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Epinardins A-D, New Pyrroloiminoquinone Alkaloids of Undetermined Deep-Water Green Demosponges from Pre-Antarctic Indian Ocean

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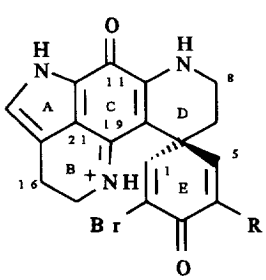
Abstract: Four novel pyrroloiminoquinone alkaloids that differ from members of the discorhabdin/prianosin family for having an allylic alcohol functionality in place of the enone system, epinardins A-D (**8-11**), have been isolated from undetermined deep-water green demosponges collected in pre-Antarctic waters near the Crozet Islands. Relative stereochemistry has been fully assigned from high-field NMR spectra. Epinardin C (**10**) proved strongly cytotoxic towards doxorubicin-resistant L1210/DX tumoural cells *in vitro*.

Recently several simple pyrroloiminoquinone alkaloids have been isolated from various demosponges and an ascidian. Poecilosclerid sponge products comprise batzellines[1] and isobatzellines[2] from *Batzella* sp., damirones A-B from *Zyzzya fuliginosa*,[3] as well as makaluvamines A-C and makaluvone from *Zyzzya* cf. *massalis*.[4] Wakayin, isolated from the polycytorid ascidian *Clavelina* sp.,[5] has a more complex structure, embodying a tryptophan moiety. Structurally related compounds bearing a tyrosine unit in place of tryptophan, have also been isolated from



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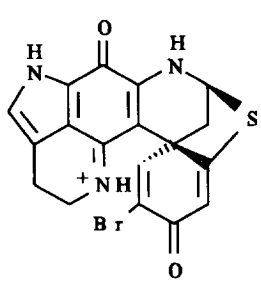
2 R = H



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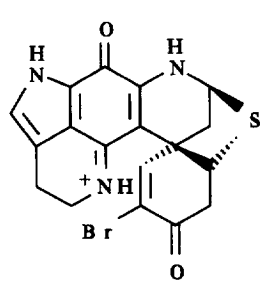
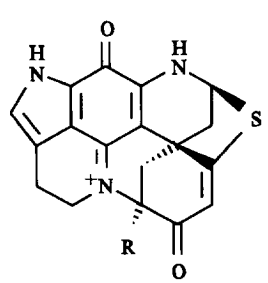


4
5 $\Delta^{16,17}$



6 R = H
7 R = OH

both Z. cf. *massalis* and two other demosponges. In makaluvamines D-F, isolated from the first,[4] the tyrosine unit is only bound through the N-atom. In discorhabdins, isolated from Hadromerid *Latruculia* spp. found in a transect between northeast New Zealand and Antarctica in Indo-Pacific, and in prianosins, isolated from Halichondrid[6] (alternatively classified as Poecilosclerid[7]) *Prianos melanos* from East China Sea near Okinawa, the tyrosine unit is spirocyclized, like in discorhabdin C (**1**) and E (**2**).[8] An additional sulphur bridge occurs in discorhabdin B (**3**),[9] discorhabdin A[9] (= prianosin A[10]) (**4**), and prianosin B (**5**),[11] with a further bond between the imino nitrogen and a tyrosine *ortho*-carbon in discorhabdin D[6] (= prianosin D)[12] (**6**) and prianosin C (**7**).[12]

Potent cytotoxic activities by these substances have stimulated both the semisynthesis of analogues, in view of establishing structure/bioactivity correlations,[8] and total synthesis of makaluvamine D,[13a,d] discorhabdin C,[13b,c] batzelline C, and isobatzelline C,[13c] and a general formal entry to these compounds[13e] to cope with their shortage from nature.

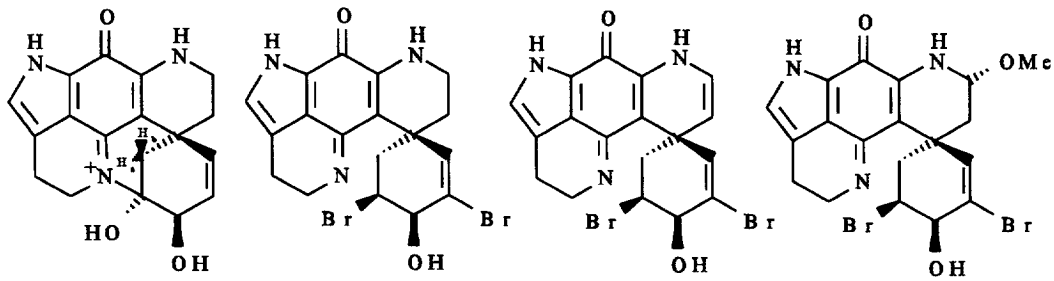
Recent examination of two samples of ours collected long ago in pre-Antarctic waters has disclosed structural variants on this theme, which are reported here under the names epinardins from the French 'dèmosponge vert èpinard' used on board to identify our spinach-green sponges.

Results and Discussion

The most polar, third in abundance, of the pigments isolated from these sponges, epinardin A, showed ^{13}C resonances for five methylenes, one *O*-deshielded sp^3 methine, three olefinic CH, two sp^3 quaternary C, six tetrasubstituted olefinic C, and a s at δ_C 168.23. Though at high-field, the latter signal is attributable to a keto C. Out of the sp^3 quaternary C's, one resonates at such low field, 89.04 ppm, that it must bear two heteroatoms. The odd number of sp^2 C's is compatible with an imine-type bond, demanding six unsaturations. Combining these data with *m/z* 324 for M^+ from FAB-MS, one obtains composition $C_{18}H_{18}N_3O_3$, implying six cycles, which was confirmed by HR-EI-MS on acetylation derivatives. Besides the spin system $-CH_2CH_2C{=}CH-$, the ^1H NMR spectrum revealed an isolated $-CH_2CH_2-$ and a $-CH_2-$ long-range coupled with a *cis* CH=CHCH-X, where X must be a heteroatom to account for methine deshielding. Both the UV spectrum and these NMR data suggest structure **8**, related to prianosin C (**7**),[11] except for the S bridge and an allyl alcohol functionality in place of the enone system. The relative configurations rest on NOESY data.

Second in polarity and least-abundant, epinardin B, differs from **8** at the tyrosine-derived ring: the deshielded δ_C 89.04 s observed for **8** is lacking, while there is a δ_H 4.59 ddd for a methine correlated (HMQC) with a C atom having ^{13}C NMR signals submerged by the solvent residual signals. A methine resonating at δ_H 4.59 ddd is part of a CH_2CHCH sp^3 spin system long-range correlated to a proton at a trisubstituted C=C bond. FAB-MS fits for a pentacyclic structure with two Br atoms (MH^+ pattern *m/z* 466 (3%), 468 (6%), and 470 (3%)); combining this with the NMR data, the composition $C_{18}H_{17}Br_2N_3O_2$ is obtained, arriving at structure **9**. $J_{H,H}$ values suggest that

ring E adopts the half-chair conformation typical of cyclohexenes,[14] with equatorial sp^3-bound Br and pseudoaxial



8 9 10 11

OH. Of the two possible half-chairs, the one in the Figure is supported by NOESY map 4.59 (2-H)/2.05 (7α-H). Mediated δ_H and $J_{H,H}$ values for 2H-16 and 2H-17 suggest that ring B undergoes rapid flipping, like in discorhabdins;[8] in contrast, ring D does not undergo conformational flipping due to restrain, presumably by steric repulsion between H_b-1 and N-18.

The least polar, most abundant, of the sponge pigments, epinardin C, lacks one $-CH_2CH_2-$ spin systems observed for **9**. Two d's at δ_C 121.00 and 105.45, as well as an AX spin system in the olefinic region, with J_{AX} 7.4 Hz, suggest a *cis*-disubstituted -CH=CH- system in place of $-CH_2-CH_2-$, i.e. structure **10**. This, and FAB-MS *m/z* 464 (1%), 466 (2%), and 468 (1%) for MH^+, support the composition $C_{18}H_{15}Br_2N_3O_2$. No stereochemical information could be provided by NOESY experiments, however, because of superimposition of the 7-H and 2-H ^{1}H-NMR signals.

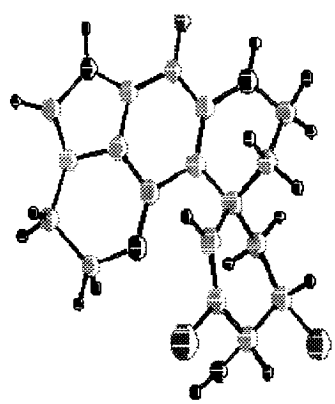


Figure. Preferred conformation of epinardin B (**9**)

The medium-polarity epinardin D proved to differ from **9** in the same area as **10** does: the $-CH_2CH_2-$ spin system is replaced by a $-CHXCH_2-$ system, characterized by a deshielded (δ_H 4.85 and δ_C 79.88) methine. This implies that X is a heteroatom. These data and FAB-MS *m/z* 496 (5%), 498 (12%), and 500 (6%) for MH^+ support the composition $C_{19}H_{19}Br_2N_3O_3$, suggesting X = OMe, which also rests on δ_H 3.31 s and δ_C 54.59 resonances. All this points to structure **11**. From NOESY data, conformational conclusions as to rings D and E of **11** are as for **9**, while preferred axial position for MeO may be a consequence of the anomeric effect. Oddly, **11** showed non-mediated δ_H and $J_{H,H}$ values for 2H-16 and 2H-17. On long standing in acetone solution, **11** lost MeOH giving

10, while the reverse could not be observed: **10** was unchanged on long standing in MeOH containing Amberlyst 15.

Epinardin A (**8**) and epinardin C (**10**) were assayed against L1210 and doxorubicin-resistant L1210/DX murine lymphocytic leukemia cells (Pharmacia) *in vitro*: epinardin C proved strongly active, albeit with poor resistant index (Table). Since the dienone form, discorhabdin C (**1**) is more cytotoxic than the corresponding dienol form by one order of magnitude,[8] we tried to oxidize **10** selectively at C-3 with a variety of reagents, albeit unsuccessfully.

The deep-green colour of our sponges [9,15] and extracts,[9] as well as the nature of the metabolites,[9,15] are compatible with the genus *Latrunculia*. It has been considered whether the strongly pigmented discorhabdins from sponges of this genus may have cyanobacterial origin.[15] Our sample 110M, coming from the darkness of 200 m depth in pre-Antarctic waters, can be assumed free of cyanobacterial symbionts.

Table. Cytotoxicity Data (IC_{50} in μg/ml) for Epinardin A (**8**) and Epinardin C (**10**)

Compound	L1210 IC_{50}	L1210/Dx IC_{50}	RI[a]
8	1.7±0.2	6.8±0.5	4
10	0.324±0.004	0.358±0.02	1
doxorubicin	0.0297±0.004	0.711±0.064	24

[a]Resistant index, as the ratio between IC_{50} values for L1210/DX cells vs.L1210 cells.

Experimental Section

General. All evaporations were carried out at reduced pressure. Flash chromatography (FC): Merck RP-18 LiChroprep (40-65 μm). TLC: Merck silica gel 60 PF_{254} plates. UV: (λ_{max} in nm, ϵ in $mol^{-1}l$ cm^{-1}): Perkin-Elmer-Lambda-3. CD: Jasco J-710 spectropolarimeter, $\Delta\epsilon(\lambda)$ in deg mol^{-1} cm^{-1}. ^{13}C NMR: Varian-XL-300 spectrometer at 75.43 MHz. ^1H NMR: Varian Unity-600 spectrometer at 599.921 MHz, probe temperature 28°; δ-values relatively to the residual CD_2HOD or $(CD_2H)_2CO$ signals taken at 3.31 and 2.05 ppm, respectively, from TMS (δ = 0), *J* values in Hz and multiplicities from DEPT.[16] Assignments were confirmed by ^{13}C-^1H correlations.[17] Relative stereochemistry was based on NOESY data,[18] acquired with 700 msec mixing time, 2048 points in F2, 400 complex increments in F1, 16 scans per increments, a final data matrix of 2K*1K points transformed with a cosine squared weighting function in both dimensions. EI-MS, HR-EI-MS, and FAB-MS spectra (the latter in *m*-nitrobenzyl alcohol with a Vacumetrics DIP gun) were taken with a Kratos MS80 mass spectrometer equipped with a home-built data system.

Collections, Isolations, and Reactions. A spinach-green sponge (104M) was collected on 21 February 1982 by beam trawl northeast of Apotres, Iles Crozet, in South Indian Ocean, during the cruise MD30 ; CP201/104M $45^0$56.2 to $45^0$55.6 S, $50^0$32.0 to $50^0$29.7 E, depth 115 m. The sponge was immediately soaked in EtOH, filling a 1.5 litres full glass jar that took a spinach-green colour with strongly fixative properties on cotton or wool cloths. The material was stored at -20^0 and worked up in Marseille in June-July 1982. Filtration, repeated extraction of the sponge with fresh EtOH, and evaporation gave a black residue (total weight 8.7 g) that on dilution with EtOH became spinach-green again. Dry sponge residue after extraction 112 g. A similar sample (110M) was obtained during the same cruise on 24 February 1982, south of Possession in the same group of islands, CP233/110M $46^0$32.8 to $46^0$33.0 S, $51^0$47.0 to $51^0$44.3 E, depth 200 m, on mud/detritus. A black residue (6.5 g) and a dry sponge residue (180 g, though including coarse detritus) were obtained. Both extracts, stored at -20^0C, were examined in 1995 obtaining identical chromatographic profiles. The extracts were therefore combined; a portion (4.9 g) was subjected to reversed-phase FC under H_2O/MeOH gradient to give, at composition H_2O/MeOH 9:1, epinardin A (**8**) (0.10 g), at H_2O/MeOH 7:3 epinardin B (**9**), and at H_2O/MeOH 2:8 a mixture of epinardin C and epinardin D (**10/11**). Epinardin B (**9**) was further purified by TLC with AcOEt/MeOH 3:2, R_f = 0.35 (0.08 g); epinardin C (**10**) and epinardin D (**11**) were separated from one another by TLC with AcOEt, R_f = 0.71 (0.30 g) and 0.60 (0.25 g), respectively. Epinardin A (**8**) (8 mg) was treated with excess Ac_2O/pyridine 3:2 at r.t. overnight, isolating by TLC with AcOEt/EtOH 9:1 a mixture (0.9 mg) of mostly tetra- (HR-EI-MS *m/z* 493.183±0.005; $[C_{26}H_{27}N_3O_7]^{\cdot+}$, calc. 493.184) and penta-acetylation (HR-EI-MS *m/z* 535.194±0.005; $[C_{28}H_{29}N_3O_8]^{\cdot+}$, calc. 535.195) products of the phenolic form[12] of epinardin A.

Biological Assays. The raw black residue from extraction showed antibacterial activity against the Gram-negative wall-defective bacteria *Escherichia coli* and *Proteus vulgaris* and the Gram-positive bacteria *Xanthomonas vescicatoria* and *Sarcina lutea*, as well as antifungal activity against the human epidermal pathogen *Trichophyton mentagrophytes* and the rice pathogen *Piricularia orizae*. No such tests were carried out on purified products, which were instead assayed in Pharmacia laboratories for *in vitro* cytotoxicity on both L1210 murine lymphocytic leukemia cell lines and on the sub-line resistant to doxorubicin (L1210/Dx), comparing the results with those for doxorubicin itself. The antiproliferative activity, obtained from dose-response curves, is expressed as IC_{50}, i.e. dose causing 50% inhibition of cell growth in treated cultures relatively to untreated controls (Table).

Epinardin A (**8**). Green powder giving a blue MeOH solution. UV(MeOH): 570(740), 367(6800), 245(9800). Strong absorption at sodium lamp wavelength -like for all epinardins- prevented measurements of optical rotation; therefore, the chirality was characterized through CD data. CD(MeOH): +1.23(580), 0.00(434), -2.47(400), 0.00(379), +3.98(354), 0.00(318), -1.51(298), 0.00(280), +1.85(266), +4.68(242), 0.00(229). δC (CD_3OD) 44.81 (t, C-1), 89.04 (s, C-2), 75.74 (d, C-3), 127.87 (d, C-4), 134.43 (d, C-5), 33.85 (s, C-6), 31.05 (t, C-7), 39.12 (t,

C-8), 147.96 (s, C-10), 168.23 (s, C-11), 125.00 (s, C-12), 126.42 (d, C-14), 120.18 (s, C-15), 20.97 (t, C-16), 47.34 (t, C-17), 153.00 (s, C-19), 99.74 (s, C-20), 124.32 (s, C-21). δ_H 2.40 (d, J_{gem} 12.5, 1a-H), 1.91 (dd, J_{gem} 12.5, $J_{1,5}$ 2.0, 1b-H), 4.58 (t, $J_{3,5}$ 2.2, $J_{3,4}$ 2.0, 3-H), 5.55 (dd, $J_{4,5}$ 10.0, $J_{4,3}$ 2.0, 4-H), 5.80 (dt, $J_{5,4}$ 10.0, $J_{5,3}$ 2.2, $J_{5,1b}$ 2.0, 5-H), 2.00 (ddd, J_{gem} 13.1, $J_{7,8\beta}$ 3.5, $J_{7,8\alpha}$ 1.6, 7β-H), 1.69 (dt, J_{gem} 13.1, $J_{7,8\beta}$ 12.5, $J_{7,8\alpha}$ 4.8, 7α-H), 3.63 (ddd, J_{gem} 15.5, $J_{8,7\alpha}$ 12.5, $J_{8,7\beta}$ 3.5, 8β-H), 3.76 (ddd, J_{gem} 15.5, $J_{8,7\alpha}$ 4.8, $J_{8,7\beta}$ 1.6, 8α-H), 7.08 (br s, $J_{14,16\beta}$ small, 14-H), 3.02 (ddd, J_{gem} 16.5, $J_{16,17\alpha}$ 9.5, $J_{16,17\beta}$ 6.5, $J_{16,14}$ small, 16β-H), 2.95 (td, J_{gem} 16.5, $J_{16,17\beta}$ 7.0, $J_{16,17\alpha}$ 6.3, 16α-H), 4.34 (dt, J_{gem} 14.5, $J_{17,16\alpha}$ 7.0, $J_{17,16\beta}$ 6.5, 17β-H), 4.14 (ddd, J_{gem} 14.5, $J_{17,16\beta}$ 9.5, $J_{17,16\alpha}$ 6.3, 17α-H). NOESY 5.80/3.63, 5.80/2.00, 4.58/2.40, 2.40/2.00, 1.91/1.69. FAB-MS: *m/z* 324 (M^+).

Epinardin B (**9**). Grey-green powder giving a red-violet MeOH solution. UV(MeOH): 390(3130), 340(4050), 248(9300), 200(11600). CD(MeOH): +0.18(453), 0.00(428), -0.50(383), 0.00(372), +2.27(340), 0.00(297), -4.46(257), -3.17(228). δ_C (CD_3OD) 36.94 (t, C-1), -48 (d, C-2), 74.49 (d, C-3), 122.23 (s, C-4), 137.95 (d, C-5), 41.11 (s, C-6), 32.71 (t, C-7), 38.93 (t, C-8), 152.92 (s, C-10), 167.23 (s, C-11), 125.09 (s, C-12), 127.54 (d, C-14), 120.93 (s, C-15), 19.41 (t, C-16), 44.53 (t, C-17), 155.65 (s, C-19), 99.88 (s, C-20), 124.68 (s, C-21). δ_H 2.28 (br dd, J_{gem} 14.5, $J_{1,2}$ 4.3, $J_{1,3}$=$J_{1,5}$ small, 1a-H), 2.62 (td, J_{gem} 14.5, $J_{1,2}$ 13.5, $J_{1,7\beta}$ 1.6, 1b-H), 4.59 (ddd, $J_{2,1b}$ 13.5, $J_{2,1a}$ 4.3, $J_{2,3}$ 2.6, 2-H), 4.44 (br d, $J_{3,2}$ 2.6, $J_{3,5}$ 0.6, $J_{3,1a}$ small, 3-H), 6.01 (br s, $J_{5,3}$ 0.6, $J_{5,1a}$ small, 5-H), 1.73 (dddd, J_{gem} 14.0, $J_{7,8\alpha}$ 12.0, $J_{7,8\beta}$ 4.5, $J_{7,1b}$ 1.6, 7β-H), 2.05 (dt, J_{gem} 14.0, $J_{7,8\alpha}$ 3.2, $J_{7,8\beta}$ 2.8, 7α-H), 3.66 (ddd, J_{gem} 15.0, $J_{8,7\beta}$ 4.5, $J_{8,7\alpha}$ 2.8, 8β-H), 3.48 (ddd, J_{gem} 15.0, $J_{8,7\beta}$ 12.0, $J_{8,7\alpha}$ 3.2, 8α-H), 7.16 (br s, $J_{14,16}$ small, 14-H), 2.92 (m, 16-H_2), 3.85 (m, 17-H_2). NOESY 7.16/2.92, 6.01/1.73, 4.59/2.05, 3.48/2.28, 2.28/2.05. FAB-MS: *m/z* 466,468,470 (1,2,1 MH^+).

Epinardin C (**10**). Green powder giving MeOH green solutions and purple-red acetone solutions. UV(MeOH): 535(374), 370(3200), 290(4400), 250(5900), 210(11400). CD(CH_3CN): +1.43(647), 0.00(414), -0.15(405), 0.00(396), +3.53(346), 0.00(302), -4.30(277), -3.77(256), -3.43(225). δ_C ($(CD_3)_2CO$) 42.56 (t, C-1), 49.38 (d, C-2), 73.63 (d, C-3), 114.83 (s, C-4), 135.88 (d, C-5), 42.79 (s, C-6), 105.45 (d, C-7), 121.00 (d, C-8), 137.10 (s, C-10), 168.41 (s, C-11), 122.26 (s, C-12), 123.21 (d, C-14), 117.48 (s, C-15), 17.43 (t, C-16), 48.44 (t, C-17), 155.64 (s, C-19), 107.84 (s, C-20), 122.18 (s, C-21). δ_H 2.45 (ddt, J_{gem} 13.7, $J_{1,2}$ 4.7, $J_{1,3}$ 1.2, $J_{1,5}$ 0.7, 1a-H), 3.40 (t, J_{gem} = $J_{1,2}$ 13.7, 1b-H), 4.70 (ddd, $J_{2,1b}$ 13.7, $J_{2,1a}$ 4.7, $J_{2,3}$ 2.4, 2-H), 4.13 (br s, $J_{3,2}$ 2.4, $J_{3,1a}$ 1.2, 3-H), 6.28 (br s, $J_{5,1a}$ 0.7, 5-H), 4.68 (d, $J_{7,8}$ 7.4, 7-H), 6.25 (d, $J_{8,7}$ 7.4, 8-H), 7.13 (t, $J_{14,16}$ small, 14-H), 2.75 (br.t, 16-H_2), 3.92 (ddd, *J* 16.8, 8.8, 7.8) and 4.04 (dt, *J* 16.8, 7.6, 7.6) 17-H_2. FAB-MS: *m/z* 464,466,468(1,2,1 MH^+).

Epinardin D (**11**). Green powder giving a red acetone solution. UV(MeOH): 480(2000), 335(24000), 242(31000), 205(35000). CD(MeOH): +2.04(543), 0.00(445), -4.26(374), 0.00(351), +8.04(328), 0.00(297), -19.16(252),

-20.42(233). δ_C ($CDCl_3$/DMSO-d_6 4:1) 34.39 (t, C-1), 50.19 (d, C-2), 74.11 (d, C-3), 117.70 (s, C-4), 137.74 (d, C-5), 40.04 (s, C-6), 37.10 (t, C-7), 79.83 (d, C-8), 54.59 (s,MeO), 139.14 (s, C-10), 169.70 (s, C-11), 122.90 (s, C-12), 123.24 (d, C-14), 118.01 (s, C-15), 17.84 (t, C-16), 48.88 (t, C-17), 154.96 (s, C-19), 110.54 (s, C-20), 122.44 (s, C-21). δ_H ($(CD_3)_2CO$) 3.18 (ddt, J_{gem} 13.8, $J_{1,2}$ 4.5, $J_{1,3} = J_{1,5}$ 1.0, 1a-H), 2.83 (td, $J_{gem} = J_{1,2}$ 13.8, $J_{1,7\beta}$ 1.5, 1b-H), 4.65 (ddd, $J_{2,1b}$ 13.8, $J_{2,1a}$ 4.5, $J_{2,3}$ 2.4, 2-H), 4.13 (br s, $J_{3,1a}$ 1.0, $J_{3,5}$ small, 3-H), 5.78 (br s, $J_{5,1a}$ 1.0, $J_{5,3}$ small, 5-H), 2.33 (dd, J_{gem} 14.3, $J_{7,8}$ 2.2, 7α-H), 1.75 (ddd, J_{gem} 14.3, $J_{7,8}$ 3.6, $J_{7,1b}$ 1.5, 7β-H), 4.85 (dd, $J_{8,7\beta}$ 3.6, $J_{8,7\alpha}$ 2.2, 8-H), 3.31 (s, MeO), 7.11 (X of ABMNX, $J_{X,A}$ 1.0, $J_{X,B}$ 0.6, 14-H), 2.70 (A of ABMNX, J_{AB} 16.0, $J_{A,M}$ 10.0, $J_{A,N}$ 7.0, $J_{A,X}$ 1.0) and 2.76 (B of ABMNX, J_{AB} 16.0, $J_{B,M}$ 8.0, $J_{B,N}$ 6.5, $J_{B,X}$ 0.6,) 16-H_2, 3.91 (M of ABMNX, J_{MN} 17.0, $J_{M,A}$ 10.0, $J_{M,B}$ 8.0) and 4.08 (N of ABMNX, J_{MN} 17.0, $J_{N,A}$ 7.0, $J_{N,B}$ 6.5) 17-H_2. NOESY 5.78/1.75, 4.65/2.33. FAB-MS: *m/z* 496,498,500 (1,2,1 MH^+).

Acknowledgments

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References

1. Sakemi, S.; Sun, H.H.; Jefford, C.W.; Bernardinelli, G. *Tetrahedron Lett.* **1989**, 30, 2517-2520.
2. Sun, H.H.; Sakemi, S.; Burres, N.; McCarthy, P. *J. Org. Chem.* **1990**, 55, 4964-4966.
3. Stierle, D.B.; Faulkner, D.J. *J. Nat. Prod.* **1991**, 54, 1131-1133; Faulkner, D.J. *Nat. Prod. Rep.* **1995** p. 240.
4. Radisky, D.C.; Radisky, E.S.; Barrows, L.R.; Copp, B.R.; Kramer, R.A.; Ireland, C.M. *J. Am. Chem. Soc.* **1993**, 115, 1632-1638.
5. Copp, B.R.; Ireland, C.M.; Barrows, L.R. *J. Org. Chem.*, **1991**, 56, 4596-4597.
6. Perry, N.B.; Blunt, J.W.; Munro, M.H.G.; Higa, T.; Sakai, R. *J. Org. Chem.* **1988**, 53, 4127-4128.
7. Ohtani, I.I.; Ichiba, T.; Isobe, M.; Kelly-Borges, M.; Scheuer, P.J. *J. Am. Chem. Soc.* **1995**, 117, 10743-10744.
8. Copp, B.R.; Fulton, K.F.; Perry, N.B.; Blunt, J.W.; Munro, M.H.G. *J. Org. Chem.* **1994**, 59, 8233-8238.
9. Perry, N.B.; Blunt, J.W.; Munro, M.H.G. *Tetrahedron* **1988**, 44, 1727-1734.
10. Kobayashi, J.; Cheng, J.-f.; Ishibashi, M.; Nakamura, H.; Ohizumi, Y.; Hirata, Y.; Sasaki, T.; Lu, H.; Clardy, J. *Tetrahedron Lett.* **1987**, 28, 4939-4942.
11. Cheng, J.-f.; Ohizumi, Y.; Wälchli, M.R.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.*

1988, 53, 4621-4624.

12. Kobayashi, J.; Cheng, J.-f.; Yamamura, S.; Ishibashio, M. *Tetrahedron Lett.* **1991**, 32, 1227-1228.
13. a) White, J.D.; Yager, K.M.; Yakura, T. *J. Am. Chem. Soc.* **1994**, 116, 1831-1838; b) Kita, Y.; Tohma, H.; Inagaki, M.; Hatanaka, K.; Yakura, T. *J. Am. Chem. Soc.* **1992**, 114, 2175-2180; c) Tao, X.L.; Cheng, J.-f.; Nishiyama, S.; Yamamura, S. *Tetrahedron* **1994**, 50, 2017-2028; d) Sadanandan, E.V.; Pillai, S.K.; Lakshmikantham, M.V.; Billimoria, A.D.; Culpepper, J.S.; Cava, M.P. *J. Org. Chem.* **1995**, 60, 1800-1805; e) Roberts, D.; Alvarez, M.; Joule, J.A., *Tetrahedron Lett.* **1996**, *37*, 1509-1512.
14. Eliel, E.L.; Wilen, S.H. *Stereochemistry of Organic Compounds*, John Wiley and Sons, Inc.: New York, 1994, p.726.
15. Kelly-Borges, M.; Mattern, R.H.; Proceedings of the 8th International Symposium on Marine Natural Products, Santa Cruz de Tenerife, 10-15 Sept. 1995, 95.
16. a) Doddrell, D.M.; Pegg, D.T.; Bendall, M.R. *J.Magn.Reson.* **1982**, 48, 323-327 ; b) Pegg, D.T.; Doddrell, D.M.; Bendall, M.R. *J. Chem. Phys.*, **1982**, 77, 2745-2752.
17. A. Bax and S. Subramanian, *J. Magn. Reson.*, **1986**, 67, 565-569; A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, **1986**, 108, 2093-2094.
18. Jeener, J.; Meier, H.B.;Bachman, P.; Ernst, R.R. *J. Chem. Phys.*, **1979**, 71, 4546-4553.

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