Tetrahedron Letters,Vol.26,No.52,pp 6449-6452,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

BIOACTIVE MARINE METABOLITES XII.[1] MORITOSIDE, AN INHIBITOR OF THE DEVELOPMENT OF STARFISH EMBRYO, FROM THE GORGONIAN EUPLEXAURA SP.

N. Fusetani*, K. Yasukawa, S. Matsunaga, and K. Hashimoto
Laboratory of Marine Biochemistry, Faculty of Agriculture
The University of Tokyo, Bunkyo-ku, Tokyo (Japan)

Abstract: Moritoside, a new hydroquinone glycoside which inhibits cell division of fertilized starfish eggs, has been isolated from the gorgonian Euplexaura sp.

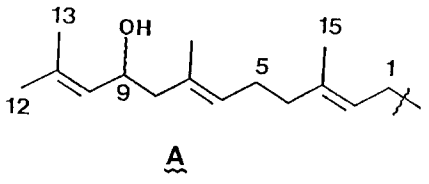
Since Weinheimer and Spraggins[2] discovered a large quantity of prostaglandins in a Caribbean gorgonian Plexaura homomalla, gorgonians have been a prime target for marine natural products chemistry. This research has led to the isolation of sesquiterpenes, diterpenes, and steroids.[3] However, only little work on Japanese gorgonians has been done. In our search for bioactive metabolites from marine invertebrates, we encountered a gorgonian Euplexaura sp. whose lipophilic extract inhibited cell division in the fertilized starfish egg assay.[4] We have isolated an active principle and have elucidated its structure as an unusual farnesyl hydroquinone glycoside.

The ethanol extract of the frozen animals (1.4 kg, wet weight), which were collected near Morito beach in the Gulf of Sagami, was partitioned between $CHCl_3$ and H_2O. The organic layer was fractionated by repeated silica gel column chromatography, HPLC on silica gel (hexane/EtOAc 2:1), and reversed phase HPLC (ODS, 72% MeOH) to yield 16 mg of the active substance which we named moritoside after the collection site. Moritoside inhibits the first cell division of fertilized starfish (Asterina pectinifera) eggs at 1 μg/ml.

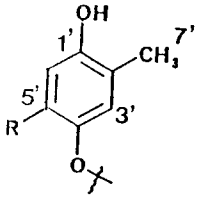
Moritoside is an optically active, colorless oil, $[\alpha]_D^{23}$ +22.6° (c= 0.1, $CHCl_3$) and has a molecular formula of $C_{34}H_{48}O_{11}$ which was established both by FAB mass spectrum [m/z 740 (M+H+diethanolamine)$^+$] and high resolution (HR) EI mass spectrum [m/z 614.3105 $(M-H_2O)^+$, $C_{34}H_{46}O_{10}$ required 614.3104]. The infrared (3400, 1745, and 1230 cm^{-1}) and ultraviolet spectra [λ_{max}(MeOH) 280 nm (ε 3580)][5] implied the presence of hydroxyl, ester, and hydroquinone functionalities. The ^1H and ^{13}C NMR spectra displayed signals assignable to 5 olefinic methyls, 3 acetates, 4 methylenes, one oxygenated methylene, 5

oxygenated methines, one acetal, 5 trisubstituted and one tetrasubstituted double bonds, and 3 exchangeable protons (Table 1). The presence of four finely-split methyl doublets (J= 1Hz) (δ 1.66, 1.66, 1.68, and 1.73), each of which was found by double resonance experiments to be coupled to one of three olefinic protons [δ 5.19 (1H,dquint, J=9,1 Hz), 5.26 (2H, brt)], was reminiscent of a farnesyl functionality.[6] It was also seen that the olefinic proton at δ5.19 was not only coupled to two olefinic methyl groups at δ1.68 and 1.66, but also to a carbinol proton at δ 4.43, indicating the presence of partial structure **A**. This was substantiated by ^{13}C NMR: olefinic methyls at 15.6, 15.8 (C-14,15),18.2 (C-13), and 25.8 (C-12), olefin carbons at δ123.6 d, 126.5 d, 128.7 d, 131.7 s, 135.1 s, and 135.5 s, methylenes at δ 28.0 (C-1), 39.1 (C-4), 25.3 (C-5), 48.0 (C-8), and the C-9 methine at δ66.0.[7]

There were two aromatic protons ascribed to a hydroquinone group at δ6.50 and 6.90, both observed as singlets. The absence of coupling between them suggested a para relationship of the two protons. Partial structure **B** was confirmed by ^{13}C NMR signals for aromatic carbons at δ148.4, 150.4 (C-1', C-4'), 130.1,122.3 (C-2',C-5'), 115.4(C-6'), and 120.0 (C-6') as well as by the presence of a benzylic methyl [δ2.19 (3H, brs), 16.2 q] and one doubly allylic methylene [C-1 of partial structure **A**, δ3.35(dd, J=15,8 Hz) 3.29(dd,J=15,6)][8] in the ^{1}H NMR spectrum. It is therefore conclusive that partial structure **A** was linked to C-5' of the hydroquinone by C-1 of the farnesyl moiety.



A



B

Since moritoside contains 12 degrees of unsaturation, of which 11 units were accounted for by the above partial structures, the remaining portion might possess a ring system including 6 oxygenated carbons, 3 acetoxyl groups, and 3 exchangeable protons. Extensive ^{1}H NMR double resonance experiments proved the presence of an altrose moiety in the molecule (Table 1). Large (J=8,10 Hz) and small (J=1,4 Hz) vicinal coupling constants are compatible with axial-axial and axial(equatorial)-equatorial relationships, respectively, expected for a 6 membered ring in a chair conformation.[9] Chemical shifts for the protons at C-3" (δ4.98), C-4" (δ 5.42), and C-6" (δ4.15,4.21) indicated that the hydroxyl groups on these carbons were acetylated. It was found that only the C-2" proton (δ 4.05) was coupled to an exchangeable proton (δ3.23). Treatment of moritoside with aq. ammonia[10] followed by enzymic hydrolysis with a glycosidase mixture (prepared from Turbo cornutus) released a monosaccharide, which was

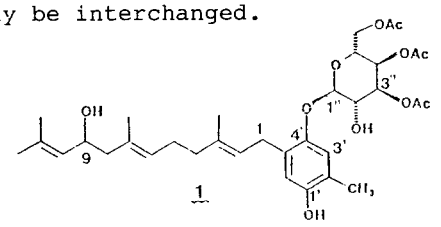
Table 1. ^{13}C NMR(25MHz) and ^{1}H NMR(500MHz) data for **1** in $CDCl_3$

NO.	Carbon*	Proton	Decoupling multiplicity
1	28.0 t[a]	3.35 dd(15,8 Hz)	{irr. 5.26}- d,(15Hz)
		3.29 dd(15,6)	{5.26}- d,(15)
2	128.7 d[b]	5.26 brt(8)	
3	135.1 s[c]		
4	39.1 t	1.6-2.3	
5	25.3 t	1.6-2.3	
6	126.5 d[b]	5.26 brt(8)	
7	135.5 s[c]		
8	48.0 t	1.6-2.3	
9	66.0 d	4.43 ddd(10,9,3.5)	{5.19}- dd,(10,3.5)
10	123.6 d[b]	5.19 dquint(9,1)	{4.43}- brs
11	131.7 s		
12	25.8 q	1.73 3H,d(1)	
13	18.2 q	1.66 3H,d(1)[h]	
14	15.6 q[d]	1.68 3H,d(1)[h]	
15	15.8 q[d]	1.66 3H,d(1)[h]	
1'	148.4 s[e]		
2'	130.1 s[f]		
3'	120.0 d	6.90 s	
4'	150.4 s[e]		
5'	122.3 s[f]		
6'	115.4 d	6.50 s	
7'	16.2 q	2.19 3H,brs	
1"	103.9 d	4.72 d(8)	{4.05}- simplified
2"	67.4 d[g]	4.05 dd(10,8)	{4.98}- d,(8),{4.72}- d,(10)
3"	72.6 d	4.98 dd(10,3.5)	{5.42}- d,(10),{4.05}- brd
4"	68.9 d	5.42 dd(3.5,1)	{4.98}- d,(1),{3.98}- d,(3.5)
5"	70.9 d[g]	3.98 ddd(7,6,1)	
6"	66.0 t	4.21 dd(11,7)	{3.98}- d,(11)
		4.15 dd(11,6)	{3.98}- d,(11)
acetyl	170.2 s(3C)		
	20.7 q(3C)	2.16 3H,s	
		2.07 3H,s	
		2.06 3H,s	
1'-OH		6.17 brs	
9-OH		2.26 brs	
2"-OH		3.23 brs	

* Assignments were made by selective proton decoupling and comparison with literature data.[11]

a: Multiplicities were determined by INEPT pulse sequence.

b-h: Assignments may be interchanged.



identical with authentic D-altrose in optical rotation ($[\alpha]_D^{23}$ +39° ; literature value[12] +33°), ^{1}H NMR spectrum and TLC. The position of glycosidation was determined by ^{1}H NMR difference nOe experiments. Irradiation of the C-7' benzylic methyl signal at δ 2.19 enhanced only the C-3' proton at δ6.90, while irradiation of the C-1 benzylic methylene signals centering at δ 3.32 enhanced both C-6' proton at δ 6.50 and the C-1" proton at δ 4.72. These experiments

revealed that the altrose moiety was β-linked to C-4' and the remaining two exchangeable protons were assignable to C-9 and C-1' hydroxyl groups. Thus the structure of moritoside was determined as **1**. Fragment ions in the HREI mass spectrum[13] also supported this structure.

Moritoside possesses unusual structural features. To the best of our knowledge, this is the first example of the occurrence of D-altrose in natural products, though the presence of free altritol in a marine brown alga has been reported.[14] Esterification of the sugar portion with three acetyl groups is also rare in nature.[15] The substitution pattern of the hydroquinone moiety in moritoside is also uncommon; a monoterpene hydroquinone glycoside with the same substitution pattern has been known from a plant[16] and a tetraprenylated benzoquinone from a soft coral.[17] The role of moritoside in the gorgonian is an interesting problem.

Acknowledgement. We thank Professor P. J. Scheuer, University of Hawaii, for reading the manuscript. We are also grateful to Dr. F. M. Bayer, Smithsonian Institution, and Dr. K. Muzik, Okinawa Expo Aquarium, for the identification of the gorgonian, and to Professor N. Ōtake and Mr. K. Furihata, Institute of Applied Microbiology of this University and Mr. K. Koseki, Japan Tobacco Inc., for measurements of ^{1}H NMR spectra.

REFERENCES AND NOTES

1. Part XI; S. Matsunaga, N. Fusetani, and K. Hashimoto, J. Am. Chem. Soc., submitted.
2. A. J. Weinheimer and R. J. Spraggins, Tetrahedron Lett., 5185 (1969).
3. "Marine Natural Products", P. J. Scheuer ed., Academic Press, New York, vol. 2 (1978).
4. S. Ikegami, K. Kawada, Y. Kimura, and A, Suzuki, Agric. Biol. Chem., **43**, 161 (1979).
5. H. H. Sun, N. M. Ferrara, O. J. McConnell, and W. Fenical, Tetrahedron Lett., **21**, 3123 (1980).
6. E.Fattorusso, S. Magno, L. Mayol, C. Santacrose, D. Sica, V. Amico, G. Orente, M. Piattelli, and C. Tringali, Tetrahedron Lett., 937 (1976).
7. B. F. Bowden and J. C. Coll, Aust. J. Chem., **34**, 2677 (1981).
8. J. C. Coll, N. Liyanage, G. J. Stokie, I. V. Altena, J. N. E. Nemorin, S. Sternhell, and R. Kazlauskas, Aust. J. Chem., **31**, 157 (1978).
9. L. M. Jackman and S. Sternhell, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry", Pergamon Press, London (1969).
10. T. Neilson and E. S. Werstiul, Can. J. Chem., **49**, 493 (1971)
11. E. Breitmaier and W. Volter, "^{13}C NMR-Spsctroscapy Monographs in Modern Chemistry" Verlag Chemie, Weinheim (1978).
12. N. K. Richtmeyer, in "Methods in Carbohydrate Chemistry", N. K. Richtmeyer ed., Academic Press, New York, vol. 1, p. 107 (1962).
13. Fragment ions in HREI mass spectrum; 289.0935($C_{12}H_{17}O_8$, calc. 289.0936), 326.2260 ($C_{22}H_{30}O_2$, calc. 326.2261)
14. J. A. Chudek, R. Foster, I. R. Davision, and R. H. Reed, Phytochemistry, **23**, 1081 (1984).
15. M. M. Bandurraga and W. Fenical, Tetrahedron, **41**, 1057 (1985).
16. H. Inouye, K. Tokura, and S. Tobita, Chem. Ber., **101**, 4057 (1968).
17. B. F. Bowden and J. C. Coll, Aust. J. Chem., **34**, 2677 (1981).

(Received in Japan 8 October 1985)