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Nanjiols D and E, Two New Uncommon Steroids from the Chinese Soft Coral *Nephthea bayeri*

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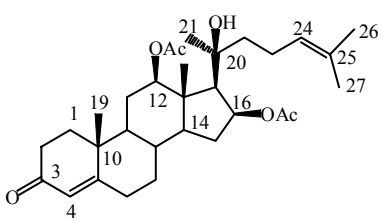
Abstract: Two new steroids, nanjiols D and E, were isolated from the soft coral *Nephthea bayeri.* Their structures were characterized by spectroscopic methods and comparison with known compounds.

Keywords: Soft coral, *Nephthea bayeri*, steroid.

Marine organisms have been found to be a storehouse of steroids, particularly in term of unique side-chain structures and unusual functionalization. Marine steroids are often found in highly oxygenated forms and possessing various biological activities[1]. Previously, we reported the isolation and structural elucidation of three new marine steroids, nanjiols A-C, which showed cytotoxicity against HL-60 and BEL 7402 cell lines, from a soft coral *Nephthea bayeri* in East China Sea[2]. In continuation of our search for minor steroid congener and biologically active compounds, we recently reexamined the soft coral *N. bayeri* collected from the Nanji Island, Zhejiang Province, during June 2003. This work has resulted in the isolation of two new steroids, named nanjiols D (**1**) and E (**2**), in addition to those previously reported[2]. This paper describes the isolation and structural determination of these new compounds.

The usual work-up[2] of the Et_2O soluble fraction of the acetone extract of the soft coral yielded the new compounds **1** (1.4 mg) and **2** (1.8 mg), respectively.



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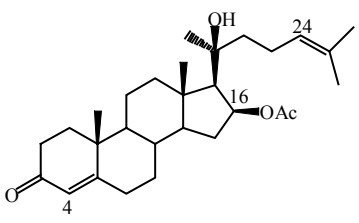

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Table 1 ^{1}H-, ^{13}C-NMR data of compounds **1** and **2** ($CDCl_3$, δ ppm)[a]

No	**1**		**2**	
	δ_H mult., *J* in Hz	δ_C mult.	δ_H mult., *J* in Hz	δ_C mult.
1	1.99, 1.72 (m)	35.6 (t)	2.03, 1.70 (m)	35.6 (t)
2	2.38, 1.60 (m)	33.8 (t)	2.40, 1.05 (m)	33.9 (t)
3	-	198.9 (s)	-	199.5 (s)
4	5.73 (s)	124.4 (d)	5.72 (s)	123.9 (d)
5	-	169.4 (s)	-	170.8 (s)
6	-	32.5 (t)	2.30 (m)	32.6 (t)
7	-	30.9 (t)	1.80 (m)	31.7 (t)
8	2.37 (m)	33.6 (d)	1.62 (m)	34.5 (d)
9	1.09 (m)	51.9 (d)	-	53.6 (d)
10	-	38.3 (s)	-	38.6 (s)
11	1.41, 1.93 (m)	26.8 (t)	-	20.7 (t)
12	4.64 (dd, 10.8, 4.8)	79.2 (d)	2.24 (m)	39.9 (t)
13	-	46.7 (s)	-	43.9 (t)
14	0.97 (m)	52.8 (d)	0.97 (m)	53.8 (d)
15	2.50, 1.30 (m)	34.2 (t)	2.51, 1.22 (m)	35.0 (t)
16	5.25 (m)	75.7 (d)	5.35 (m)	77.7 (d)
17	1.62 (m)	65.6 (d)	1.53 (m)	59.9 (d)
18	1.22 (s)	11.3 (q)	1.16 (s)	14.6 (q)
19	1.18 (s)	17.2 (q)	1.19 (s)	17.4 (q)
20	-	74.8 (s)	-	76.5 (s)
21	1.21 (s)	26.8 (q)	1.28 (s)	26.3 (q)
22	1.73 (m)	41.0 (t)	-	43.8 (t)
23	2.06 (m)	22.9 (t)	1.98 (m)	23.1 (t)
24	5.11 (brt, 7.0)	124.7 (d)	5.07 (brt, 7.0)	124.1 (d)
25	-	131.7 (s)	-	132.0 (s)
26	1.60[b] (s)	17.6[d] (q)	1.59[f] (s)	17.6[g] (q)
27	1.70[b] (s)	25.7[d] (q)	1.67[f] (s)	25.6[g] (q)
12-OCO**CH$_3$**	2.06[c] (s)	21.4[e] (q)	-	-
12-O**CO**CH$_3$	-	170.4 (s)	-	-
16-OCO**CH$_3$**	2.07[c] (s)	21.7[e](q)	2.09 (s)	21.6 (q)
16-O**CO**CH$_3$	-	169.2 (s)	-	169.5 (s)

[a] δ values are reported in ppm referenced to TMS as internal standard. ^{1}H and ^{13}C assignments were based on 2D NMR and comparison with model compounds.
[b-g] The resonances with the same superscript may be reversed.

Nanjiol D (**1**)[3] was obtained as an amorphous powder. Its molecular formula, $C_{31}H_{46}O_6$, was deduced from its HRESIMS (*m/z* 537.3224 $[M+Na]^+$, calcd. 537.3192). A comparison of overall ^{1}H- and ^{13}C-NMR data revealed similarities between compound **1** and co-occurring nanjiol B. In fact, compound **1** differs from nanjiol B only in the side chain. The presence of an olefin in side chain, instead of two saturated carbons**,** was evident by the peak at δ 5.11 (brt, 7.0 Hz) in its ^{1}H-NMR spectrum and signals at δ

124.7 (d) and 131.7 (s) in its ^{13}C-NMR spectrum. $^{2}J_{CH}$ HMBC cross-peaks of H_3-26/C-25 and H_3-27/C-25, as well as $^{3}J_{CH}$ HMBC cross-peaks of H_3-26/C-24 and H_3-27/C-24, allowed the unambiguous location of the olefin at $\triangle^{24}$. Comparison of ^{1}H- and ^{13}C-NMR data of side chain of **1** with those of solasteroside A[4] further confirmed the structure of side chain. Finally, the absolute configuration at C-20 was confirmed to be the same as that of nanjiol B by the significant NOE cross-peak for Me-21 and H_{eq}-12, and comparison of ^{13}C-NMR data of compound **1** with those of nanjiol B [2,5], showing almost identical chemical shift values for C-17, C-20 and C-21. Compound **1** was therefore established as (20*S*)-12β, 16β, 20-trihydroxycholesta-4, 24-diene-3-one 12, 16-diacetate.

Nanjiol E (**2**)[6] had a molecular formula of $C_{29}H_{44}O_4$ as determined by HRESIMS (*m/z* 479.3161$[M+H]^+$, calcd. 479.3137), two mass units less than that of nanjiol C. In a similar manner, the ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of nanjiol C, suggesting the presence of an α, β-unsaturated system in the A ring and an β-acetoxyl at C-16, while the side chain was the same as that of **1** possessing also a double bond at $\triangle^{24}$. Therefore, compound **2** is the 24, 25-didehydro derivative of nanjiol C.

Compounds **1** and **2** were tested for the anti-tumor activity against HL-60, BEL 7402 and A-549 cell lines. In the bioassay, compound **1** showed weak cytotoxicity toward the growth of HL-60 cells while **2** was found inactive against all three cell lines. Other bioassays, such as anti fungi, anti hCOX-2 *etc*, for these compounds are currently ongoing. Further study should be conducted to understand the biosynthesis and biological role of these metabolites in the lift cycle of the soft coral.

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References and Notes

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2. Z. Y. Shao, D. Y. Zhu, Y. W. Guo, *J. Nat. Prod.*, **2002**, *65*, 1675.
3. Spectral data of compound **1**: $[\alpha]^{25}_{D}$+ 125 (*c* 0.20, $CHCl_3$); IR ν(KBr) cm^{-1}: 3477, 2962, 1733, 1673, 1259, 1027, 798; UV λ_{max} (MeOH): 240.0 nm (log ε 4.23); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm): see **Table 1**; ^{13}C-NMR ($CDCl_3$, 100MHz): see **Table 1**.
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6. Spectral data of compound **2**: $[\alpha]^{25}_{D}$+ 66 (*c* 0.20, $CHCl_3$); IR ν(KBr) cm^{-1}: 3417, 2923, 1739, 1664, 1228, 1026, 756; UV λ_{max} (MeOH): 240.5 nm (log ε 4.18); ^{1}H-NMR ($CDCl_3$, 400 MHz, δ ppm): see **Table 1**; ^{13}C-NMR($CDCl_3$, 100MHz): see **Table 1**.

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