Nanjiols A−C, New Steroids from the Chinese Soft Coral *Nephthea bayeri*

Zhi-Yu Shao, Da-Yuan Zhu, and Yue-Wei Guo*

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, People's Republic of China

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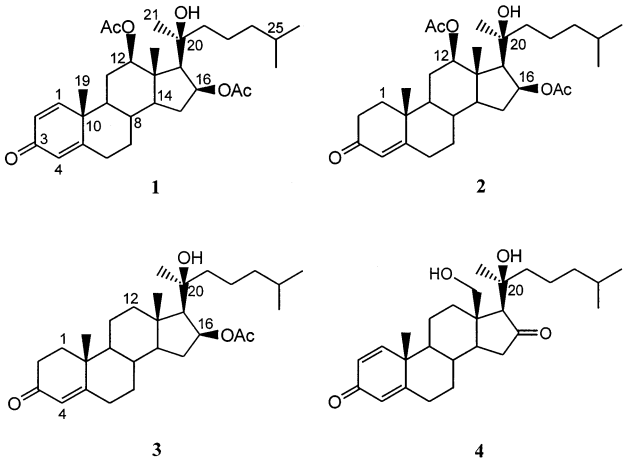
Three new polyoxygenated steroids, nanjiol A (**1**), B (**2**), and C (**3**), were isolated from an East China Sea soft coral *Nephthea bayeri*, and their structures were characterized by spectroscopic methods and comparison with known compounds. The new molecules were structurally related to (20*S*)-cholesta-1,4-diene-18,20-diol-3-one (**4**), a typical metabolite from the black coral *Antipathes subpinnata*.

Soft corals (phylum Coelenterata) are a rich source of various sesqui- and diterpenoids and polyoxygenated steroids with interesting biological activitives.[1−3] In the course of our research for discovering biologically active substances from marine organisms, a sample of the alcyonacean *Nephthea bayeri* Verseveldt (Nephtheidae) collected off Nanji Island, China, was investigated. Three novel uncommon sterols, named nanjiols A−C (**1**−**3**), together with six known compounds, have been isolated from this organism. In the present paper, we report the isolation and structural determination by spectroscopic methods of these new compounds.



The specimens were collected at Nanji Island (the locality suggested the name assigned to the new steroids), Zhejiang Province, China, and extracted exhaustively with Me_2CO. The EtOAc-soluble fraction from the Me_2CO extract was chromatographed on a silica gel column eluting with light petroleum ether with increasing amounts of EtOAc. The fractions eluted with light petroleum ether/EtOAc (3:2) were further purified by repeated column chromatography to afforded pure nanjiol C (**3**, 6.4 mg). The same treatment of fractions eluted with light petroleum ether/EtOAc (1:1) gave nanjiol A (**1**, 25 mg) and B (**2**, 198 mg), respectively.

Nanjiol A (**1**) was isolated as a UV-absorbing [λ_{max} 242 nm, log ϵ 4.17] amorphous powder. Its molecular formula, $C_{31}H_{46}O_6$, was deduced from its HRESIMS {*m*/*z* 537.3196 $[M + Na]^+$, corresponds to $C_{31}H_{46}O_6Na$ (calcd, 537.3192)}. A comparison of overall ^1H and ^{13}C NMR data (Table 1, **2**) revealed similarities between the model compound **4**, previously isolated from Meditereanean black coral *Antipathes subpinnata*,[4] and compound **1**, possessing the same cross-conjugated enone system in the A ring and the same saturated side chain. In fact, **1** differs from **4** only in the functionalities present in the C and D rings. The presence of two acetoxyl groups in the C/D rings of **1** was evident by the peaks at δ 170.4, 169.9, 78.6, and 75.7 in its ^{13}C NMR spectrum. Careful analysis of the 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC) (Figure 1) allowed the location of two acetoxyl groups at C-12 and C-16, respectively. Moreover, the coupling constants and splitting pattern of H_{ax}-12 (δ 4.58, dd, J = 10.8, 4.8 Hz) indicated that the 12-*O*-acetyl was β oriented, while the β orientation of the 16-*O*-acetyl was inferred by observation of a significant NOE between H_α-16 (δ 5.19, m) and H_α-17 (δ 1.58, m) in the NOESY spectrum (see Figure 2). Finally, the absolute stereochemistry at C-20 was assigned *S*, the same as that of model compound **4**, through comparison of ^{13}C NMR data (see Table 2) of compound **1** with those of **4**, showing almost identical chemical shift values for C-17,

Table 1. ^1H NMR Data of Compounds **1**−**3**

H	**1**[a] (mult., *J* in Hz)	**2**[a] (mult., *J* in Hz)	**3**[a] (mult., *J* in Hz)
1	6.93 (d, 10.1)	1.72 (m), 1.96 (m)	1.68 (m), 2.15 (m)
2	6.19 (dd, 10.1, 1.7)	2.38 (m)	2.35 (m)
4	6.03 (d, 1.7)	5.72 (s)	5.72 (s)
6	2.36 (m)	2.33 (m)	2.37 (m)
7	1.87 (m), 0.98 (m)	1.01 (m), 1.82 (m)	1.01 (m), 1.80 (m)
8	1.67 (m)	1.62 (m)	1.63 (m)
9	1.16 (m)	1.11 (m)	0.94 (m)
11α	2.01 (m),	1.89 (m)	1.56 (m)
11*β*	1.60 (m)	1.40 (*m*)	1.56 (m)
12α	4.58 (dd, 10.8, 4.8)	4.64 (dd, 10.8, 4.8)	1.24 (m),
12*β*			2.22 (m)
14	0.91 (m)	0.97 (m)	0.94 (m)
15α	2.42 (m)	2.50 (m)	2.47 (m)
15*β*	1.25 (m)	1.30 (m)	1.21 (m)
16α	5.19 (m)	5.25 (m)	5.34 (m)
17	1.58 (m)	1.63 (m)	1.45 (m)
18	1.22 (s)	1.25 (s)	1.16 (s)
19	1.19 (s)	1.18 (s)	1.19 (s)
21	1.17 (s)	1.22 (s)	1.28 (s)
22	1.63 (m)	1.64 (m)	1.49 (m)
23	1.32 (m)	1.34 (m)	1.34 (m)
24	1.13 (m)	1.17 (m)	1.13 (m)
25	1.52 (m)	1.55 (m)	1.52 (m)
26	0.85[c] (d, 6.5)	0.86[e] (d, 6.5)	0.86[g] (d, 6.5)
27	0.84[c] (d, 6.5)	0.87[e] (d, 6.5)	0.85[g] (d, 6.5)
12-OAc	2.02[d] (s)	2.06[f] (s)	
16-OAc	2.04[d] (s)	2.07[f] (s)	2.09 (s)

[a] The ^1H NMR spectra were measured in $CDCl_3$ at 400 MHz. δ values are reported in ppm referenced to TMS as internal standard. [b] Assignments were made with the aid of the C−H correlation spectroscopy spectra. [c−g] The resonances with the same superscript may be reversed.

* Corresponding author. Tel: 86-21-64311833, ext. 349. Fax: 86 21 64370269. E-mail: ywguo@mail.shcnc.ac.cn.

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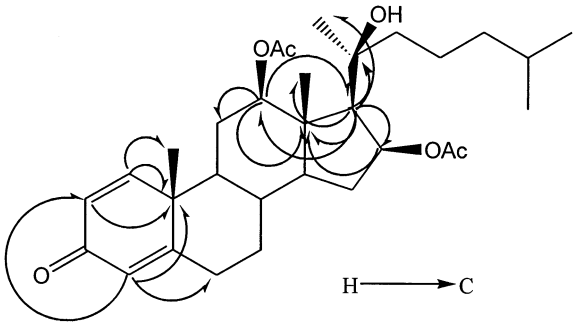

Figure 1. Selected key HMBC correlations of compound **1**.

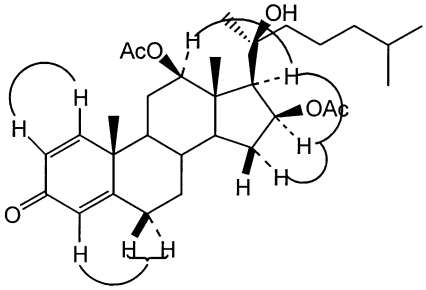

Figure 2. Selected key NOESY correlations of compound **1**.

Table 2. ^{13}C NMR Data[a] ($CDCl_3$) of Compounds **1**−**4**

carbon	**1**	**2**	**3**	**4**[5]
1	154.6 (d)	35.6 (t)	35.7 (t)	154.9 (t)
2	127.8 (d)	33.8 (t)	34.0 (t)	127.8 (d)
3	186.0 (s)	199.1 (s)	199.4 (s)	186.0 (s)
4	124.4 (d)	124.4 (d)	124.0 (d)	124.2 (d)
5	167.5 (s)	169.4 (s)	170.8 (s)	168.0 (s)
6	32.4 (t)	32.5 (t)	32.7 (t)	33.6 (t)
7	32.2 (t)	30.9 (t)	31.7 (t)	34.3 (t)
8	33.5 (t)	33.6 (t)	34.5 (t)	35.5 (t)
9	49.9 (d)	51.9 (d)	53.4 (d)	52.1 (d)
10	42.9 (s)	38.3 (s)	38.6 (t)	43.4 (d)
11	28.2 (t)	26.8 (t)	20.8 (t)	22.1 (t)
12	78.6 (d)	79.1 (d)	40.0 (t)	32.5 (t)
13	46.8 (s)	46.7 (t)	43.4 (s)	48.7 (s)
14	52.2 (d)	52.8 (d)	53.9 (d)	49.7 (d)
15	34.3 (t)	34.2 (t)	35.0 (t)	39.2 (t)
16	75.5 (d)	75.8 (d)	77.8 (d)	216.9 (s)
17	65.4 (d)	65.2 (d)	59.9 (d)	68.7 (d)
18	11.3 (q)	11.3 (q)	14.6 (q)	62.1 (t)
19	18.6 (q)	17.2 (q)	17.4 (q)	18.7 (q)
20	74.6 (s)	74.8 (s)	75.8 (s)	74.2 (s)
21	26.7 (q)	26.8 (q)	26.5 (q)	24.8 (q)
22	41.4 (t)	41.8 (t)	44.5 (t)	42.7 (t)
23	21.7 (t)	21.9 (t)	22.4 (t)	22.4 (t)
24	39.9 (t)	39.9 (t)	39.7 (t)	39.1 (t)
25	27.8 (d)	27.9 (d)	27.9 (d)	27.9 (d)
26	22.5[b] (q)	22.5[d] (q)	22.6[f] (q)	22.6[g] (q)
27	22.7[b] (q)	22.7[d] (q)	22.7[f] (q)	22.5[g] (q)
12-OCO*C*H$_3$	21.6[c] (q)	21.5[e] (q)		
12-O*C*OCH$_3$	170.4 (s)	170.9 (s)		
16-OCO*C*H$_3$	21.7[c] (q)	21.7[e] (q)	21.7 (q)	
16-O*C*OCH$_3$	169.9 (s)	169.9 (s)	169.5 (s)	

[a] δ values are reported in ppm from the residual solvent signal (δ 77.0). ^{13}C assignments were based on DEPT and two-dimensional ^{1}H−^{13}C correlation experiments and comparison with model compounds.[4,5] [b−g] The resonances with the same superscript may be reversed.

C-20, and C-21.[4] Compound **1** was therefore established as (20*S*)-12β,16β,20-trihydroxycholesta-1,4-diene-3-one 12,16-diacetate.

Nanjiol B (**2**), the most abundant metabolite of *N. bayeri*, was shown to be the 1,2-dihydro derivative of **1**. Its molecular formula, deduced from HRFABMS(*m/z* 517.3560 [M + H]$^+$), corresponds to $C_{31}H_{49}O_6$ (calcd, 517.3529), two mass units more than that of **1**. Comparison of its spectral data [UV λ_{max} 239 nm, log ϵ 4.18; IR ν_{max} 3450, 1737, 1676 cm^{-1}; ^{1}H and ^{13}C NMR (Table 1, 2)] with those of **1** clearly indicated that it differs only in the A ring, where the olefin at C-1−C-2 was reduced. The rest of the structure **2** is the same as in compound **1**.

Nanjiol C (**3**) had a molecular formula of $C_{29}H_{46}O_4$ as determined by HRESIMS {*m/z* 481.3288 [M + Na]$^+$, corresponding to $C_{29}H_{46}O_4Na$ (calcd, 481.3294)}. Its UV (λ_{max} 241 nm, log ϵ 4.07), IR (ν_{max} 3448, 1745, 1675 cm^{-1}), and ^{1}H and ^{13}C NMR data (Table 1, 2) are very similar to those of **2**, suggesting the presence of an α,β-unsaturated system in the A ring, a saturated side chain at C-17, and also an acetoxyl at C-16. In fact, lack of the 12-*O*-acetyl as compared to **2** is the only difference recognizable in the spectroscopic data of **3**. The ^{13}C NMR resonance for C-12 was reasonably upfield shifted from δ 78.6 to δ 40.0, as expected for the absence of the 12-*O*-acetyl group. In addition, the significant NOE cross-peak for H_{eq}-12 resonating at δ 2.22 and Me-21 (δ 1.28) observed in the NOESY spectrum of **3** requires a preferential conformation, having Me-21 and H_{eq}-12 spatially very close, indicating the *S* configuration at C-20.[4,5] Therefore, compound **3** is the 12-deacetoxyl derivative of **2**.

Compounds **1**−**3** were tested for the cytotoxicity against HL-60 human promyelocytic leukemic cells and BEL 7404 hepatocellular carinoma cells. Compound **2** exhibited moderate cytotoxicity (IC_{50} = 5 μg/mL) toward the growth of HL-60 cells. Further study should be conducted to understand the biological role of these metabolites in the life cycle of the soft coral.

Experimental Section

General Experimental Procedures. Optical rotations were determined in $CHCl_3$ on a Perkin-Elmer 241MC polarimeter. UV spectra were obtained on a Varian CARY 300 BIO spectrophotometer, and IR spectra were recorded on a Nicolet Magna FT-IR 750 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Bruker DRX 400 NMR spectrometer at 400 and 100.6 MHz, respectively. ESIMS were obtained with a Finnigan LCQ-DECA mass spectrometer, HRFABMS were obtained with a Finnigan MAT 95 mass spectrometer, and HRESIMS were obtained with a Micromass Q-Tof micro mass spectrometer.

Biological Material. The soft coral *N. bayeri* Verseveldt (Nephtheidae) was collected at Nanji Island, Zhejiang Province, China, in May 2000, at a depth of −2 m and was immediately frozen and transferred to SIBS, where it was kept at −20 °C until extraction. A voucher specimen is stored for inspection at the SIBS (voucher no. NJ02-25).

Extraction and Isolation. The bodies of the soft coral *N. bayeri* (800 g dry wt after extraction) was chopped, then soaked in acetone, and extracted at room temperature (2000 mL × 4). The combined acetone extracts were concentrated in vacuo, thus obtaining an aqueous suspension, which was extracted with EtOAc (600 mL × 4). Evaporation of EtOAc extracts gave an oil (20 g), which was subjected to silica gel column chromatography using a petroleum ether/EtOAc gradient as eluent. The fraction eluted with petroleum ether/EtOAc (3:2) afforded compound **3** (6.4 mg). The fraction eluted by petroleum ether/EtOAc (1:1) yielded compounds **1** (25 mg) and **2** (198 mg), respectively.

Compound 1: amorphous powder; $[\alpha]^{25}_D$ +43.8° (*c* 0.35, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 242 (4.17) nm; IR (KBr) ν_{max} 3467, 2954, 1736, 1664 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1 and 2; ESIMS (%) *m/z* 515 [M + H]$^+$ (5), 537 [M + Na]$^+$ (30), 1051 [2M + Na]$^+$ (100); HRESIMS *m/z* 537.3196 (calcd for $C_{31}H_{46}O_6Na$ 537.3192).

Compound 2: colorless oil; $[\alpha]^{25}_D$ +75.8° (*c* 1.02, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 239 (4.18) nm; IR (KBr) ν_{max} 3450, 2956,

1737, 1676 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and 2; ESIMS (%) *m*/*z* 539 $[M + Na]^+$ (100); HRFABMS *m*/*z* 517.3560 (calcd for $C_{31}H_{49}O_6$ 517.3529).

Compound 3: colorless oil; $[\alpha]^{25}_D$ +73.8° (*c* 0.69, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 241 (4.07) nm; IR (KBr) ν_{max} 3448, 2950, 1745, 1675 cm^{-1}; ^1H and ^{13}C NMR, see Table 1 and 2; ESIMS (%) *m*/*z* 459 $[M + H]^+$ (100), 481 $[M + Na]^+$ (16), 917 $[2M + H]^+$ (30), 939 $[2M + Na]^+$ (37); HRESIMS *m*/*z* 481.3288 (calcd for $C_{29}H_{46}O_4Na$ 481.3294).

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Supporting Information Available: Color photograph of the soft coral *Nephthea bayeri*.

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