Tetrahedron Letters,Vol.28,No.47,pp 5837-5840,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

ACALYCIXENIOLIDES, NOVEL NORDITERPENES WHICH INHIBIT CELL DIVISION OF FERTILIZED STARFISH EGGS, FROM THE GORGONIAN ACALYCIGORGIA INERMIS[1]

Nobuhiro Fusetani,* Minao Asano, Shigeki Matsunaga,
and Kanehisa Hashimoto

Laboratory of Marine Biochemistry, Faculty of Agriculture,
The University of Tokyo, Bunkyo-ku, Tokyo (Japan)

Summary: Novel norditerpenes, acalycixeniolides A (**1**) and B (**2**), have been isolated from the gorgonian A. inermis. Their structures have been determined by spectroscopic study.

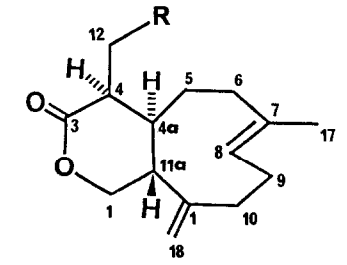
Coelenterates, particularly soft corals and gorgonians, are a rich source of biologically active diterpenes which include cembranoids, asbestinins, briareins, and xenicanes.[2,3] In the course of our continuing studies on bioactive metabolites of Japanese marine invertebrates, we encountered a gorgonian whose lipophilic extract showed considerable activity in the starfish egg assay. The bioassay-guided isolation afforded two norditerpenes of the xenicane group.

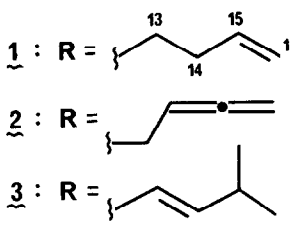
The ether-soluble portion of the ethanol extract of the gorgonian Acalycigorgia inermis (Hedlund) (500 g, wet weight) that was collected by using SCUBA (-15m) in the Gulf of Suruga was fractionated on a SiO_2 column with stepwise elution using benzene and EtOAc. The active fractions were further purified by HPLC on SiO_2 with n-hexane-EtOAc-MeCN (94.5:5:0.5), then on C_{18} with 90% aqueous MeOH, to yield two active components, named acalycixeniolide A (10 mg) and B (6 mg). Acalycixeniolide A and B inhibit the cell division of fertilized starfish (Asterina pectinifera) eggs with ED_{50}'s of 20 and 5 μg/mL, respectively.

Acalycixeniolide A (**1**) is a colorless amorphous powder, $[\alpha]^{23}_D$ +143° (c 0.31, CH_2Cl_2), with a molecular formula of $C_{19}H_{28}O_2$ which was established by HREIMS (M^+, m/z 288.2085, Δ -0.5mmu). It was transparent above 220 nm. The ^1H and ^{13}C NMR spectra revealed the presence of an olefinic methyl, eight methylenes including one oxygenated, three sp^3 methines, one each mono-, 1,1-di-, and trisubstituted olefin, and an ester carbonyl (IR 1750 cm^{-1}). These features required the bicyclic nature of **1**.

The gross structure of 1 was deduced by interpretation of a COSY spectrum in conjunction with decoupling experiments in its ^{1}H NMR study, leading to assignments of all the proton signals as shown in Table 1. To start with, the isolated methine signal at δ 2.78 was coupled to both the 4a-H methine signal and the 12-H_2 methylene signals. The latter signals were correlated to the terminal vinyl through two successive pairs of methylene signals (12-and 13-H_2). On the other hand, the 4a-H signal was coupled to methylene signals (5-H_2) which were in turn correlated with another pair of methylene signals (6-H_2) with no further coupling in one direction, and to a methine signal (11a-H) which was further coupled to the oxygenated methylene signals (1-H_2) in the other. Independently, the olefinic methine signal (8-H) correlated to the methyl signal at δ 1.68 was also coupled to the methylene signals (9-H_2) which were further coupled to another pair of methylene signals (10-H_2). Decoupling of these signals together with that of 11a-H by irradiation of the exo-methylene signals (18-H_2) placed this exo-olefin between C-10 and C-11a, while the chemical shift for 4-H indicated that this methine was adjacent to a carbonyl, thus linked to the oxygenated C-11 to form a δ-lactone ring. The dead-end methylene (C-6) was therefore linked to the remaining quaternary carbon of the trisubstituted olefin (C-7), forming a cyclononene ring. The E-geometry of this olefin was shown by the ^{13}C chemical shift of the olefinic methyl (δ 16.4). These data together with other spectroscopic features - except for the side chain - were in good agreement with those of coraxeniolide A (3), which is a metabolite of a deep-sea gorgonian.[4]

Relative stereochemistry was deduced by difference NOE experiments as shown in Fig. 1. These results suggested that the δ-lactone was in a boat conformation and trans-fused to the cyclononene ring at C-4a and C-11a with 4-H to be cis to 4a-H. This stereochemistry also coincided with that of coraxeniolide A, which was determined by X-ray diffraction, as indicated by the analoguous NMR data of the two.





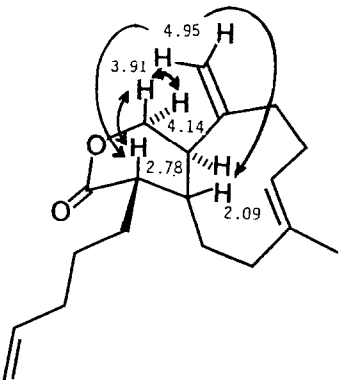


Fig.1 NOE's observed for acalycixeniolide A

Table 1. ^{1}H and ^{13}C NMR Chemical Shifts for Acalycixeniolide A and B (**1** and **2**)

	1		**2**	
C	^{1}H ($CDCl_3$)	^{13}C ($CDCl_3$)	^{1}H ($CDCl_3$)	^{13}C ($CDCl_3$)
1	3.91 (1H, dd, J=12.2, 12.0Hz)	70.5t	3.92 (1H, dd, 11.5, 11.5)	70.5t
	4.14 (1H, dd, 12.2, 6.8)		4.17 (1H, dd, 11.5, 7.0)	
3		175.3s		175.3s
4	2.78 (1H, brq, 7.5)	45.3d	2.92 (1H, brq, 7.0)	44.9d
4a	2.09 (1H, ddd, 10.7, 7.5, 2.9)	42.2d	2.08 (1H, m)	41.1d
5	1.08 (1H, tdd, 14.3, 11.0, 4.0)	30.1t	1.08 (1H, tdd, 14.0, 11.0, 4.0)	30.1t
	1.69 (1H, dt, 14.3, 4.0)		1.65 (1H, m)	
6	1.97 (1H, ddd, 14.3, 12.5, 4.0)	39.9t	1.95 (1H, m)	39.7t
	2.19 (1H, dt, 12.5, 4.0)		2.20 (1H, dt, 13.0, 4.0)	
7		135.8s		135.8s
8	5.34 (1H, brt, 7.6)	123.9d	5.34 (1H, brt, 8.0)	123.8d
9	2.08 (1H, m)	24.9t	2.08 (1H, m)	24.9t
	2.47 (1H, m)		2.47 (1H, m)	
10	2.33 (1H, m)	35.5t	2.33 (1H, m)	35.5t
	2.10 (1H, m)		2.10 (1H, m)	
11		153.0s		153.0s
11a	1.96 (1H, ddd, 12.2, 6.8, 2.9)	49.7d	1.99 (1H, ddd, 11.5, 7.0, 2.9)	49.8d
12	1.48 (1H, m)	26.9t*	1.52 (1H, m)	26.2t**
	1.92 (1H, m)		2.02 (1H, m)	
13	1.38 (1H, m)	26.7t*	2.10 (2H, m)	25.8t**
	1.52 (1H, m)			
14	2.12 (1H, m)	33.6t	5.12 (1H, quint, 6.5)	89.0d
15	5.82 (1H, ddt, 16.8, 10.3, 6.7)	138.3d		208.6s
16	4.98 (1H, dq, 10.3, 2.0)	114.8t	4.70 (2H, m)	75.3t
	5.04 (1H, dq, 17.3, 2.0)			
17	1.68 (3H, d, 1.0)	16.4q	1.68 (1H, brs)	16.3q
18	4.95 (1H, brs)	112.1t	4.96 (1H, brs)	112.1t
	4.96 (1H, brs)		4.98 (1H, brs)	

*,** : These assignments may be interchanged.

Acalycixeniolide B (**2**) possesses a molecular formula of $C_{19}H_{26}O_2$ as secured by HREIMS (M^+, m/z 286.1888, Δ -4.4mmu). The IR spectrum suggested the presence of allene functionality (1960 cm^{-1}) in addition to ester (1750 cm^{-1}). The ^{13}C NMR signals for **2** were similar to those of **1** except for the

terminal portion of the side chain, where the allyl (33.6t, 138.3d, 114.8t) was substituted by an allenyl (89.0d, 208.6s, 75.3t). Interpretation of the COSY spectrum led to assign the gross structure for **2** (Table 1). Relative stereochemistry of **2** was found to be same as **1** in a similar manner.

Several xenicane diterpenes have been isolated from soft corals,[5,6,7] gorgonians,[4,8] and brown algae.[9] Our compounds are the first example of norxenicane diterpenoids, whose biosynthesis poses an interesting problem. The presence of the monosubstituted allene is also unique among natural products, especially in isoprenoids.

Acknowledgment: We are grateful to Professor P. J. Scheuer, University of Hawaii, for reading this manuscript and for providing the ^{1}H NMR chart of **3**. We thank Professor K. Yamazato, University of the Ryukyus, for the identification of the gorgonian. Thanks are also due to Professor N. Otake, and Mr. K. Furihata, Institute of Applied Microbiology of this University, Mr. H. Kaniwa of the Central Reserch Laboratories, Yamanouchi Pharmaceutical Co.,Ltd., Mr. K. Kataoka of Faculty of Pharmaceutical Sciences, this University, and Ms. H. Onoue, the Central Research Laboratories, Ajinomoto Co., Ltd., for measurements of 400 and 500 MHz ^{1}H NMR spectra. We are also indebted to Dr. T. Kusumi, University of Tsukuba, for valuable discussion and measurements of 500 MHz ^{1}H NMR spectra.

REFERENCES AND NOTES

1. Part 22 of Bioactive Marine Metabolites series. Part 21 : N. Fusetani, M. Sugano, S. Matsunaga, and K. Hashimoto, Teterahedron Lett., in press.
2. B. Tursch, J. C. Braekman, D. Daloze, and M. Kaisin, "Terpenoids from Coelenterates" in "Marine Natural Products: Chemical and Biological Perspectives," (Edited by P. J. Scheuer), Vol. 2, pp.247-296. Academic Press, New York (1978).
3. D. J. Faulkner , Nat. Prod. Rep., **1**, 551 (1984); **3**, 1 (1986).
4. R. E. Schwartz, P. J. Scheuer, V. Zabel, and W. H. Watson, Tetrahedron, **37**, 2725 (1981).
5. D. J. Vanderah, P. A. Steudler, L. S. Ciereszko, F. J. Schmitz, J. D. Ekstrand, and D. van der Helm, J. Am. Chem. Soc., **99**, 5780 (1977).
6. J. C. Braekman, D. Daloze, B. Tursch, J. P. Declercq, G. Germain, and M. van Meerssche, Bull. Soc. Chem. Belg., **88**, 71 (1979).
7. A. Groweiss and Y. Kashman, Tetrahedron, **39**, 3385 (1983).
8. M. D'Ambrosio, A. Guerriero, and F. Pietra, Z. Naturforsch., **39C**, 1180 (1984).
9. J. Finer, J. Clardy, W. Fenical, L. Minale, R. Riccio, J. Battaile, M. Kirkup, and R. E. Moore, J. Org. Chem., **44**, 2044 (1979).

(Received in Japan 6 June 1987)