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Structure Chacterization By Two-Dimensional NMR Spectroscopy, of Two Marine Triterpene Oligoglycosides From A Pacific Sponge of The Genus *Erylus*

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Abstract: The isolation and characterization of two novel triterpene glycosides from a sponge of the genus *Erylus*, collected at a depth of 500 m in the South of New Caledonia, are described. The structures are characterized by the presence of a branched oligosaccharide chain, composed of three (1) and four (2) D-galactopyranose units, respectively. Analysis of the oligosaccaride structures was achieved by {^{1}H, ^{1}H} correlation spectroscopy, two-dimensional homonuclear Hartmann-Hahn, and ^{1}H-detected {^{1}H, ^{13}C} one bond (HMQC) and multiple-bond (HMBC) shift correlation NMR experiments.
The novel lanostane derived aglycone features a rare 14-carboxyl group and a 24-methylene, 25-methyl side chain.

INTRODUCTION

Steroidal and triterpene oligoglycosides are the predominant metabolites of starfishes and sea cucumbers, respectively.[1] Besides these echinoderms, only a limited number of marine organisms have been shown to contain glycosides. For example, pregnane- or cholestane-type steroidal monoglycosides or diglycosides have been reported from soft corals[2], ophiuroids[3], gorgonians[4,5] and fishes[6]. More recentely glycosides have also been isolated from some sponges; several nor-lanostane-triterpenoid oligoglycosides from *Asteropus sarasinosum*[7,8], a 4-methyl steroidal diglycoside from *Erylus lendenfeldi*[9] and a steroidal saponin from *Pachastrella* genus[10]. In search of novel bioactive marine natural products from new-caledonian organisms, we have isolated two new lanostane derived triterpene oligogalactosides, from a sponge of the genus *Erylus* collected at a depth of 500 m in the South of New Caledonia.

In the present work we report the complete structural assignments of Eryloside C (1) and D (2)[a]. Structure elucidation of oligosaccharide portions has been accomplished exclusively on the basis of two-dimensional proton-proton and proton-carbon chemical shift correlation spectroscopy.

a) Y. Kashman *et al.* gave the name eryloside A to the 3β-*O*-[β-D-galactopyranosyl-(1—>2)-β-D-galactopyranosyl]-23ξ-hydroxy-4α-methyl-5α-cholesta-8,14-diene, isolated from *Erylus lendenfeldi* [9]. In the same paper the Authors claim the isolation of a second glycoside, assigned as eryloside B, whose structure is not yet determined (Y. Kashman, personal information to one of the Authors, Luigi Minale).

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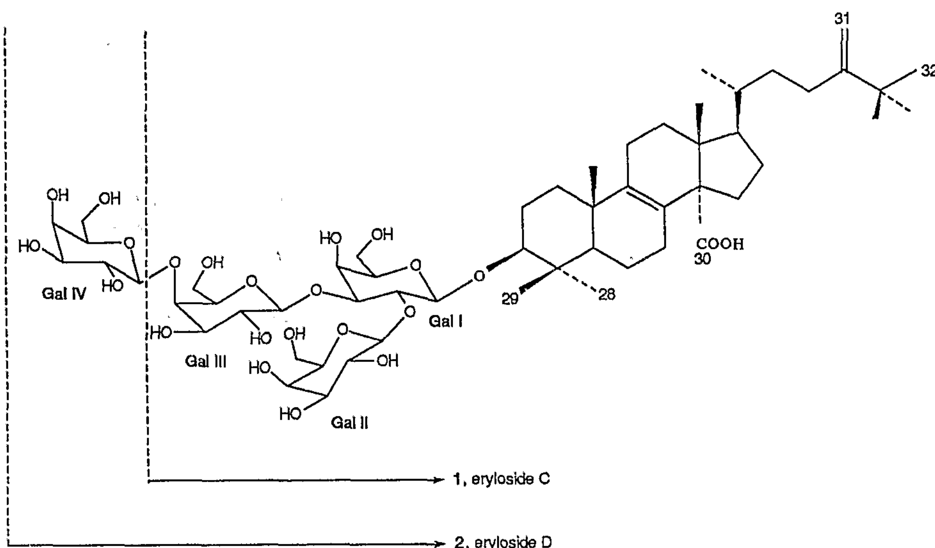

RESULTS AND DISCUSSION

The freeze-dried subject sponge was extracted with *n*-hexane, dichloromethane and finally with methanol (r. t.). The methanol extract was partitioned into a mixture of *n*-butanol and water to afford the *n*-butanol soluble portion, which was subjected to Sephadex LH-20 column chromatography and then to droplet counter current chromatography ($CHCl_3$-MeOH-H_2O, 7 : 13 : 8, ascending mode) to give two saponins : eryloside C (1, 0.00144 % dry weight) and eryloside D (2, 0.00200 % dry weight). ^{1}H NMR spectra indicated that both saponins 1 and 2 posses identical aglycone but differ in the saccharide moiety.

Our structural assignments began with the most aboundant tetragalactoside eryloside D (2) . The molecular formula of this white amorphous powder was determined as $C_{56}H_{92}O_{23}$ by ^{13}C-NMR data and negative ion fast atom bombardment (FAB) mass spectrum, which gave a molecular ion species [M-H]$^-$ at *m/z* 1131 and a weak fragment ion at *m/z* 483 which was assigned to the aglycone moiety.

On anhydrous acid methanolysis Eryloside D (2) gave methyl galactosides (GC)[b]. Its ^{13}C NMR spectrum showed 56 resonance lines, 24 of which could be assigned to four β-D-galactopyranose units (four anomeric carbon atoms at δ 106.9, 105.9, 105.7, 104.3 ppm). Four anomeric protons were also observed in the ^{1}H NMR spectrum (Table II). This implied a $C_{32}H_{52}O_3$ molecular formula for the aglycone portion.

^{13}C NMR signals assigned to the tetracyclic nucleus of the aglycone(Table I) showed a close similarity to those reported for penasterol, a lanostane-related natural triterpene with a 14-carboxy group, recently isolated from the sponge *Penares sp.*[12]. Lanostane-related natural triterpenes with a 14-carboxy group are very rare, the two only examples being lyofolic acid from a terrestrial plant[13] and the mentioned penasterol[12]. The ^{13}C NMR spectrum of 2 (Table I) featured quaternary carbon signals at 177.3 and 63.8 ppm indicative of the presence of a carboxy group at C-14, and two low field resonances at 128.8 and 140.2 ppm, assigned to the double bond at the 8,9 position.

b) The D- configuration of galactose was determined by the positive exciton split CD curve of the methyl-2,3,4,6-tetra-*O*-(*p*-bromobenzoyl)-α-D-galactopyranoside[11] [CD: 236/254 nm, Δε -29.9/+ 50.1, A:+ 80.0]

The ^{1}H chemical shift and coupling constants of the 3-H methine signal (δ_H 2.96, J=10.3, 5.0 Hz) were consistent with those of a 3β-hydroxy-4,4'-dimethyl sterol. The substitution pattern of the side chain was deduced from the ^{1}H and ^{13}C chemical shifts at δ_H 4.69 and 4.88 (each 1H) and δ_C 106.6 (CH_2) and 159.8 (C) ppm, indicative of the presence of an exo-methylene double bond, and from one low field methyl singlet (9H) at δ_H 1.09 ppm (δ_C 29.8 ppm), assigned to a tert-butyl group adjacent to the double bond. The attachment of the glycosidic chain at C-3 was at first deduced by the significant downfield shift (δ_C 91.7 ppm in d_4-methanol) observed for this carbon resonance in 2, relative to the corresponding signal in penasterol (δ_C 76.9 in d_6-DMSO), and subsequently confirmed by NOE and 2D NMR experiments.

Table I. ^{13}C NMR data for the aglycone of eryloside D (2) in d_4-methanol.

C	δ_C	C	δ_C	C	δ_C	C	δ_C
1	35.1	9	140.2	17	51.8	25	37.5
2	28.2	10	36.5	18	16.7	26	29.8
3	91.7	11	23.3	19	19.2	27	29.8
4	40.6	12	27.8	20	36.3	28	27.7
5	52.0	13	46.6	21	19.3	29	14.4
6	18.2	14	63.8	22	36.0	30	177.3
7	28.4	15	30.8	23	30.3	31	106.6
8	128.8	16	27.5	24	159.8	32	29.8

OLIGOSACCHARIDE CHAIN

Owing to the presence of four galactopyranosyl units, the complete structural analysis by conventional degradation methods would have required the obtaining and subsequent structural study of at least two different prosapogenols, through the alternative removal of the two terminal units. Uncertainty in succeeding in such a task with the limited amount of sample available and need to preserve as much as possible the natural product made the use of 2D-NMR techniques the selected tool in structural analysis of this oligosaccharide chain. The position of the interglycosidic linkages was determined using a combination of {^{1}H, ^{1}H} correlation spectroscopy (COSY), 2D homonuclear Hartmann-Hahn (HOHAHA)[4], and proton detected {^{1}H,^{13}C} one bond (HMQC)[15] and multiple bond (HMBC)[16] shift correlation NMR experiments. The chemical shifts assignments of the proton and carbon signals are summarized in Table II. The four galactopyranosyl residues are connected by β-glycosidic linkages [anomeric's: δ_H 4.45 (d, J=7.0 Hz), 4.50 (d, J=7.0 HZ), 4.62 (d, J=7.0 Hz), 4.80 (d, J=7.0 Hz); δ_C 104.3, 105.7, 105.9, 106.9 ppm]. The COSY experiment allowed the sequential assignment of most of the resonances for each galactosyl ring, starting from the anomeric signals. Nevertheless not all proton resonances could be successfully assigned with confidence, because of the overlapping of some signals in the one dimensional spectrum, *e.g.* that for proton pairs H4 Gal III / H4 Gal I, H3 Gal II / H3 Gal IV and H3 Gal I / H4 Gal IV.

Complete assignments were then achieved by combination of COSY and HOHAHA results. Indeed the 2D-HOHAHA experiment (Fig. I) clearly showed correlation signals for the H-1 to H-4 spin system of every galactosyl residue. The coherence transfer to H5 was not obtained because of the small coupling H4-H5 ($J_{4,5}$ <1.5 Hz)[17]. Crosspeaks in both experiments displayed full coupling informations, which helped assignments and allowed identification of multiplet patterns and measurement of coupling constants.

A proton-carbon one bond chemical shift correlation experiment via heteronuclear multiple quantum

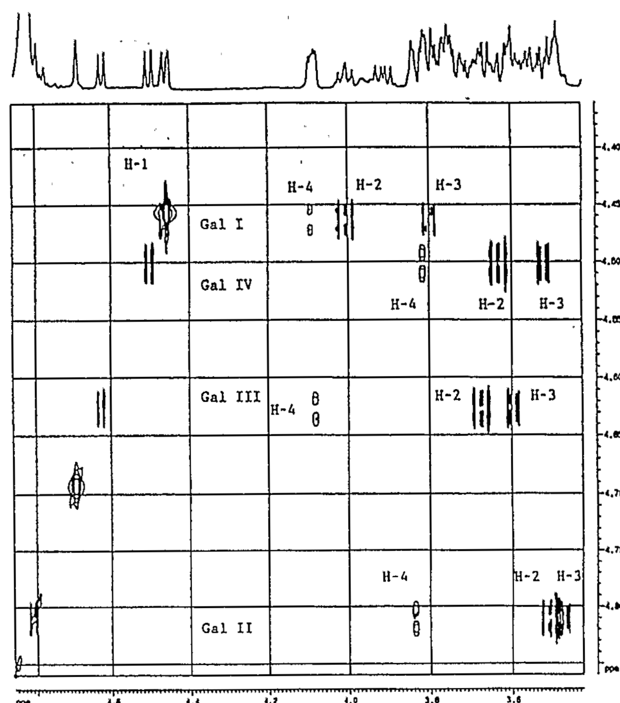

Fig. 1. Expanded region of 2D-HOHAHA of eryloside D (2) in d_4-methanol.

Table II. NMR data[a] for the oligosaccharide moiety of eryloside D (2) in d_4-methanol.

	Gal I		Gal II		Gal III		Gal IV	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	4.46 d	105.9	4.80 d	104.3	4.63 d	105.7	4.50 d	106.9
2	4.01 t	77.3[b]	3.53 t	73.5	3.68 t	73.5	3.65 t	73.7
3	3.80 dd	85.2[b]	3.52 dd	75.2	3.60 dd	75.4	3.53 dd	75.3
4	4.09 bs	70.0	3.85 bs	70.0	4.08 bs	79.8[b]	3.82 bs	70.3
5	3.55 t	75.8	3.52 t	76.9	3.62 t	75.8	3.57 t	76.9
6	3.75dd-3.82dd	62.6[c]	3.65dd-3.77dd	62.4[c]	3.72dd-3.92dd	62.6[c]	3.75dd-3.78dd	62.6[c]

a) from 2D-COSY, 2D-HOHAHA and HMQC experiments; ^{1}H-^{1}H coupling constants in the galactopyranosyl spin systems were measured from COSY and HOHAHA spectra as follows: $J_{1,2}$=7.0 Hz, $J_{2,3}$=7.0 Hz, $J_{3,4}$=4.2 Hz, $J_{5,6}$=6.8 Hz, $J_{6,6}$=11.3 Hz.; b) glycosidated carbons; c) values can be interchanged.

coherence (HMQC) correlated all proton resonances with those of their corresponding carbons (Table II). Data from the above experiment determined the position of interglycosidic linkages by the comparison of the carbon chemical shifts observed with those of the corresponding methyl galactopyranosides[18] and taking into account the known effects of glycosidation. Thus two galactosyl residues showed to be terminal units, as suggested by the absence of any ^{13}C glycosidation shifts for these sugars (Gal II and Gal IV), while glycosidation shift on C-2 (+3.8 ppm) and C-3 (+10.3 ppm) of Gal I and on C-4 (+9.4 ppm) of Gal III established the presence of both a nodal galactopyranosyl residue glycosidated at C-2 and C-3 and a C-4 glycosidated galactopyranosyl unit.

A strong negative NOE observed between H1 of Gal I and the C-3α proton of the aglycone proved direct attachment of Gal I to the aglycone and leaved two possible sequences for the tetragalactoside chain of eryloside D (2): i.e. β-D-gal (1 – >4)-β -D-gal (1 – >3)-[β-D-gal (1 – >2)]-β-D-gal-aglycone or β-D-gal (1 – >4)-β-D-gal (1 – >2)-[β-D-gal (1 – >3)]-β-D-gal-aglycone.

A proton-detected multiple bond heteronuclear correlation (HMBC) experiment allowed us to differentiate between the two hypotheses. Key correlation peaks through glycosidic linkages were observed (Table III), proving linkage of the terminal Gal IV residue to the C-4 of the inner Gal III unit and linkage of this latter to the C-3 of the nodal Gal I. Linkage of the terminal Gal II unit to the C-2 of the nodal Gal I and linkage of Gal I to the C-3 of the aglycone were also established.

Hence, the oligosaccharide moiety has the structure shown in 2 and eryloside D (2) is 3β -*O* - { β - D - galactopyranosyl (1 – >4) - β - D - galactopyranosyl (1 – >3) - [β - D - galactopyranosyl (1 – >2)] - β - D - galactopyranosyl } - 14 - carboxy - 24, 25 - dimethyl - lanosta-8(9), 24(31)-diene.

Table III. Selected data from HMBC experiment of eryloside D (2) in d_4-methanol. Connectivities observed across the glycosidic linkages[a]

C-atom	HMBC (^{1}H)
91.7 (C-3 aglycone)	4.46 (H-1 Gal I)
104.3 (C-1 Gal II)	4.01(H-2 Gal I)
105.7 (C-1 Gal III)	3.80 (H-3 Gal I)
106.9 (C-1 Gal IV)	4.08 (H-4 Gal III)
85.2 (C-3 Gal I)	4.63 (H-1 Gal III)
79.8 (C-4 Gal III)	4.50 (H-1 Gal IV)

a) The experiment was optimized for long range couplings with a fixed delay Δ=60 ms. The low pass J-filter in the experiment to eliminate responses from direct ($^1J_{CH}$) pairs was optimized for 150 Hz.

Eryloside C (1), negative ion FAB MS, *m/z* 969 [M-H]⁻, posses the same aglycone as 2 and contains three moles of galactose. In addition to the signals for the aglycone already observed in the spectrum of 2, the ^{13}C NMR spectrum contains signals for three galactose units. A detailed comparison of the ^{13}C NMR data (Table IV)[c] with those of eryloside D (2) showed that virtually superimposable signals, due to the 2,3-disubstituted galactose unit (Gal I), were present in both spectra. The remaining signals in 1 were assigned to two terminal galactose residues. ^1H NMR and COSY measurements were consistent with the above deductions.

Therefore the structure of eryloside C (1) is 3β-*O*-{β-D-galactopyranosyl (1 – >3)-[β-D-galactopyranosyl (1 – >2)]-β-D-galactopyranosyl} - 14-carboxy- 24, 25- dimethyl- lanosta-8(9), 24(31)-diene.

c) The low solubility of eryloside C (1) in CD_3OD required comparison of ^{13}C NMR data in Pyr-d_5.

Table IV. ^{13}C data for the oligosaccharide moieties of eryloside C (1) and D (2) in d_5-pyridine[a].

		Eryloside C (1)	Eryloside D (2)
Gal I	1	105.8	105.8
	2	77.5	77.8
	3	84.8	85.0
	4	69.6	69.8
	5	76.1	75.7
	6	63.0	62.5
Gal II	1	105.5	104.9
	2	72.8	73.8
	3	75.6	75.4
	4	69.6	70.4
	5	76.8	76.3
	6	61.9	60.7
Gal III	1	104.7	105.7
	2	73.6	73.8
	3	75.2	75.2
	4	70.0	80.6
	5	76.1	76.3
	6	61.4	61.6
Gal IV	1		108.0
	2		73.7
	3		75.7
	4		70.4
	5		77.4
	6		62.5

a) Assignments based on 2D-COSY and HMQC experiments.

EXPERIMENTAL

All NMR measurements were performed on a Bruker AMX-500 spectrometer equipped with a Bruker X-32 computer, using the UXNMR software package. The eryloside D (2) samples were prepared dissolving 8 mg in 0.4 ml of d_5-pyridine or in 0.4 ml of d_4-methanol; spectra of eryloside C (1) were performed with 10 mg dissolved in 0.4 ml of d_5-pyridine.

Two-dimensional homonuclear proton chemical shift correlation (COSY) experiment was measured by employing the conventional pulse sequence. The COSY spectrum was obtained using a data set (t_1 X t_2) of 1024 X 1024 points for a spectral width of 1165 Hz (relaxation delay 1 s) . The data matrix was processed using a unshifted sine bell window function, following transformation to give a magnitude spectrum with symmetrization (digital resolution in both F2 and F1 dimensions 1.13 Hz/pt) .

The 2D-HOHAHA experiment was performed in the phase-sensitive mode (TPPI) using a MLEV-17 sequence for mixing[14]. The spectral width (t_2) was 1002 Hz; 512 experiments of 40 scans each (relaxation delay 1.5 s, mixing time 100 ms) were acquired in 2K data points. For processing, a unshifted sine bell window function was applied in both dimension before transformation. The resulting digital resolution in F2 was 0.48 Hz/pt .

The ^1H-detected {^1H, ^{13}C} shift correlation experiments (at 305 K) utilized a 5-mm probe with reverse geometry and the sample was not spun.

^1H-detected heteronuclear multiple quantum coherence (HMQC) experiments were performed according to Bax and Subramanian[15], using an initial BIRD pulse to suppress ^1H resonances not coupled to ^{13}C and a GARP sequence for ^{13}C decoupling during data acquisition. The spectral width in ^1H dimension was 2300 Hz; 512 experiments of 48 scans each (relaxation delay 1.5 s, delay after BIRD pulse 0.4 s, fixed delay t_1 3.3 ms) were acquired in 1K data points . A sine square window function was applied in t_2 dimension and a trapezoidal window

(TM_1 0.03 Hz, TM_2 O.6 Hz) in t_1 dimension before Fourier transformation (digital resolution in F2 dimension 2.25 Hz/pt).

^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectroscopy was performed according to Bax and co-workers[16]. The spectral width in the ^{1}H dimension was 2300 Hz; 256 FIDs of 80 scans in 1K data points each (relaxation delay 3 s, low pass J-filter delay 3.3 ms, long range couplings evolution delay 60 ms) were collected. The data processing was identical to that used for the HMQC experiment and the final digital resolution in F2 dimension was 2.25 Hz/pt).

Optical rotation were measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Infrared spectra were recorded on a Bruker IFS-48 spectrometer . Fast atom bombardment mass spectra (FAB MS) were recorded in a glycerol matrix in the negative ion mode on a VG ZAB instrument (Xe atoms of energy of 2-6 kV). CD spectra were measured with a JASCO 500A polarimeter. Droplet counter current chromatography (DCCC) was performed on an apparatus manufactured by Tokyo Rikakikai Co., equipped with 300 tubes. GLC analysis were performed on a Supelco SP200 capillary column (30 m, i.d. 0.32 mm, film thickness 0.25 mm, carrier gas He, 5 ml min^{-1}, 156 °C)

Extraction and isolation .

The sponge (3 Kg) was collected in the south of New Caledonia at depth of 500 m and freeze dried. A sample is kept at the ORSTOM Centre de Nouméa under de reference R1363. The lyophilized material (0.9 Kg) was extracted in a Soxhlet apparatus with *n*-hexane (1.5 l) and then with CH_2Cl_2 (1.5 l) . The residual material was then extracted at room temperature with methanol (1 l for three times). The methanol extract was partitioned between *n*-BuOH and water to afford the *n*-BuOH soluble portion (3 g), which was chromatographed on a Sephadex LH-20 (180 g) column with methanol as eluent. Fractions (4 ml) were collected and analyzed by TLC on SiO_2 with *n* -BuOH-acetic acid-water (60:15:25).

Fractions 10-33 (0.8 g) mainly contained the glycosides and were submitted to DCCC with chloroform-methanol-water (7:13:8) [ascending mode; fractions of 4ml were collected and analized by TLC as above] to give two main fractions: 66-88 containing Eryloside D (18 mg) and 143-165 containing Eryloside C (16 mg). This latter was further purified by precipitation from methanol as a white amorphous powder (10 mg).

Methanolysis of eryloside D (2). Carbohydrate constituents and configuration of D-Galactose.

A solution of eryloside D (2, 2 mg) in anhydrous 2N HCl-MeOH (0.5 ml) was heated at 80 °C in a stoppered reaction vial for 15 h. After cooling, the solution was neutralized with Ag_2CO_3 and centrifuged, then the supernatant was evaporated to dryness under N_2. A minor portion of the residue was reacted with TRISYL-Z (Pierce) and analysed by GLC, the retention times were identical to those of autentic methyl D-galactosides. The remaining part (major) of residue was treated in dry pyridine (1 ml) with *p*-bromobenzoyl chloride (15 mg) and a catalytic amount of 4-dimethylaminopyridine, the mixture was stirred overnight at 60 °C under nitrogen, treated with chilled water and then extracted with chloroform. The extract was washed with saturated aqueous $NaHCO_3$, water and then evaporated off under reduced pressure. The benzoate mixture was separated by HPLC using a Whatman Partisil PXS M9 10/25 column; 10% ethyl acetate in hexane, flow rate 2 ml min^{-1}. Methyl 2,3,4,6-tetra-*O*-(*p*-bromobenzoyl)-α-D-galactopyranoside eluted after 22 min and was identified by its ^{1}H NMR spectrum ($CDCl_3$, 500 MHz), δ_H 7.91, 7.85, 7.82, 7.63, 7.61, 7.57, 7.52, 7.40 (each 2 H, d, J 8.8 Hz, ArH), 5.97(1 H, d, J 3.4 Hz, 4-H), 5.92 (1 H, dd, J 10.5 and 3.4 Hz, 3-H), 5.62 (1 H, dd, J 10.5 and 3.6 Hz, 2-H), 5.28 (1 H, d, J 3.6 Hz, 1-H), 4.58 (2 H, m, 5- and 6-H), 4.37 (1 H, m, 6-H); CD (hexane) $\Delta\varepsilon_{254}$ + 50.1, $\Delta\varepsilon_{236}$ -29.9; A + 80.0.

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REFERENCES AND NOTES

1. Stonik, V. A.; Elyakov, G. B. *Biorganic marine chemistry*; ed. P.J. Scheuer; Springer Verlag: New York, 1988, vol.2, p.43.
2. Kobayashi, M.; Kiyota,Y.; Orito, S.; Kyogoku, Y. ; Kitagawa, I. *Tetrahedron Lett.*, 1984, *25*, 3731.
3. Riccio, R.; D'Auria, M. V.; Minale, L. *J .Org. Chem*, 1986, *51*, 533.
4. Bandurraga, M. M. ; Fenical, W. *Tetrahedron*, 1985, *41*, 1057.
5. Fusetani, N. ; Yasukawa, K.; Matsunaga, S. ; Hashimoto, K. *Tetrahedon Lett.* 1987, *28*, 1187.
6. Tachibana, K.; Yasukawa, K.; Matsunaya, S.; Hashimoto, K. *Tetrahedron*, 1985, *41*, 1027.
7. Kitagawa, I.; Kobayashi, M.; Okamoto, Y.; Yoshikawa, M.; Hamamoto, Y. *Chem. Pharm. Bull.*, 1987, *35*, 5036.
8. Schmitz, F. J.; Ksebati, M. B.; Gunasekera, S. P.; Agarwal , S. *J. Org. Chem.*, 1988, *53*, 594.
9. Carmely, S.; Roll, M.; Loya, Y.; Kashman, Y. *J. Nat. Prod.*, 1989, *52*, 167.
10. Hirota, H.;Takayama, S. ; Miyashiro, S.; Ozaki, Y. ; Ikegami, S. *Tetrahedron Lett.*, 1990, *31*, 3321.
11. Liu, H.W.; Nakanishi, K. *J. Am. Chem. Soc.*, 1982, *104*, 1178
12. Cheng, J.; Kobayashi, J.; Nakamura, H.; Ohizumi, Y.;Hirata, Y.; Sasaki, T. *J. Chem. Soc. Perkin Trans I*, 1988, 2403.
13. Sakakibara, J.; Hotta, Y.;Yasuc, M. *Yakugaku Zasshi*, 1971, *91*, 1318. Sakakibara, J.; Hotta, Y.;Yasuc, M.; Iitaka, Y.; Yamazaki, K. *J. Chem. Soc. Chem. Comm.*, 1974, 839.
14. Bax, A. ; Davis, D. G. *J. Magn. Res.*, 1985, *65*, 355.
15. Bax, A.; Subramanian, S. *J. Magn. Res.*, 1986, *67*, 565.
16. Bax, A.; Summers, F. *J. Am. Chem. Soc.* 1986, *108*, 2093. Bax, A.; Aszalos, A.;Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.*, 1986, *108*, 8056.
17. Homans, S. W. *Progress in NMR Spectroscopy*, 1990, *22*, 55.
18. Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*, ed. VCH Verlagsgesellschaft GmbH, D-6940, Weinheim (Federal Republic of Germany), 1987, p. 380-393.