Chemistry of Natural Compounds, Vol. 46, No. 2, 2010

TWO NEW NATURAL KETO-ACID DERIVATIVES FROM *Sargassum pallidum*

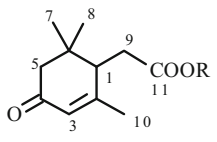
Chang-Yun Wang,[1*] Xin Liu,[1] Li-Min Guo,[1] Chang-Lun Shao,[1] Yu-Chun Fang,[1] Yu-Xi Wei,[2] Cai-Juan Zheng,[1] Qian-Qun Gu,[1] Wei-Ming Zhu,[1] and Hua-Shi Guan[1]

UDC 547.594.3+547.596

Sargassum pallidum (Turn.) C. Ag., a kind of brown algae distributed mainly in the Shandong and Liaoning Provinces of China, has been used in folk medicine for a long time in China [1]. Only several polysaccharides from *S. pallidum* with antitumor and antioxidant activities have been reported [2]. However, there are no reports on phytochemical analysis of the secondary metabolites.

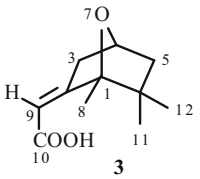
In this paper, two new natural keto-acid derivatives, 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-acetic acid (**1**) and 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-acetic acid methyl ester (**2**), were reported from the whole plant of *S. pallidum* (Turn.) C. Ag.. Their structures were established by comprehensive analysis of the spectral data, especially 2D NMR spectral results. Compound **3** was isolated for the first time from the marine enviroment.

Compound **1** was obtained as a yellow oil, $[\alpha]_D^{21}$ –7.96° (*c* 9.15, MeOH). The molecular formula of **1** was established as $C_{11}H_{16}O_3$ from the results of ESI-MS ($[M + H]^+$ at *m*/*z* 197) and its NMR data. The molecular formula indicated four degrees of unsaturation within the molecule. The IR spectrum showed the presence of carbonyl groups (1653, 1651 cm^{-1}). The ^{13}C NMR and DEPT spectra showed that **1** has 11 carbon signals, attributable to 3 methyl, 2 methylene, 2 methine, and 4 quaternary carbons, including 1 ketone carbonyl at δ_C 202.1 and 1 carboxyl at δ_C 180.0. The ^1H NMR spectrum together with HMQC data displayed one olefinic proton signal at δ_H 5.79 (s), one triple signal due to one methine proton at δ_H 2.70, two methylene group signals at δ_H 2.02, 2.40 and δ_H 2.17, 2.49, and three methyl group signals at δ_H 1.03 (s, CH_3), 1.04 (s, CH_3), 2.03 (s, CH_3). The whole structure of **1** was mainly determined by the HMBC spectra (Table 1). The correlations from H-9 proton to C-1, C-2, C-6, C-11 and the correlations from H-1 to C-2, C-3, C-6, C-9, C-11 suggested that C-1 was directly connected with C-2, C-6, and C-9. The correlations from H-5 to C-1, C-3, C-4, C-6, C-7, C-8, and the correlations from H-7, H-8 to C-1, C-5, C-6 indicated that C-5 was linked with C-4 and C-6. The correlations from H-3 to C-1, C-2, C-10 and the correlations from H-5 to C-3 confirmed that there were linkages between the C-3 and C-2, C-4 positions. Thus protons of 10-CH_3 showed the HMBC correlations to the C-1, C-2 and C-3, placing the methyl group at the C-2 position. Thus, the structure of compound **1** was identified as 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-acetic acid. As is known, compound **1** has not been reported previously as a natural product and was only obtained as a synthetic compound [3, 4].



1, 2

1: R = H; **2:** R = CH_3



3

1) Key Laboratory of Marine Drugs, Ministry of Education, the school of Medicine and Pharmacy, Ocean University of China, Qingdao 266003, China, e-mail: changyun@ouc.edu.cn; 2) School of Medicine, Qingdao University, Qingdao 266071, China. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 246–247, March–April, 2010. Original article submitted November 24, 2008.

 0009-3130/10/4602-0292

TABLE 1. NMR Data for Compound **1** (600 and 150 MHz, in CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC
1	2.70 (1H, t, J = 6.0)	50.0	2, 3, 6, 9, 11
2	–	169.1	–
3	5.79 (1H, s)	125.7	1, 2, 10
4	–	202.1	–
5	2.02 (1H, d, J = 17.0)	48.3	1, 3, 4, 6, 7, 8
	2.40 (1H, d, J = 17.0)		
6	–	37.3	–
7	1.03 (3H, s)	26.3	1, 5, 6, 8
8	1.04 (3H, s)	28.4	1, 5, 6, 7
9	2.17 (1H, dd, J = 15.6, 6.0)	38.2	1, 2, 6, 11
	2.49 (1H, dd, J = 15.6, 6.0)		
10	2.03 (3H, s)	24.0	1, 2, 3
11	–	180.0	–

Compound **2**, isolated as a yellow oil, gave a $[M + H]^+$ ion at *m*/*z* 211 by ESI-MS, thereby establishing the molecular formula of **2** as $C_{12}H_{18}O_3$. The ^{13}C NMR and DEPT spectra showed that **2** had 12 carbon signals, attributable to 3 methyl, 1 methoxyl, 2 methylene, 2 methine, and 4 quaternary carbons. The ^1H and ^{13}C NMR spectra confirmed that compound **2** has a similar structure as **1** but with one more methoxyl group. Thus, the difference between these two structures is that there is one methyl ester group in compound **2**. According to the above data, compound **2** was determined to be 2,6,6-trimethyl-4-oxo-2-cyclohexene-1-acetic acid methyl ester. To the best of our knowledge, this is the first report of **2** as a natural product, though the compound has previously been synthesized [4].

Compound **3** was isolated as a white powder. Its structure was identified as ficusic acid by comparison of the spectroscopic data with those in the literature [5]. This is the first report on ficusic acid isolated from the marine material.

The antitumor bioassay tests indicated that compounds **1**–**3** showed no cytotoxic activities against tumor cell lines, including K562 and P388.

Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. IR (KBr) spectra were obtained on a Nicolet Nexus 470 infrared spectrophotometer. ^1H, ^{13}C NMR, and 2D NMR spectra were recorded on a JEOLJNM-ECP600 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on a Micromass Q-TOF spectrometer for ESI-MS. Silica gel (200–300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qingdao Marine Chemical Factory, Qingdao, China.

Plant Material. The Plant was collected in 2003 from the Yellow Sea, near Weihai, Shandong Province, China. The species was identified by Prof. Baoren Lu of the Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen was deposited in the Key Laboratory of Marine Drugs, Ministry of Education, School of Medicine and Pharmacy, Ocean University of China.

Extraction and Separation of Metabolites. The air-dried whole plant of *S. pallidum* (5.3 kg) was cut and extracted with 70% EtOH four times at room temperature. The ethanolic extract was evaporated to dryness, suspended in water, and partitioned successively with cyclohexane, ethyl acetate, and *n*-butanol. The extracts were then concentrated under reduced pressure. The ethyl acetate extract was subjected to silica gel column chromatography eluting with petroleum–acetone (100:0 to 0:100) to yield five fractions (fractions B1–B5). Fraction B2 was chromatographed on Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) and then purified by reversed phase HPLC (MeOH–H_2O, 1:1) to afford **3** (6.0 mg), **1** (18.3 mg) and **2** (11.4 mg).

2,6,6-Trimethyl-4-oxo-2-cyclohexene-1-acetic acid (1): yellow oil, $[\alpha]_D^{21}$ –7.96° (*c* 9.15, MeOH). IR spectrum (KBr, ν, cm^{-1}): 3418 (OH), 2964, 2730, 2494, 1653 (C=O), 1651 (C=O), 1561, 1455, 1386, 1304, 1057, 787, 683. Mass spectrum (ESI-MS^+ *m*/*z*, I_{rel}, %): $[M + H]^+$ 197 (100).

2,6,6-Trimethyl-4-oxo-2-cyclohexene-1-acetic acid methyl ester (2): yellow oil, $[\alpha]_D^{21}$ –0.51° (*c* 5.70, MeOH). ^1H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.66 (1H, t, J = 6.9, 5, H-1), 5.86 (1H, s, H-3), 2.11 (1H, d, J = 17.0, H-5), 2.31 (1H, d, J = 17.0, H-5), 0.97 (3H, s, H-7), 1.07 (3H, s, H-8), 2.29 (1H, dd, J = 16.5, 4.6, H-9), 2.59 (1H, dd, J = 16.4, 6.9, H-9), 1.96 (3H, s, H-10), 3.73 (3H, s, H-12). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 47.0 (C-1), 162.8 (C-2), 126.1 (C-3), 198.7 (C-4), 47.2 (C-5), 36.1 (C-6), 25.8 (C-7), 28.0 (C-8), 33.9 (C-9), 23.4 (C-10), 173.2 (C-11), 52.1 (C-12).

Ficusic acid (3): white powder, $[\alpha]_D^{21}$ +47.930 (*c* 1.00, MeOH). Mass spectrum (ESI-MS$^+$ *m*/*z*, I_{rel}, %): $[M + H]^+$ 197 (100). ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.54 (1H, ddd, J = 11.9, 3.7, 2.3, H-3), 2.04 (1H, ddd, J = 12.8, 4.1, 2.3, H-3), 4.14 (1H, m, H-4), 1.51 (1H, t, J = 11.9, H-5), 1.33 (1H, t, J = 12.4, H-5), 1.59 (3H, s, H-8), 5.72 (1H, s, H-9), 1.27 (3H, s, H-11), 1.32 (3H, s, H-12). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 86.4 (C-1), 171.5 (C-2), 49.8 (C-3), 65.1 (C-4), 47.9 (C-5), 35.0 (C-6), 30.0 (C-8), 113.3 (C-9), 180.7 (C-10), 25.1 (C-11), 25.6 (C-12).

ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation of China (No. 30572314; 40776073), the Program for New Century Excellent Talents in Universities of the Ministry of Education of China (No. NCET-05-0600), the Basic Research Program of Science and Technology, Ministry of Science and Technology of China (No. 2007FY210500), the Cultivation Fund of the Key Scientific and Technical Innovation Project of the Ministry of Education of China (No. 706038), and the Science and Technology Program of Shandong Province, China (No. 03BS109). We wish to thank Prof. Baoren Lu for identification of the plant material.

REFERENCES

1. Editorial Board of China Herbal, State Administration of Traditional Chinese Medicine, *China Herbal*, Shanghai Science and Technology Press, Shanghai, **1**, 463 (1999).
2. H. Ye, K. Q. Wang, C. H. Zhou, J. Liu, and X. X. Zeng, *Food Chem.*, **111**, 428 (2008).
3. S. Shibata, H. Matsushita, K. Kato, H. Kaneko, M. Noguchi, M. Saburi, and S. Yoshikawa, *Agric. Biol. Chem.*, **45**, 315 (1981).
4. W. Takayuki, *Chem. Pharm. Bull.*, **13** (1), 43 (1965).
5. Y. C. Li and Y. H. Kuo, *Phytochemistry*, **49**, 2417 (1998).