Tetrahedron Letters,Vol.28,No.11,pp 1187-1190,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

DIMORPHOSIDES A AND B, NOVEL STEROID GLYCOSIDES FROM THE GORGONIAN *ANTHOPLEXAURA DIMORPHA*[1]

Nobuhiro Fusetani,* Kenji Yasukawa, Shigeki Matsunaga, and Kanehisa Hashimoto

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

Abstract: Dimorphosides A (1) and B (2) have been isolated from the Western Paciffic gorgonian *Anthoplexaura dimorpha* as the cell-division inhibitors in the sea urchin egg assay The structures were determined by chemical and spectrometric means

Animal saponins had been accepted as unique echinoderm metabolites [2-3] until recent isolations of steroid glycosides from a soft coral,[4] a gorgonian[5], and a fish.[6] In the course of our search for bioactive metabolites from Japanese marine invertebrates,[1] we examined the methanolic extract of a gogonian *Anthoplexaura dimorpha* , which is widely distributed along the southern coast of Japan, and found that it inhibited the development of fertilized sea urchin eggs.[7] From this gorgonian we have isolated two steroid glycosides named dimorphosides A (**1**) and B (**2**) as the major active constituents.

The gorgonian (1.5 kg wet weight) collected in the Gulf of Sagami was extracted with MeOH; the extract was partitioned between ether and water. The ether phase was again partitioned between *n*-hexane and 20 % aq MeOH, while the aqueous phase was partitioned between *n*-BuOH and water. The 20 % aq. MeOH layer and the *n*-BuOH layer were combined and subjected to successive chromatographies on silica gel ($CHCl_3$-MeOH-H_2O), Toyopearl HW40 ($CHCl_3$-MeOH,1:1), ODS open column (13 % aq. MeOH), and reverse-phase HPLC (ODS, 12 % aq MeOH) to yield dimorphoside A (**1**, 640 mg) and dimorphoside B (**2**, 50 mg) as colorless amorphous solids. Both compounds inhibit the development of fertilized sea urchin (*Hemicentrotus pulcherrimus*) eggs at a concentration of 6 μg/mL.

Dimorphoside A ($[\alpha]^{23}_D$-59°, *c* 0.38, MeOH) possesses a molecular formula of $C_{38}H_{62}O_{13}$ which was established by FABMS [*m/z* 832 (M + H + diethanolamine)$^+$] and ^{13}C NMR (Table 1). The presence of hydroxyl and carboxylic acid groups was inferred from the IR spectrum (3500-3000, 1690 cm^{-1}) The ^1H and ^{13}C NMR spectra revealed the presence of four methyls, 10 methylenes, 6 methines, two quaternary carbons, 2 oxygenated methylenes, 9 oxygen-bearing methines, two ketals, one trisubstituted double bond, and a carboxylic acid. Interpretation of the COSY spectrum[8] starting from the two ketal protons revealed a pentose and a hexose unit Acid hydrolysis (10 % HCl in aq. MeOH, reflux) of **1** followed by cellulose column chromatography (*n*-BuOH saturated with water) gave D-arabinose and D-glucose in a ratio of 1 1, which were identified by ^1H NMR and optical rotation as well as by TLC. Thus, the aglycone must possess a molecular formula of $C_{27}H_{44}O_4$.

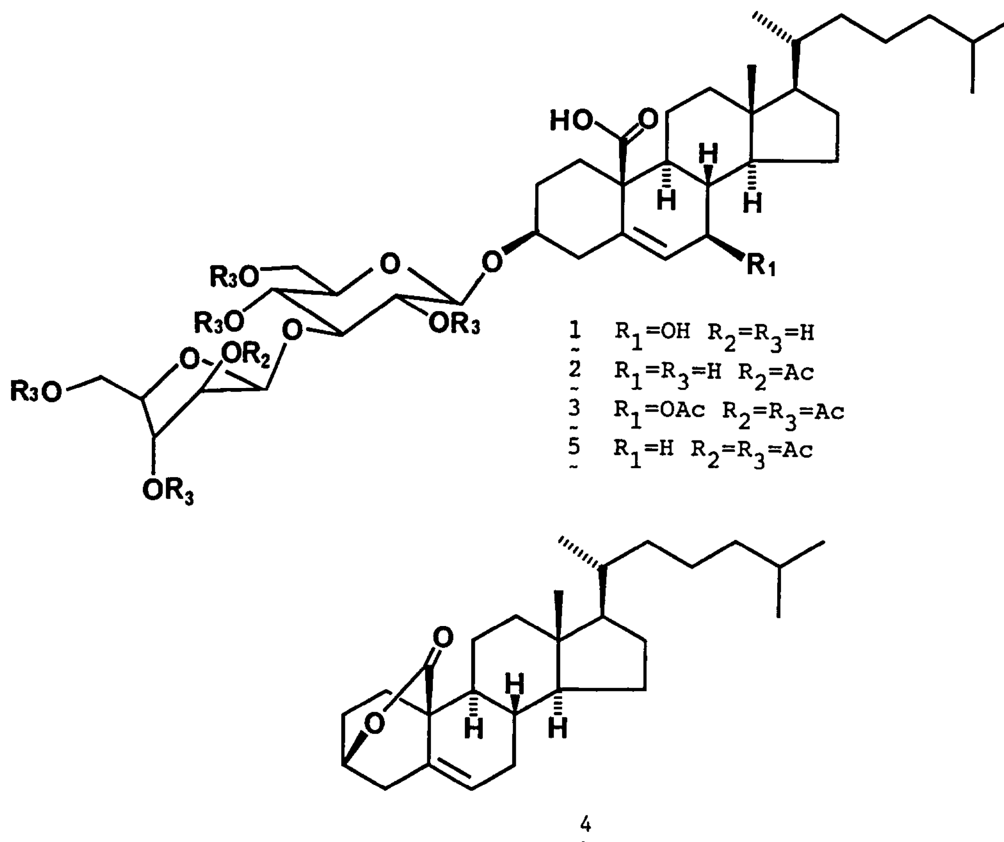

Table 1 ^{13}C NMR Spectral Data for the Dimorphosides

	1*1	2*2		1	2		1	2
1	35.9*3	35 0	14	58 5	57 9	27	24.0	23 2
2	33 2	32 2	15	28 2	25 0	1'	103 2	102 5
3	80 0	79 2	16	30.5	29 2	2'	75 5	74 8
4	42 2	41 7	17	57.7	57 5	3'	70.8	69 9
5	139.6	136.3	18	13 4	12.5	4'	86 5	85 4
6	130.6	125.5	19	178 7	177 5	5'	78.2	77 3
7	74.1	32 2	20	37.9	37.0	6'	63.5	62.5
8	42.3	33 2	21	20 2	19.3	1"	104 4	101 4
9	48 5	50 0	22	38 2	37 3	2"	79.6	79 9
10	53 3	-*4	23	25.7	25 1	3"	75.4	71 4
11	25.2	24 4	24	41 5	40 7	4"	84 5	82 9
12	41.9	41 0	25	29 9	29 1	5"	63 2	61.8
13	45 0	43.6	26	23.8	23 0	OAc	-	20.7, 172.6

*1 100 MHz in CD_3OD Assignments were made by (C,H)COSY spectrum and by comparison with those for a model compound.[10]

*2 25 MHz in CD_3OD Assignments were made by comparison with those for 1

*3 Multiplicities were determined by INEPT spectra

*4 Burried under the solvent signal

Since the glycoside and its methyl ester (CH_2N_2,MeOH) gave no genuine aglycone by chemical and enzymic hydrolysis,[9] further structural study was performed on the intact molecule mainly by extensive NMR experiments. The presence of one methyl singlet (δ 0 69, 13 4 q), 3 methyl doublets [δ 0.87, 0 88, 0 94, (each, 3H, d , J=7Hz),20 2 q, 23 8 q, 24.0 q], 4 methine carbons (three of which were appearing at lower field), 2 quaternary carbons, and a carboxylic acid group was reminiscent of a steroid skeleton with a C10 carboxylic acid group [10] The 6 degrees of unsaturation for the aglycone were accounted for by the 4 carbocyclic rings, a carboxylic acid, and an olefin There were also two oxygenated methines in the aglycone. The position of these functional groups was determined by a COSY spectrum. The H6 olefinic proton (δ 5.48 br s) was not only correlated with the H7 carbinol proton (δ 3 72), but also with one of the H4 methylene protons at δ 1.97 (H4a), which was in turn coupled to H7 and H4b (δ 2 59) The H4b signal was correlated with H3 at δ 3 61, which was also coupled to H2b at δ 2 00. The H2b signal was coupled to both protons of the C1 methylenes [δ 0 91 (H1a), 2 56 (H1b)], the latter was further coupled to the H2a signal at δ 1.67. There was a cross peak between H7 and H8 (δ 1 79) These results allowed us to establish the connectivities from H1 through H8, which was compatible with a 3,7-diol-5-en system. The stereochemistry at C3 was not defined at this time due to overlapping signals, while the β-orientation of the C7 hydroxyl group was estimated from a small coupling (ca 1 Hz) observed between H6 and H7 signals.[11] These assignments were supported by a (C,H)COSY spectrum[12] enhancing long-range couplings, which gave the following correlations H6→C8 and C10, H4b→C2, C5 and C6, H4a→C5 and C6 This long-range (C,H)COSY spectrum also revealed cross peaks between C18 methyl protons and C12, C13, C14, and C17; between C21 methyl protons and C17, C20, and C22, between C26 and C27 methyl protons and C24 and C25 Therefore, the C19 methyl must be oxidized to a carboxylic acid

Upon treatment with Ac_2O/pyridine **1** gave the heptaacetate **3**,[13] which showed H3 and H7 signals at δ 3 47 and 4 99, respectively, indicating that the sugar portion is attached to C3. The shape of the H3 signal was characteristic of a C3 axial proton.[14] A ^{1}H NMR double resonance experiment allowed us to assign all the signals for the arabinofuranose and the glucopyranose residues.[13] Absence of ^{1}H NMR acetylation shift[15] for the H3' signal revealed that arabinose was attached to glucose at C3' position A coupling constant of 8.1 Hz for the glucose H1' proton and ^{13}C NMR chemical shifts for the arabinose residue in **1**[16] (Table 1) showed that both glucose and arabinose were β-linked

Dimorphoside B (**2**, $[\alpha]^{23}_D$ -12°, *c* 0.3, MeOH) possessed a molecular formula of $C_{40}H_{64}O_{13}$ [FABMS *m/z* 858 (M+H+diethanolamine)$^+$] ^{1}H and ^{13}C NMR spectra (Table 1) indicated that **2** was also composed of D-glucose, D-arabinose,[17] and a steroid bearing a carboxylic acid at C10 Dimorphoside B lacked the C7 hydroxyl group, while it contained one acetyl group Upon treatment with HCl/aq MeOH dimorphoside B gave the lactone **4**,[18] whose optical rotation and ^{1}H NMR data were in good agreement with those cited in the literature [10] ^{1}H NMR decoupling experiments revealed that the H2" signal for the arabinose residue appeared at δ 4 83 (dd, J= 4 9, 8 5 Hz),[19] thereby suggesting that the acetoxyl group is attached at the C2'' position of the arabinose residue. Dimorphoside B was easily converted to pentaacetate **5**, whose ^{1}H NMR spectrum was identical with that of **3** within the region between 3 4 and 5 4 except for the H7 signal Thus, the structure of dimorphoside B was assigned as **2**.

To the best of our knowledge this is the first isolation of steroid glycosides bearing a carboxylic acid at C10, though related steroid sulfates have been isolated from a sponge [10]

Acknowl edgment We thank Professor Paul J. Scheuer of the University of Hawaii for valuable editorial comments. We also thank Dr. Y. Ichikawa of The Institute of Physical and Chemical Research for valuable discussion. We are indebted to Dr. O. Kamo of JEOL Ltd. and H. Kaniwa of Yamanouchi Pharmaceutical Co., Ltd. for measurements of NMR spectra We are grateful to Y. Kato of our laboratory for the sea urchin egg assay.

References and Notes

1. Part 17 of the bioactive marine metabolites series. Part 16. Y. Kato, N. Fusetani, S. Matsunaga, K. Hashimoto, S. Fujita, and K. Furuya, *J. Am. Chem. Soc.*, **1986,** *108*, 2780.
2. D. J. Burnell and J. W. ApSimon. *Marine Natural Products. vol V.* P. J. Scheuer Ed., Academic Press, New York, 1983, p. 287, L. Minale, C. Pizza, R. Ricco, and F. Zollo, *Pure Appl. Chem.*, **1982,** *54*, 1935.
3. D. J Faulkner, *Nat. Prod. Rep.*, **1984,** *1*, 551.
4. M. Kobayashi, Y. Kiyota, S. Orito, Y. Kyogoku, and I. Kitagawa, *Tetrahedron Lett.*, **1984,** *25*, 3731.
5. M. M. Bandurraga and W. Fenical, *Tetrahedron,* **1985,** *41*, 1057
6. K. Tachibana, M Sakaitani, and K. Nakanishi, *Tetrahedron,* **1985,** *41*, 1027.
7. R. S Jacobs, S. White, and L. Wilson, *Fed. Proc.*, **1981,** *40*, 26.
8. A. Bax and R. Freeman, *J. Magn. Reson.*, **1981,** *44*, 542.
9. By treatment with either base or acid the aglycone underwent decarboxylation followed by aromatization to yield several non-polar UV-active compounds, which have not been characterized.
10. T. Nakatsu, R. P. Walker, J. E. Thompson, and D. J. Faulkner, *Experientia,* **1983,** *39*, 759
11. E. Fattorusso, V. Lanzotti, S. Magno, and E. Novellino, *J Org. Chem.*, **1985,** *50*, 2868 The 7β orientation of the hydroxyl group was also supported by the ^{13}C NMR shift for the C15 signal
12. G. A. Morris and L. D. Hall, *J. Am. Chem. Soc.*, **1981,** *103*, 4703.
13. **3**, FABMS *m/z* 1126 (M + H + diethanolamine)$^+$; ^{1}H NMR δ 5.53 (1H, br s, H6), 5.28 (1H, d, *J*=4.9Hz, H1''), 5.22 (1H, dd, 6.8, 6.0, H3''), 5.11 (1H, dd, 4.9, 6.0, H2''), 4.99 (1H, br d, 8, H7), 4.99 (1H, dd, 9.0, 9.8, H4'), 4.84 (1H, dd, 8.1, 9.0, H2'), 4.42 (1H, d, 8.1, H1'), 4.24 (1H, dd, 4.9, 11.5, H5''a), 4.14 (1H, dd, 4.9, 12.5, H6a), 4.10 (1H, dd, 3.0, 12 5, H6'b), 4.09 (1H, dd, 4.9, 11.5, H5''b), 4.00 (1H, ddd, 4.9, 4.9, 6.8, H4''), 3.87 (1H, dd, 9.0, 9.0, H3'), 3.55 (1H, ddd, 3.0, 4.9, 9.8, H5'), 3.47 (1H, m, H3), 2 10 (6H, s), 2.09 (3H, s), 2.07 (6H, s), 2.06 (6H, s), 0.89 (3H, d, 6.4, H21), 0.86 (3H, d, H26), 0 85 (3H, d, H27), 0.66 (3H, s, H18).
14. J. E. Bridgeman, P C. Cherry, A. S. Clegg, J. M. Evans, S. E. R. H. Jones, A. Kasal, V. Kumur, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J Chem. Soc. (C)*, **1970,** 250.
15. Y. Kawazoe, Y. Sato, T. Okamoto, and K. Tsuda, *Chem. Pharm. Bull.*, **1963,** *11*, 328.
16. R. G. S. Ritchie, N. Cyr, B. Korsch, H. J. Koch, and A. S. Perlin, *Can. J. Chem.*, **1975,** *53*, 1424.
17. The chirality of the glucose and the arabinose moieties were determined both as D by chiral GC analysis (Chirasil Val III column, Applied Science) on the acid hydrolysate of **2** as the pertrifluoroacetyl methyl glycoside derivatives. (W. A. Konig, I. Benecke, and H. Bretting, *Angew. Chem. Int. Ed. Eng.*, **1981,** *20*, 693)
18. **4**: $[\alpha]^{23}_D$ -105°, *c* 0.4 ($CHCl_3$), ^{1}H NMR δ 5.61 (1H, m, H6), 4.69 (1H, m, H3), 0.91 (3H, d, H21), 0.85 (6H, d, H26, H27), 0.83 (3H, s, H18).
19. The H1'' and H3'' signals for **2** were observed at δ 5.42 (1H, d, *J*=4.9Hz) and 4.50 (1H, dd, 7.8,8.5), respectively.

(Received in Japan 11 November 1986)