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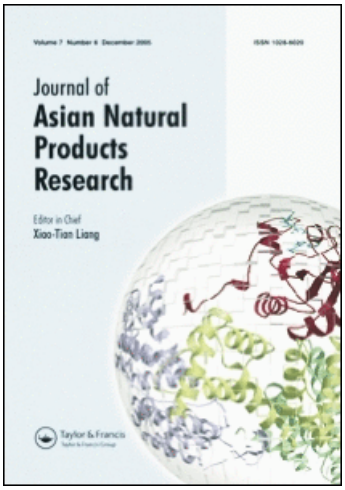

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X. -P. Huang[a]; Z. -W. Deng[b]; L. V. Ofwegen[c]; J. Li[d]; H. -Z. Fu[d]; X. -B. Zhu[e]; W. -H. Lin[d]
[a] Graduate School of the Chinese Academy of Science, Beijing, China [b] Test and Analytic Centre, Beijing Normal University, Beijing, China [c] National Museum of Natural History Naturalis, RA Leiden, The Netherlands [d] State Key Laboratories of Natural and Biomimetic drugs, Peking University, Beijing, China [e] Institute of Oceanology, The Chinese Academy of Science, Qingdao, China

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Two new pregnane glycosides from soft coral *Cladiella krempfi*

X.-P. HUANG†‡¶, Z.-W. DENG§, L. V. OFWEGEN‖, J. LI‡, H.-Z. FU‡, X.-B. ZHU† and W.-H. LIN‡

†Institute of Oceanology, The Chinese Academy of Science, Qingdao 266071, China
‡State Key Laboratories of Natural and Biomimetic drugs, Peking University, Beijing 100083, China
¶Graduate School of the Chinese Academy of Science, Beijing 100039, China
§Test and Analytic Centre, Beijing Normal University, Beijing 100073, China
‖National Museum of Natural History Naturalis, 2300, RA Leiden, The Netherlands

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Two new pregnane-type steroidal glycosides, pregna-5,20-dien-3-*O*-α-fucopyranoside (**1**) and pregna-20-en-3-*O*-α-fucopyranoiside (**2**), along with two known pregnane derivatives, were isolated from the soft coral *Cladiella krempfi*. Their structures were determined by spectroscopic data analysis.

Keywords: Soft coral; *Cladiella krempfi*; Pregna-5,20-dien-3-*O*-α-fucopyranoside; Pregna-20-en-3-*O*-α-fucopyranoiside

1. Introduction

The genus *Cladiella* of the soft corals are widely distributed in tropical and subtropical shallow-water habitats. Previous chemical investigation on this genus led to the isolation of members of structural diversity metabolites, involving cembranes [1,2], eunicellane diterpenes [3–12], sesquiterpenes [13], steroids and glycolipids [14–17]. Pregnane-type steroids are a class of natural products mainly occurring in soft corals, and various plant species mostly belonging to the Asclepiadaceae family [19]. Zheng *et al.* have isolated two anti-tumour active pregnane glycosides from *Cladiella krempfi* [16] and two pregnenones from *Cladiella* sp. [17]. In our continuation to search for bioactive novel metabolites from Chinese marine organisms, the soft coral *C. krempfi* was collected by SCUBA from Hainan Island in the South China Sea. From the MeOH extract, two new pregnane glycosides (**1**, **2**), together with two known pregnane derivatives, pregna-1,20-dien-3-one (**3**) and 1,4,20-pregnatien-3-one (**4**), were isolated, respectively. In this paper, we report the structural elucidation of compounds **1** and **2**.

*Corresponding author. E-mail: whlin@bjmu.edu.cn

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2. Results and discussion

The molecular formula of **1** (figure 1) was established as $C_{27}H_{42}O_5$ on the basis of HRFAB-MS, indicating seven degrees of unsaturation. The ^{1}H NMR spectrum displayed the signals for three methyls at δ 0.58 (3H, s), 0.97 (3H, s) and 1.06 (3H, d, $J = 6.5$ Hz); four olefinic protons at δ 5.33 (1H, br), 5.77 (1H, ddd, $J = 7.7$, 11.7 and 17.4 Hz), 4.99 (1H, brd, $J = 11.7$ Hz), and 4.97 (1H, brd, $J = 17.4$ Hz); and six oxygenated methine protons between δ 3.29–4.74 (m), along with three D_2O exchangeable protons at δ 4.44 (1H, d, $J = 5.0$ Hz), 4.36 (1H, d, $J = 4.5$ Hz) and 4.22 (1H, d, $J = 6.5$ Hz). The ^{13}C NMR and DEPT spectra exhibited 27 carbons including three methyls, nine methylenes, 12 methines, and three quaternary carbons. The ^{1}H NMR and ^{13}C NMR spectral data of **1** were characteristic of a steroidal glycoside, of which the aglycon was identical to 3-*O*-5,20-dien-pregnene based on a detailed 2D NMR elucidation and by comparison of the NMR data with those already reported [18]. The partial structure of sugar moiety was accomplished by COSY and HMBC spectra. The DQF-COSY correlations between H-1′ (δ 4.74)/H-2′ (δ 3.51), H-2′/H-3′ (δ 3.48), H-3′/H-4′ (δ 3.46), H-4′/H-5′ (δ 3.85), and H-5′/H-6′ (δ1.06) implied a 6-deoxy-hexose unit. The J value between H-2′/H-3′ (10.0 Hz) indicated the axial-orientation for both protons, and J value (3.5 Hz) for H-3′/H-4′ enabled to assign an equatorial for H-4′. The configuration of H-5′ was determined as an axial due to the NOESY correlation between H-3′/H-5′ (figure 2). Thus, the sugar moiety was concluded to be a fucopyranose. The small coupling constant of the anomeric proton H-1′ (δ 4.74, d, $J = 2.9$ Hz) confirmed it as an equatorial proton, which was clarified to annex to C-3 from the HMBC correlations between H-1′/C-3 (δ 76.47, d) and H-3 (δ 3.29, m)/C-1′ (δ 97.67, d). Additionally, the relative configuration of H-3 was assigned to be in axial orientation (α-configuration) according to the J value (12.0 Hz) of H-3/H-2β. Thus, the structure of **1** was determined as pregna-5,20-dien-3-*O*-α-fucopyranoside.

The molecular formula ($C_{27}H_{44}O_5$) of **2** (figure 1) was determined by HRFAB-MS, 2 mass units higher than that of **1**. The ^{1}H NMR and ^{13}C NMR data of **2** closely resembled those of **1**, and was in good agreement with those of **1** in the sugar moiety (table 1), indicating that both **2** and **1** possessed the same sugar unit. The ^{13}C NMR and DEPT spectra of **2** differed from those of **1** in that the signals assigned for the double bond C-5/C-6 of **1** were replaced by a methylene at δ 28.35 (t) and a methine at δ 44.15 (d) of **2**. The HMBC correlations of H-19 (δ 0.77, s, 3H) to δ 36.62 (t, C-1), 35.29 (s, C-10), 53.96 (d, C-9) and 44.15 (d, C-5),

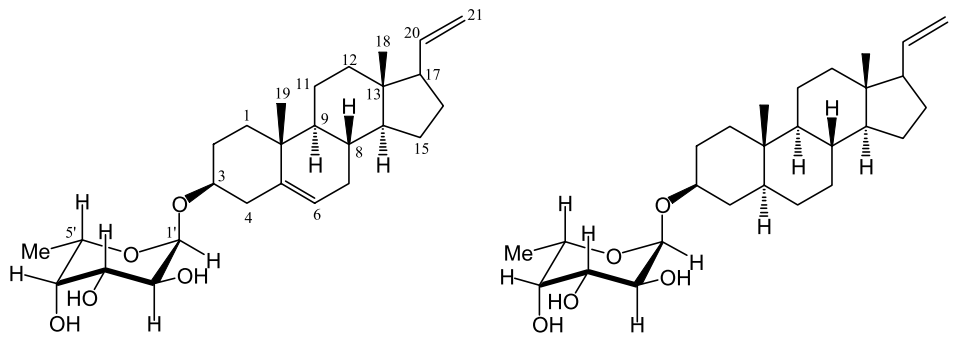

Figure 1. The structures of **1** and **2**.

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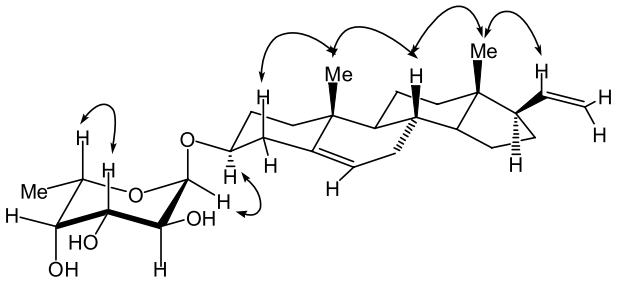

Figure 2. The key NOE correlations of **1**.

confirmed that **2** was a 5,6-dihydro-derivative of **1**. Therefore, the structure of **2** was determined as pregna-20-en-3-*O*-α- fucopyranoside.

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3. Experimental

3.1 General experimental procedures

Melting points were measured on a XT-4A-micromelting point apparatus without correction. Optical rotations were measured on a Perkin-Elmer 243B Polarimeter using a sodium lamp

Table 1. ^{1}H NMR and ^{13}C NMR data of **1** and **2** in DMSO-d_6

	1		***2***	
	δ_C	δ_H	δ_C	δ_H
1	36.88, t	1.80, m; 1.04, m	36.62, t	1.62, m; 1.10, m
2	29.30, t	1.76, m; 1.48, m	29.08, t	1.74, m; 1.24, m
3	76.47, d	3.29, m	75.67, d	3.35, m
4	38.24, t	2.31, dd, 12.5, 2.0 2.13, dd, 12.5, 12.0	33.98, t	1.54, m
5	140.61, s		44.15, d	1.17, m
6	121.05, d	5.33, m	28.35, t	1.25, m
7	31.53, t	1.93, m; 1.39, m	31.75, t	1.64, m; 1.37, m
8	31.47, d	1.50, m	35.11, d	1.66, m
9	49.89, d	0.91, m	53.96, d	0.98, m
10	36.96, s		35.29, s	
11	20.23, t	1.36, m; 1.54, m	20.35, t	1.37, m; 1.54, m
12	36.39, t	1.60, m; 1.05, m	37.07, t	1.63, m
13	43.03, s		43.24, s	
14	55.27, d	0.98, m	54.72, d	0.97, m
15	24.49, t	1.16, m; 1.61, m	24.38, t	1.12, m; 1.70, m
16	26.51, t	1.70, m; 1.53, m	26.54, t	1.54, m; 1.24, m
17	54.67, d	1.94, m	55.04, d	1.95, m
18	12.68, q	0.58, s	12.08, q	0.55, s
19	19.16, q	0.97, s	12.82, q	0.77, s
20	139.54, d	5.77, ddd, 7.7, 11.7, 17.4	139.50, d	5.76, m
21	115.01, t	4.99, brd, 11.7 4.97, brd, 17.4	114.87, t	4.98, m; 4.96, m
1′	97.67, d	4.74, d, 2.9	97.35, d	4.73, br
2′	69.63, d	3.51, dd, 2.9, 10.0[a]	69.64, d	3.51, m
3′	67.95, d	3.48, dd, 3.5, 10.0[a]	67.95, d	3.49, m
4′	71.67, d	3.46, brd, 3.5[a]	71.66, d	3.46, m
5′	66.00, d	3.85, brq, 6.5[a]	65.89, d	3.84, m
6′	16.55, q	1.06, d, 6.5	16.52, q	1.07, d, 6.5

[a] Coupling constants were measured after adding a trace of D_2O

operating at 589 nm. The ^{1}H NMR and ^{13}C NMR experiments were carried out on Brucker Avance 500X and Varian INOVA 500 spectrometers, and chemical shifts were referenced to the solvent (DMSO-d_6) signals as internal standard. HRFAB mass spectra were measured on Brucker FTICR APEXII mass spectrometer. Column chromatography was carried with silica gel (200–300 mesh), and HF254 silica gel for TLC was obtained from Qingdao Marine Chemistry Co. Ltd., Qingdao, China. Sephadex LH-20 (18–110 μm) was provided by Pharmacia.

3.2 Animal material

Soft coral *Cladiella krempfi* was collected by SCUBA in the inner reef of Hainan Island, in November 2003. A voucher specimen (HSE-7A) is deposited in the National Research Laboratories of Natural and Biomimetic Drugs, Peking University. The soft coral species was identified by Dr Leen van Ofwegen at the Institute of Systematic Population Biology, Amsterdam University, the Netherlands.

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3.3 Extraction and isolation

The soft coral (1.87 kg, wt.) was homogenised and then extracted with MeOH. The MeOH extract was concentrated *in vacuo*, and partitioned between H_2O and petroleum ether, EtOAc and n-BuOH, successively. The petroleum ether extract (5.0 g) was subjected to silica gel column chromatography eluting with petroleum ether-acetone as a gradient. Fraction 1 (70 mg) collected from the eluent (5:1), was chromatographed on silica gel column and eluted with petroleum ether-acetone (20:1) to afford compounds **3** (30 mg) and **4** (4.0 mg). Fraction 7 (50 mg) eluted by petroleum ether-acetone (1:1), was separated on a Sephadex LH-20 column with 99.5% aqueous MeOH as eluent to yield compounds **1** (8.0 mg) and **2** (1.1 mg).

3.3.1 Pregna-5,20-dien-3-*O*-α-fucopyranoside (1). White amorphous solid, mp 198–200°C, $[\alpha]_D^{25}$ -72.5 (*c* 0.52, MeOH). IR (KBr) 3452 (br), 2944, 1635, 1436, 1378, 1169, 1135, 1105, 1082, 1031 cm^{-1}. ^{1}H NMR and ^{13}C NMR data, see table 1. HRFAB-MS *m/z* 469.2919 $[M + Na]^+$ (calcd for $C_{27}H_{42}O_5Na$, 469.2924).

3.3.2 Pregna-20-en-3-*O*-α-fucopyranoside (2). White amorphous solid, mp 270–272°C, $[\alpha]_D^{25}$ -16.36 (*c* 0.32, MeOH). IR (KBr) 3419 (br), 2918, 2849, 1539, 1465, 1108, 1006 cm^{-1}. ^{1}H NMR and ^{13}C NMR data, see table 1. HRFAB-MS *m/z* 471.30801 $[M + Na]^+$ (calcd for $C_{27}H_{44}O_5Na$, 471.3073).

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References

[1] C.A. Gray, M.T. Davies-Coleman, M.H. Schleyer. *J. Nat. Prod.*, **63**, 1551 (2000).
[2] M.Y. Wang, Y. Luo, J.Y. Su. *Chin. Chem. Lett.*, **11**, 783 (2000).
[3] R. Kazlauskas, P.T. Murphy, R.J. Wells, P. Schueholzer. *Tetrahedron. Lett.*, 4643 (1977).
[4] J.E. Hochlowski, D.J. Faulkner. *Tetrahedron. Lett.*, **21**, 4055 (1980).
[5] K. Yamada, N. Ogata, K. Ryu, T. Miyamoto, T. Komori, R. Higuchi. *J. Nat. Prod.*, **60**, 393 (1997).
[6] Y. Uchio, M. Nakatani, T. Hase, M. Kodama, S. Usui, Y.A. Fukazawa. *Tetrahedron Lett.*, **30**, 3331 (1989).
[7] Y. Uchio, M. Kodama, S. Usui, Y. Fukazawa. *Tetrahedron Lett.*, **33**, 1317 (1992).
[8] J.K. Liu, L.M. Zeng, D.G. Wu. *Chin. Sci. Bull*, **37**, 1627 (1992).
[9] N.S. Sarma, R. Chavakula, I.N. Rao, R. Kadirvelraj, T.N.G. Row, I. Saito. *J. Nat. Prod.*, **56**, 1977 (1993).
[10] D.S. Rao, C. Sreedhara, D.V. Rao, C.B. Rao. *J. Chem. Sect. B*, **33**, 198 (1994).
[11] J. Liu, L. Zeng, Y. Huang. *Zhongshan Daxue Xuebao Ziran Kexueban*, **32**, 131 (1993).
[12] C.B. Rao, D.S. Rao, C. Satyanaraayana, D.V. Rao, K.E. Kassuhlke, D.J. Faulkner. *J. Nat. Prod.*, **57**, 574 (1994).
[13] A. Ata, J. Ackerman, P. Radhika. *Tetrahedron. Lett.*, **44**, 6951 (2003).
[14] N.S. Sarma, R. Chavakula, S.R. Rao. *Indian J. Chem. Sect. B*, **34**, 264 (1995).
[15] V. Anjaneyulu, P.V. Subba, P. Radhika, H. Laatsch, R.N. Asolkar. *Indian J. Chem. Sect. B*, **40**, 405 (2001).
[16] W. Lan, C.W. Lin, J.Y. Su, L.M. Zeng. *Chem. J. Chin. Univ*, **24**, 2019 (2003).
[17] G.W. Zhang, X. Ma, L. Zeng, J. Su. *Chin. J. Appl. Chem*, **20**, 1021 (2003).
[18] O.M. Coebar, A.D. Rodriguez, O.L. Padilla. *J. Nat. Prod.*, **60**, 1186 (1997).
[19] D. Deepak, S. Srivastav, A. Khare. *Prog. Chem. Nat. Prod.*, **71**, 169 (1997).

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