ORGANIC LETTERS

2005
Vol. 7, No. 6
991−994

New Hemiketal Steroid from the Soft Coral *Cladiella* sp.

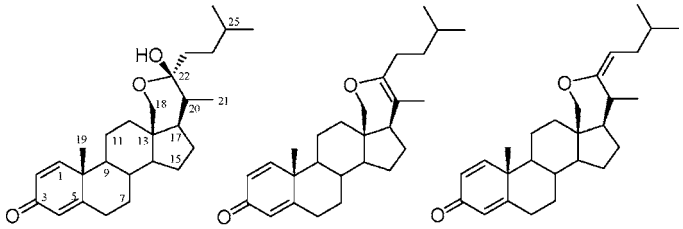
Guang-Wen Zhang,[†,‡] Xiang-Quan Ma,[†] Hiroshi Kurihara,[‡] Cui-Xian Zhang,[†] Xin-Sheng Yao,[‡] Jing-Yu Su,[†] and Long-Mei Zeng*[,†]

School of Chemistry and Chemical Engineering, Sun Yat-Sen (Zhongshan) University, Guangzhou 510275, China, and Institute of Traditional Chinese Medicine & Natural Products, Jinan University, Guangzhou 510632, China

ceszlm@zsu.edu.cn

Received November 3, 2004

ABSTRACT



1 **1a** **1b**

A new hemiketal steroid, named cladiellin A (1), was first isolated from the soft coral *Cladiella* sp. Its structure was determined by spectroscopic methods and X-ray analysis. Compound 1 easily converted to 1a when NMR spectra were measured in $CDCl_3$ solution and quickly changed to 1b when pyridine was used. The structures of dehydrated products 1a and 1b were determined by spectroscopic analysis. Bioassay showed that all these three compounds showed antioxidant activity.

Free radicals play important roles in many physiological and pathological conditions.[1] Usually, the generation and scavenging of oxygen free radicals is balanced in the human body. If there is an imbalance, excessive amounts of active oxygen radicals may be generated. It has been found that free radical reactions can produce deleterious modifications in membranes, proteins, enzymes and DNA,[2] increasing the risk of diseases such as Alzheimer's,[3] Parkinson's,[4] cancer, angiocardiopathy,[5] arthritis,[6] asthma, diabetes, and degenerative eye disease.[7] Therefore, it is important to find effective scavengers of active oxygen radicals. In recent years, the oxygen radical absorbance capacity (ORAC) assay has been widely accepted as a tool for antioxidant assessment.[8]

In our continuing study on discovery of potential drug leads from marine invertebrates,[9] the ethanolic extract of the titled soft coral exhibited antioxidant activity evaluated by ORAC as described in the literature.[8] Bioassay-guided fractionation led to isolation of a new hemiketal steroid, named cladiellin A (**1**), with antioxidant activity. Compound **1** is very unstable. It easily converted to dehydrate product **1a** or **1b** under weak acid or base condition. This paper describes the isolation and structural elucidation of natural product **1** and dehydration products **1a** and **1b**.

* To whom correspondence should be addressed. Tel: +86-20-84036447. Fax: +86-20-84112245.
† Sun Yat-Sen (Zhongshan) University.
‡ Jinan University.

(1) Vendemiale, G.; Grattagliano, I.; Altomare, E. *Int. J. Clin. Lab. Res.* **1999**, *29*, 49−55.
(2) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. *FASEB J.* **2003**, *17*, 1195−1214.
(3) Terranova, R.; Sorace, R.; Romeo, A.; Mauro, C. D.; Romeo, R.; Luca, S. *Minerva Med.* **2001**, *92*, 405−410.
(4) Olanow, C. W. *Ann. Neurol.* **1992**, *32*. Suppl, S2−9.
(5) Bankson, D. D.; Kestin, M.; Rifai, N. *Clin. Lab. Med.* **1993**, *13*, 463−480.
(6) Jaswal, S.; Mehta, H. C.; Sood, A. K.; Kaur, J. *Clin. Chim. Acta* **2003**, *338*, 123−129.
(7) Florence, T. M. *Aust. N. Z. J. Ophthalmol.* **1995**, *23*, 3−7.
(8) Kurihara, H.; Fukami, H.; Asami, S.; Toyoda, Y.; Nakai, M.; Shibata, H.; Yao, X. S. *Biol. Pharm. Bull.* **2004**, *27*, 1093−1098.
(9) (a) Xu, X. H.; Yao, G. M.; Kong, C. H.; Li, Y. M.; Lin, C. J.; Wang, X.; Su, J. Y.; Zeng, L. M. *Chin. J. Org. Chem.* **2003**, *23*, 953−957. (b) Ma, X. Q.; Yan, S. J.; Su, J. Y.; Zeng, L. M. *Chem. J. Chin. Univ.* **2004**, *25*, 479−481. (c) Su, J. Y.; Long, K. H.; Peng, T. S.; He, C. H.; Jon, C. *J. Am. Chem. Soc.* **1986**, *108*, 177−178. (d) Zeng, L. M.; Lan, W. J.; Su, J. Y.; Zhang, G. W.; Feng, X. L.; Liang, Y. J.; Yang, X. P. *J. Nat. Prod.* **2004**, *67*, 1915−1918.

10.1021/ol0477475 CCC: $30.25
Published on Web 02/16/2005

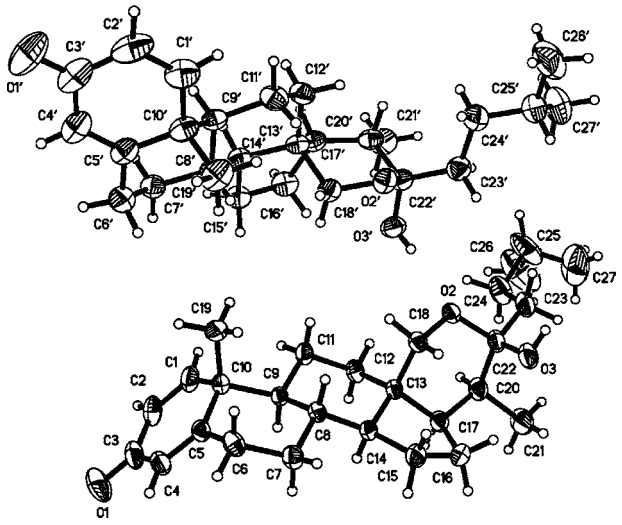


Figure 1. Perspective drawing of the X-ray structure of **1**.

The soft coral *Cladiella* sp. (500 g, dried wt) collected from Sanya bay Hainan Island of China was extracted with 95% ethanol. The ethanol extract (48 g) was partitioned between petroleum ether and water to obtain a petroleum ether soluble portion (32 g), which was chromatographed on silica gel eluting with a gradient elution of ethyl acetate-petroleum ether to afford **1** (5 mg).

Compound **1** was obtained as colorless crystals: mp 169.5−171.5 °C; $[\alpha]^{20}_{D}$ = +18.2 (*c* 1.0 MeOH). Its HREIMS exhibited a molecular ion peak at *m*/*z* 413.3031 $[M + H]^+$, corresponding to the molecular formula $C_{27}H_{40}O_3$ (calcd 413.3056), indicating 8 degrees of unsaturation. The IR spectrum showed absorption bands of hydroxyl (3445 cm^{-1}, br), double bond (3039 cm^{-1}) and α,*β*-α′,*β*′-unsaturated carbonyl (1658 cm^{-1}) groups. FABMS of **1** exhibited peak at *m*/*z* 395 $[M + H - H_2O]^+$, confirming the presence of a hydroxyl group in **1**.

The chemical structure of **1** was established (Figure 1) by X-ray diffraction analysis[10] since good quality crystals of **1** were obtained by slow crystallization in mother liquor. According to IUPAC sequence rule[11] the chiral center with the lowest locant, carbon-8, has the (*R*)-chirality, the relative stereochemistry of the eight chiral carbons was assigned as 8*R**,9*S**,10*S**,13*R**,14*S**,17*R**,20*S**,22*R**.

When the NMR spectra of compound **1** were measured in $CDCl_3$, six olefinic carbon signals were found in the ^{13}C NMR spectrum (see Table 1 column 2), two more than that determined by X-ray structural analysis, and the expected signal for the anomeric carbon was lacking. We supposed

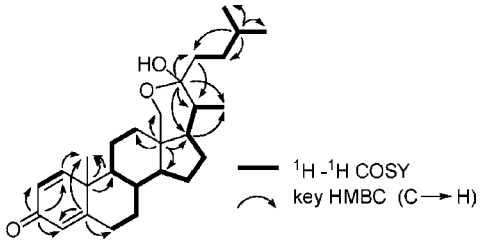


Figure 2. ^{1}H-^{1}H COSY, key HMBC for compound **1**.

that dehydration had happened during the process of NMR measurement. As we know, $CDCl_3$ might contain trace of HCl, related to the acidity of the solvent. To obtain complete NMR spectra of compound **1**, the spectra were measured in CD_3OD again, (Table 1, column 1). The ^{13}C NMR and DEPT spectra of **1** showed signals for four olifinc carbons, four methyl groups, nine methylene carbons, nine methine carbons, and five quaternary carbons including an anomeric carbon (δ_C 101.1 s) and a conjugated dienone group [δ_C 159.8 (d), 127.7 (d), 188.9 (s), 124.1 (d), 173.9 (s)]. These spectral data, coupled with 8 degrees of unsaturation, suggested that **1** was a tetracyclic compound with a conjugated dienone moiety and a hemiketal group.

Detailed analysis of the ^{1}H and ^{13}C NMR data associated with HSQC, HMBC spectra, all the signals of H and C could be assigned (Table 1 column 1). The correlations of ^{1}H-^{1}H COSY revealed three proton−proton networks, as depicted by the bold lines in Figure 2. Its HMBC spectrum showed many informative ^{1}H−^{13}C long-range correlations, such as C-1/H-19; C-3/H-2, H-1, H-4; C-5/H-4, H-6, H-7a, Me-19; C-10/H-1, H-9, Me-19; C-13/H-12, H-14, H-17, H-18b; and C-22/H-17, H-18, H-20, H-23, Me-21; etc. (Figure 2). Combination of the analysis led to the establishment of the

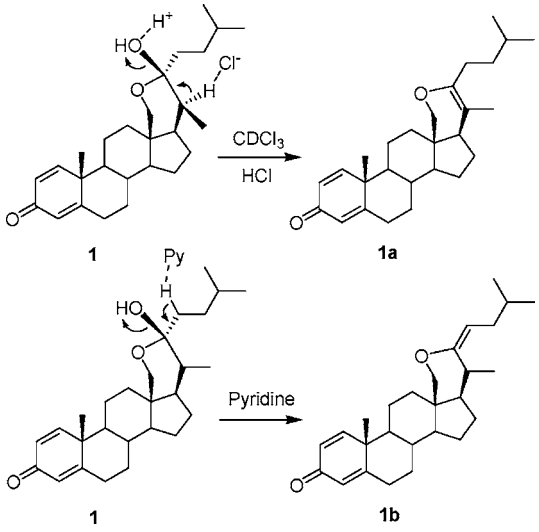


Figure 3. Dehydration reaction of **1** under weak acid or base condition.

(10) Crystallographic data for cladiellin A (**1**) (deposition no. CCDC 262864) have been deposited at the Cambridge Crystallographic Data Centre. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, U.K.; fax: +44 1223 336033.

(11) IUPAC Commission on Nomenclature of Organic Chemistry, **1974**. Recommendation for section E, fundamental stereochemistry. *Pure Appl. Chem.* **1976**, *45*, 11−15.

Table 1. NMR Data for Compound **1**, **1a**, and **1b***

	1				**1a**		**1b**	
No.	^{13}C	^{1}H (m. *J* in Hz)	^{1}H−^{1}H COSY	HMBC	^{13}C	^{1}H (m. *J* in Hz)	^{13}C	^{1}H (m. *J* in Hz)
1	159.8 d	7.38 d (10.5)	H-2	H-19	155.8 d	7.07 d (10.0)	156.2 d	7.00 d (9.8)
2	127.7 d	6.27 dd (10.5,1.5)	H-1		127.5 d	6.23 dd (10.0,1.5)	127.9 d	6.42 d (9.8,1.5)
3	188.9 s			H-1, 2, 4	186.3 s		186.1 s	
4	124.1 d	6.12 d (1.5)		H-6	123.9 d	6.07 d (1.5)	123.7 d	6.26 d (1.5)
5	173.9 s			H-1, 4, 6, 7a, 19	168.9 s		169.3 s	
6a	33.96 t	2.56 td (12.8, 3.8)	H-7	H-4, 7b	33.9 t	2.46 td (12.5, 4.0)	32.8 t	2.26 m
b		2.39 m	H-7b			2.36 m		2.17 m
7a	35.4 t	2.02 m	H-6a	H-6a	32.7 t	1.98 m	34.0 t	1.72 m
b		1.01 m	H-6, 8	H-6		1.05 m		0.84 m
8	36.8 d	1.55 m	H-7b, 9, 14	H-6b, 7b, 9, 14	35.4 d	1.55 m	35.6 d	1.33 m
9	54.7 d	1.11 m	H-8, 11	H-1, 11, 14, 19	52.6 d	1.15 m	53.1 d	0.98 m
10	45.7 s			H-1, 2, 4, 6b, 9, 19	43.6 s		44.0 s	
11	23.7 t	1.86 m	H-9, 12	H-9, 12	22.4 t	1.80 m	22.5 t	1.67 m
12a	33.4 t	2.26 m	H-11	H-11	31.4 t	2.06 m	32.5 t	2.44 m
b		0.90 m	H-11			0.90 m		0.90 m
13	43.7 s			H-11,12,14,15a,17,18b	41.2 s		43.0 s	
14	55.7 d	1.12 m	H-8, 15	H-9, 12b, 15	53.5 d	1.30 m	54.7 d	0.97 m
15a	25.6 t	1.67 m	H-14, 16a		24.5 t	1.72 m	24.9 t	1.60 m
b		1.40 m	H-14, 16			1.38 m		1.40 m
16a	24.3 t	2.41 m	H-15, 17	H-20	27.9 t	2.07 m	23.6 t	2.86 m
b		1.64 m	H-15b, 17			1.53 m		1.74 m
17	50.8 d	1.49 m	H-16a, 20	H-18b, 21	47.6 d	1.58 m	50.1 d	1.54 m
18a	58.4 t	3.91 d (11.3)		H-12b, 14, 17	63.6 t	3.95 dd (10.5; 2.0)	57.5 t	4.16 dd (11.3)
b		3.54 d (11.3)				3.35 dd (10.5; 1.5)		3.67 dd (11.3)
19	19.2 q	1.34 s		H-1, 9	18.7 q	1.23 s	18.9 q	1.05 s
20	34.01 d	1.88 m	H-17, 21	H-17, 21, 23a	106.0 s		33.5 d	2.06 m
21	14.8 q	0.94 d (7.5)	H-20	H-20	16.7 q	1.59 s	15.1 q	1.22 d (6.8)
22	101.1 s			H-17,18,20,21,23,24	146.3 s		150.1 s	
23a	39.6 t	1.68 m	H-24a	H-20, 24, 25	30.5 t	2.14 m	100.2 d	6.61 br s
b		1.52 m	H-24b			1.50 m		
24a	33.96 t	1.32 m	H-23a	H-25, 26, 27	36.8 t	1.34 m	39.4 t	2.02 m
b		1.19 m	H-23b, 25					1.93 m
25	29.8 d	1.48 m	H-24b, 26, 27	H-26, 27, 24	27.9 d	1.53 m	28.9 d	1.53 m
26	23.4 q	0.90 d (6.8)	H-25	H-24, 25, 27	22.5 q	0.90 d (7.0)	23.1 q	0.92 d (6.8)
27	23.0 q	0.90 d (6.8)	H-25	H-24, 25, 27	22.5 q	0.90 d (7.0)	23.1 q	0.92 d (6.8)

*Spectra of compound **1** were measured in CD_3OD (^{1}H, 750 MHz and ^{13}C, 188 MHz), compound **1a** were measured in $CDCl_3$ (^{1}H, 500 MHz and ^{13}C, 125 MHz), while compound **1b** were measured in pyridine-d_5 (^{1}H, 750 MHz and ^{13}C, 188 MHz).

structure of **1**, which accords with the structure determined by X-ray single-crystal analysis.

As mentioned above, compound **1a**, $[\alpha]^{20}_{D} = +9.4$ (*c* 1.0 MeOH), was obtained as white solid in the NMR tube when $CDCl_3$ was removed by N_2 flow. The molecular formula of **1a** was found to be $C_{27}H_{38}O_2$ by HREIMS data [$(M + H)^+$, 395.2956, calcd 395.2950] indicating 9 degrees of unsaturation. By comparison of the HREIMS data of compound **1** and **1a**, we supposed that compound **1** might convert to **1a** by dehydration under weak acid condition as shown in Figure 3. All the H and C signals had been assigned (Table 1 column 3). The ^{13}C NMR exhibited signals at δ_C 106.0 (s, C-20) and δ_C 146.3 (s, C-22) instead of δ_C 101.1 (s, C-22) and δ_C 34.0 (d, C-20) in **1** indicating the double bond generated located between C-20 and C-22. In the ^{1}H NMR spectrum, the 21-Me signal at δ_H 1.59 (s, 3H) showed no splitting and was in rather downfield than **1** [δ_H 0.94 (3H, d, $J = 7.5$ Hz, Me-21)], indicating C-21 was linked with an olefinic quaternary carbon. Thus, the position of the double bond generated could be determined as shown. It was reasonable that the dehydration was triggered by trace HCl in the $CDCl_3$ solvent under Zaitsev's rule (Figure 3). All the H and C signals of compound **1a** were assigned by the analysis of the ^{1}H, ^{13}C, ^{1}H−^{1}H COSY, HMQC, and HMBC data.

It is interesting that when the ^{13}C NMR spectrum of **1** was measured in pyridine-d_5, dehydration did happen immediately. As shown in Table 1, column 3, the ^{13}C NMR spectrum in pyridine-d_5 also exhibited six olefinic carbon signals as **1a**, but the chemical shifts were different. We considered that compound **1** might convert to another dehydrate product **1b** via another route **(**Figure 3). The specific optical rotation of **1b** was $[\alpha]^{20}_{D} = +7.6$ (*c* 1.0 MeOH). All the H and C signals had been assigned (Table 1 column 3). The ^{13}C NMR exhibited signals at δ_C 150.1 (s, C-22) and δ_C 100.2 (d, C-23) and the ^{1}H NMR showed

double split at Me-21 (δ_H 1.22, d, $J = 6.8$ Hz) indicating the double bond generated locate between C-22 and C-23.

The ORAC values of compounds **1**, **1a**, and **1b** showed 3.15, 4.78, and 5.17 units (1 μM of Trolox equiv)/0.31 μg/mL, respectively. It indicated **1b** is a better antioxidant or free radical scavenger than **1**, and **1a**.

Acknowledgment. This work was supported by the National High Technology Developing Project (2001AA620403) of China, the National Natural Science Foundation of China (29932030), and the Natural Science Foundation of Guangdong province, China (04300810). We thank Xiao-Long Feng of Sun Yat-Sen (Zhongshan) University for X-ray crystal structure analysis. We also thank Dr. Harukazu Fukami of the Institute for Health Care Science, Suntory, Ltd., for NMR spectra data obtained in CD_3OD and pyridine-d_5 solution.

Supporting Information Available: Experimental section and selected ^1H, ^{13}C, COSY, HSQC, HMBC, and TOCSY spectra for **1**, **1a**, and **1b**. X-ray data for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0477475