5a
7	137.6	1	7.28	d	6.5	133.9	1	7.10	d	7.5	C5a, C6, C9
9	146.7	0				146.1	0				
10a	151.2	0				149.4	0				
2'	156.6	0				162.3	0				
4'	55.6 (br)	1	5.38	br s		57.3	1	5.53	dl	12, 7.5	C4a, C5, C5a, C5'
5'	30.8 (br)	2	2.28	br s		31.3	2	2.53	m	13, 12, 12, 3	
			2.48	br s				2.24	m	13, 7.5, 3, 2	
6'	37.7 (br)	2	3.40	m		48.8	2	4.01	ddl	13, 12, 2	
			3.32	br s				3.39	dt	13, 3	
4OCH_3						57.2	3	4.13	s		C4
8NCH_3						37.6	3	3.48	s		C7, C9
9NH			11.77	br s				9.67	br s		C9
9NH			9.49	br s							
1'NCH_3						39.5	3	2.94	s		C2', C6'
2'NH_2			7.67	br s							
2'NCH_3						40.1	3	3.13	br s		
2'NCH_3						40.1	3	2.91	br s		
3'NCH_3	35.5 (br)	3	2.88	br s		41.3	3	3.38	s		C2', C4'

[a]Recorded in CD_3OD at 50 MHz. [b]From DEPT experiment. [c]Recorded in $(CD_3)_2SO/(CD_3)_2CO$, 1:1, at 500 MHz at 25°C. [d]Recorded in $(CD_3)_2CO$ at 500 MHz. [e]Recorded in $(CD_3)_2CO$ at 50 MHz.

The structure assigned to hexamethyl tetrahydrovariolin B (6) was elucidated by interpretation of spectral data and is the only solution for which a 4-bond long-range coupling does not have to be invoked. The 4-methoxypyridine ring was established as follows: the ^1H NMR signal at δ 8.28 (d, 1 H, J = 6 Hz, H2) was coupled to a signal at δ 7.05 (d, 1 H, J = 6 Hz, H3) that showed a 7% enhancement on irradiation of the

methoxy signal at δ 4.13 (s, 3 H). The long-range couplings from H2 to C10a and from H3 to C4a define the remaining carbon signals for the pyridine ring. Although there are no data that confirm the bond from C4a to C10a, the chemical shifts of the ^{1}H and ^{13}C NMR signals require a pyridine ring. A second pair of aromatic proton signals occurred at δ 6.71 (d, 1 H, J = 7.5 Hz, H6) and 7.10 (d, 1 H, J = 7.5 Hz, H7). Irradiation of the *N*-methyl signal at δ 3.48 (s, 3 H) caused an 8% enhancement of the H7 signal. There are long-range couplings from both the H7 and CH_3N8 signals to a carbon signal at δ 146.1 (s, C9), which also has a long-range coupling to the HN9 signal at 9.67 (br s). The H6 signal shows a long-range coupling to a carbon signal at δ 134.4 (s, C5a). The long-range coupling data for the N1' and N3' methyl groups, together with the NOEDS experiment described in the previous paragraph, define the presence of the N1',N3'-dialkyltetramethylguanidinium group. The -H_2C6'-H_2C5'-HC4'< moiety was assigned from the COSY spectrum and the long-range correlations between H_3C3' and C4' and between H_3C1' and C6' completed the assignment of the tetrahydropyrimidine ring. The long-range couplings from H4' to C4a, C5, and C5a can only be explained if C5 is connected to C4a, C5a and C4'. Furthermore, irradiation of the H_3CO4 signal caused a 2% enhancement of the H_3C3' signal, irradiation of the H4' signal caused a 12% enhancement of the H6 signal together with the expected enhancements of signals due to H6'(ax), H5'(eq) and H_3C3', and irradiation of the H6 signal caused enhancements of the H7 and H4' signals. The remaining bonds at C5a, C9, and C10a must all be joined to the remaining nitrogen atom to form a tricyclic pyridopyrrolopyrimidine ring system.

Having defined the structure of **6**, we returned to the structural elucidation of *N*(3')-methyl tetrahydrovariolin B (**3**). The *N*-methyl group was placed at N(3') because the H4' signal showed an 8% enhancement on irradiation of the *N*-methyl signal. The signal broadening in the ^{1}H NMR spectrum at room temperature was found to be caused by equilibration of axial and equatorial conformers at C4', as indicated by analysis of the $J_{4',5'}$ coupling constants for the two conformers in the -80°C spectrum.

Variolin A (**2**) showed weak *in vitro* activity against the P388 cell line (IC_{50} = 3.8 μg/mL). *N*(3')-Methyl tetrahydrovariolin B (**3**) inhibited the growth of *Saccharomyces cerevisiae* (36 mm zone at 2 mg/mL), showed *in vitro* activity against the HCT 116 cell line (IC_{50} = 0.48 μg/mL), and only modest *in vivo* activity against P388 leukemia (T/C 125% at 10 mg/Kg).

EXPERIMENTAL

General: Infrared spectra were recorded on a Perkin-Elmer series 1000 FT-IR spectrophotometer. Ultraviolet spectra were obtained on a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer. ^{1}H NMR experiments were recorded on Varian 500 MHz, XL300 and UNITY300 spectrometers. ^{13}C NMR spectra were recorded on an IBM WP-200 SY spectrometer with an Aspect 3000 data station, or on Varian XL300 and UNITY300 spectrometers. Carbon chemical shifts are reported in ppm relative to δ = 39.6 ppm for $(CD_3)_2SO$, δ = 29.2 ppm for $(CD_3)_2CO$, and δ = 49.3 ppm for CD_3OD, while proton chemical shifts are reported in ppm relative to δ = 2.62 ppm for $(CHD_2)_2SO$, δ = 2.17 ppm for $(CD_3)_2CO$, and δ = 3.30 ppm for CHD_2OD. Mass spectra were obtained from regional facilities at UC Riverside. The optical rotation was measured on a Perkin Elmer model-201 polarimeter using a 5 cm microcell. Melting points were determined on a Mel-temp. melting point apparatus and are uncorrected.

Collection, extraction and chromatography: For the SIO investigation, the red sponge *Kirkpatrickia varialosa* was collected by hand using SCUBA (-27 m) at Cape Armitage in McMurdo Sound, Antarctica (77°51'S, 166°33'E) during October–December 1989 (voucher 90-172, SIO Benthic Invertebrate Collection

#P1144). The sponge (88 g dry wt.) was soaked in methanol, sonicated for one hour and extracted overnight. The solvent was filtered and evaporated, and the sponge was reextracted in the same manner. The combined extract, after evaporation of the solvent, was partitioned between water and *n*-butanol. The organic layer was separated and the solvent removed in *vacuo*. The deep red colored extract was chromatographed on Sephadex LH-20 using methanol as eluant. Fractions 5-9, which showed a UV active spot, were combined and purified on Sephadex LH-20 using first methanol then 1:1 MeOH/CH_2Cl_2 to obtain *N*(3')-methyl tetrahydrovariolin B (**3**, 0.24% dry weight). Slow evaporation of the fractions 19-33 resulted in separation of variolin A (**2**, 0.024% dry weight) as a red solid.

For the UCant investigation, the red sponge *Kirkpatrickia varialosa* was collected by hand using SCUBA in the shallow water of Cape Armitage in McMurdo Sound, Antarctica in 1988 and 1991 and frozen immediately until extraction. Voucher specimens (88ANT02-04 and 91ANT01-03) are held in the University of Canterbury collection. A typical isolation was as follows: the sponge (wet weight 1040 g) was extracted, by blending and filtering, with MeOH (1 x 1 L), 3:1 MeOH/CH_2Cl_2 (2 x 0.5 L) and 100:1 MeOH/HCl (aq) (2 x 0.5 L). The combined extracts (54.9 g) were subjected to reverse phase C18 flash chromatography.[10] Variolin A (**2**) was isolated from C18 fractions by chromatography on a Baker Bond® Diol column (1:9 MeOH/CH_2Cl_2), then on Sephadex LH20 (1:1 MeOH/CH_2Cl_2) and finally on a Baker Bond® NH_2 column (1:9 MeOH/CH_2Cl_2) to give 244 mg of the TFA salt after addition of TFA to prevent degradation (0.0234% w/w sponge). Silica tlc (8:2 CH_2Cl_2/MeOH) showed an orange spot which changed to pink on standing, presumably due to aerial oxidation.

Variolin A (2): red colored solid; mp 196°(dec); UV (MeOH) 469 nm (ε 14570), 372 (sh, ε 2850), 353 (sh, ε 3840), 326 (sh, ε 5720), 315 (ε 5375), 254 (ε 8945); IR (KBr) 3420, 3365, 3325, 3135, 1633, 1585, 1445, 1275, 1085, cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; HRFABMS: *m/z* 324.1200 $(M + H)^+$, $C_{15}H_{14}N_7O_2$ requires 324.1209.

N(3')-methyl tetrahydrovariolin B (3): light yellow solid; mp 226° (dec); $[\alpha]_D$ - 22.4° (c 3.5, MeOH); UV (MeOH) 385 nm (ε 3190), 367 (ε 6840), 351 (ε 6955), 324 (ε 9585), 311 (ε 8660), 256 (ε 9680); IR (KBr) 3600-2500 (br), 1633, 1572, 1490, 1300 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; HRFABMS: *m/z* 312.1579, $C_{15}H_{18}N_7O$ requires 312.1570.

Methylation of N(3')-methyl tetrahydrovariolin B (3): To a solution of *N*(3')-methyl tetrahydrovariolin B (**3**) (32 mg) in acetone (10 mL) were added anhydrous K_2CO_3 (100 mg) and CH_3I (0.1 mL). The resulting solution was stirred under nitrogen at 45°C for 72 hr. The reaction mixture was diluted with CH_2Cl_2 (10 mL), filtered through celite, and the solvents evaporated under vacuum. The crude reaction product was purified on a small Sephadex LH-20 column, eluting with 1:1 MeOH/CH_2Cl_2 to obtain pure hexamethyl tetrahydrovariolin B (**6**, 21 mg): UV (MeOH) 352 nm (ε 6685), 336 (ε 8575), 319 (ε 9900), 309 (ε 9235), 253 (ε 8640); IR (KBr) 3420, 1655, 1595, 1405, 1290 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; HRFABMS: *m/z* 382.2365(MH^+), $C_{20}H_{28}N_7O$ requires 382.2355.

Methylation of Variolin A (2): Variolin A (5 mg) was dissolved in dry DMF (1 mL) and added to a flask containing silver oxide (25 mg). The mixture was stirred under nitrogen for 5 min, CH_3I (0.015 mL) was added. After 10 hr, a further portion of CH_3I (0.005 mL) was added and stirring was continued for 15 hr, until the starting material had all reacted, as judged by tlc. The reaction mixture was filtered under vacuum, $CHCl_3$ (1 mL) was added to the filtrate and the resulting suspension was centrifuged to remove insoluble material. The organic layer was washed with water (1 mL), evaporated in *vacuo* and purified by chromatography on Sephadex LH-20 using methanol as eluant and by HPLC on a Selectosil 10 amino column using 1:4 MeOH/CH_2Cl_2 as eluant to obtain the permethylated product (**5**, 1.1 mg). ^1H NMR ($(CD_3)_2SO$) δ 9.91 (q, 1 H, J = 4.6 Hz),

8.37 (d, 1 H, J = 5.7 Hz), 8.00 (d, 1 H, J = 6.2 Hz), 7.54 (d, 1 H, J = 6.2 Hz), 7.24 (d, 1 H, J = 5.7 Hz), 3.99 (s, 3 H), 3.84 (s, 3 H), 3.19 (d, 3 H, J = 4.6 Hz); ^{13}C NMR ($(CD_3)_2SO$) δ 186 (weak), 174 (weak), 149.0 (d), 143.3 (d), 109.2 (d), 101.1 (d), 56.5 (q), 51.9 (q), 27.8 (q), the remaining signals were not clearly visible; HRFABMS: *m/z* = 315.1116 (MH^+), $C_{15}H_{15}N_4O_4$ requires 315.1093. LREIMS: *m/z* (int., %) 314 (21), 255 (81), 198 (100), 182 (9), 59 (25).

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