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A Novel Ansamycin, Naphthomycin K from *Streptomyces* sp.

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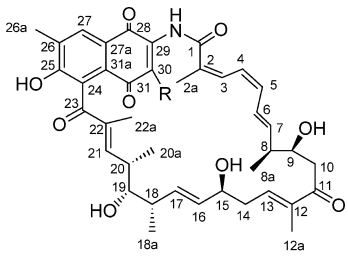
Abstract One novel ansamycin, namely naphthomycin K, together with two known naphthomycins A and E, were isolated from the commensal strain *Streptomyces* sp. CS of the medicinal plant *Maytenus hookeri*. Their structures were elucidated by the analysis of NMR and MS data. Naphthomycin K showed evident cytotoxicity against P388 and A-549 cell lines, but no inhibitory activities against *Staphylococcus aureus* and *Mycobacterium tuberculosis*.

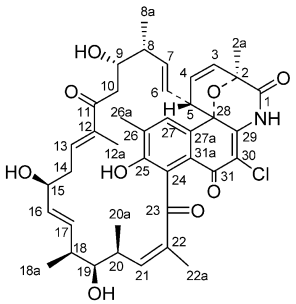
Keywords ansamycin, naphthomycin K, *Streptomyces* sp. CS

In our continuous search for maytansine-producing commensal microorganisms from the medicinal plant *Maytenus hookeri*, we found that the culture extract of *Streptomyces* sp. CS [1], one of the commensal microorganisms isolated from the tissue cultures of *M. hookeri*, showed potent antifungal activity against *Penicillium avellaneum* UC-4376 by the diffusion assay on agar plates and produced an array of chloride-containing compounds as indicated by LC-ESI-MS detection (data not shown). Naphthomycins A and E (**1**, **2**) were isolated from the fermentation extracts of the strain CS cultivated on ISP2 agar medium (data not shown), and antifungal activity-guided fractionation afforded a new macrolide antibiotic, named 24-demethyl-bafilomycin C_1, obtained from the extracts of large scale submerged fermentations in ISP2 liquid medium [1]. The presence of chlorine-containing compounds such as **1** in this strain drew our attention because maytansinoids contain chlorine, and both belong to the type I polyketide family. Guided by analysis of extracts from fermentations in nine different culture media for antifungal activity and LC-ESI-MS profile, ISP3 was selected and used for large-scale solid state fermentation. A novel ansamycin, naphthomycin K (**3**, Fig. 1) as well as **1** and **2** were isolated from the extract of 14 liters of agar plate fermentation of strain CS.

1 was determined to have the molecular formula $C_{40}H_{46}{}^{35}ClNO_9$ (*m/z* 719.2866, calcd.: 719.2861), and $C_{40}H_{46}{}^{37}ClNO_9$ (*m/z* 721.2812, calcd: 721.2832), which indicated the presence of chlorine in this molecule. The ^{13}C-NMR and DEPT spectra showed forty carbon signals



R=Cl Naphthomycin A (1)
R=H Naphthomycin E (2)



Naphthomycin K (3)

Fig. 1 Structures of naphthomycins A, E and K isolated from *Streptomyces* sp. CS.

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Table 1 The NMR data for naphthomycin A (**1**)[a]

No	δ^{13}C	δ^{1}H[b]	HMBC	^{1}H-^{1}H COSY
1	168.8	—	—	—
2	131.5	—	—	—
3	128.7	6.57 (dd, 1.5, 9.9)	C-5, C-2, C-2a	H-4
4	123.0	6.04 (d, 10.0)	C-7, C-5, C-3, C-6	H-3, H-5
5	132.0	6.04 (d, 10.0)	C-7, C-3, C-6	H-4, H-6
6	126.9	6.47 (dd, 10.0, 15.0)	C-8	H-5, H-7
7	139.9	5.42 (dd, 10.0, 15.0)	C-5, C-9, C-8, C-8a	H-6, H-8
8	44.9	2.24 (m)	C-9	H-7 H-9, H-8a
9	72.8	3.51 (m)	C-7, C-8, C-8a	H-10, H-8
10	40.5	2.46 (dd, 7.3, 17.1)	C-9, C-8	H-9, H-10
		3.24 (dd, 2.8, 17.1)	C-9, C-8	H-10
11	203.7	—	—	—
12	137.6[c]	—	—	—
13	142.9	6.72 (t, 5.7)	C-11, C-15, C-14, C-12a	H-14
14	36.1	2.26 (m)	C-13, C-12, C-15	H-13, H-15
15	72.2	3.94 (m)	C-13, C-17, C-14	H-16, H-14
16	136.5	5.61 (dd, 8.0, 15.0)	C-15, C-18, C-14, C-18a	H-17, H-15
17	134.0	5.37 (dd, 9.7, 15.1)	C-15, C-18, C-18a	H-16, H-18
18	41.3	2.15 (m)	C-19	H-17, H-19, H-18a
19	76.9	3.04 (dd, 2.2, 9.8)	C-21, C-17, C-18a, C-20a	H-18
20	33.6	2.65 (m)	C-21, C-20a	H-21, H-20a
21	147.1	5.89 (dd, 1.3, 10.2)	C-23, C-22a	H-20
22	137.8[c]	—	—	—
23	201.8	—	—	—
24	119.9	—	—	—
25	161.4	—	—	—
26	133.6	—	—	—
27	131.4	7.90 (s)	C-25, C-29, C-26a	—
27a	121.7	—	—	—
28	178.9	—	—	—
29	134.3[d]	—	—	—
30	136.3[d]	—	—	—
31	178.1	—	—	—
31a	134.3	—	—	—
2a	20.5	2.07 (d, 1.2, 3H)	C-1, C-2, C-3, C-4	—
8a	17.2	1.13 (d, 6.5, 3H)	C-7, C-9, C-8	H-8
12a	11.0	1.65 (s, 3H)	C-11, C-13, C-12	—
18a	16.1	0.89 (d, 6.7, 3H)	C-17, C-19, C-18	H-18
20a	10.5	0.78 (d, 6.7, 3H)	C-21, C-19, C-20	H-20
22a	12.5	1.97 (d, 1.1, 3H)	C-23, C-21, C-22	—
26a	16.4	2.33 (s, 3H)	C-25, C-26, C-27	—
NH	—	8.40, s	C-1, C-29	—

[a] ^{1}H- and ^{13}C-NMR spectra were obtained at 400 and 100 MHz on Bruker AM-400 with TMS as internal standard, 2D NMR spectra were obtained at 500 MHz on Bruker DRX-500, respectively, and measured in $CDCl_3$ at room temperature.

[b] Coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H.

[c, d] The assignments are interchangeable.

Table 2 The NMR assignments for naphthomycin K (**3**)[a]

No	^{13}C	^{1}H[b]	HMBC	^{1}H-^{1}H COSY
1	168.6	—	—	—
2	76.3	—	—	—
3	127.1	5.91 (d, 10.3)	C-2, C-5	H-4
4	130.2	5.99 (dd, 4.7, 10.2)	C-2	H-3, H-5
5	52.5	2.84 (dd, 4.5, 9.0)	C-4, C-6	H-4
6	130.0	5.03 (dd, 8.9, 15.5)	C-6, C-5, C-8	H-7, H-5
7	133.8	5.35 (dd, 7.7, 16.6)	C-5, C-8, C-8a	H-6
8	41.7	2.32 (m)	C-6	H-7, H-8a
9	71.2	3.86 (br d, 9.8)	—	H-8
10	38.5	2.44 (m), 2.65 (br d, 17.8)	C-11	H-10
11	202.3	—	—	—
12	140.5	—	—	—
13	137.8	6.55 (br s)	C-11, C-12a	H-14
14	35.3	2.77 (m), 2.44 (m)	C-11, C-15	H-14
15	68.8	4.56 (br s)	C-13	H-14
16	133.9	5.59 (br d, 7.9)	C-15, C-18	—
17	133.2	5.59 (br s)	C-15, C-18	H-18
18	41.9	2.32 (m)	—	H-17, H-18a
19	75.5	3.15 (d, 9.5)	C-20a	H-18
20	34.3	2.77 (m)	C-20a, C-21, C-22	H-21, H-20a
21	142.0	6.12 (d, 8.6)	C-23, C-22a	H-20
22	139.8	—	—	—
23	201.5	—	—	—
24	123.1	—	—	—
25	154.6	—	—	—
26	130.9	—	—	—
27	130.0	7.30 (s)	C-25, C-31a, C-28	—
27a	136.0	—	—	—
28	75.4	—	—	—
29	107.6	—	—	—
30	122.1	—	—	—
31	176.2	—	—	—
31a	127.5	—	—	—
2a	20.4	1.68 (s, 3H)	C-1, C-3, C-2	—
8a	14.2	0.57 (d, 7.2, 3H)	C-7, C-9, C-8	H-8
12a	11.5	1.74 (s, 3H)	C-11, C-12a, C-13	—
18a	17.3	0.99 (d, 6.7, 3H)	C-17, C-19, C-18	H-18
20a	10.8	0.83 (d, 6.6, 3H)	C-21, C-19, C-20	H-20
22a	12.4	2.07 (d, 1.1, 3H)	C-23, C-21, C-22	H-22
26a	16.6	2.30 (s, 3H)	C-25, C-26	—
NH	—	7.81, s	C-28, C-2, C-1	—

[a] ^{1}H- and ^{13}C-NMR spectra were obtained at 400 and 100 MHz on Bruker AM-400 with TMS as internal standard, 2D NMR spectra were obtained at 500 MHz on Bruker DRX-500, respectively, and measured in $CDCl_3$ at room temperature.
[b] Coupling constants are presented in Hz. Unless otherwise indicated, all proton signals integrate to 1H.

for seven methyl, two methylene, sixteen methine, and fifteen quaternary carbon atoms, respectively. Inspection of the NMR data (proton, carbon, DEPT, HMQC and HMBC) and ^{1}H-^{1}H COSY spectra (Table 1) assigned the planar structure of **1** to be naphthomycin A [2∼6]. The complete NMR assignments were unambiguously carried out on the basis of HMQC and HMBC experiments (Table 1).

2 was determined to have the molecular formula $C_{40}H_{47}NO_9$ by HREI-MS (*m*/*z* 685.3294, calcd.: 685.3299) and was determined to be naphthomycin E by comparison of its ^{1}H- and ^{13}C-NMR spectral data with those of **1** and reference 7.

3, $[\alpha]_D^{24}$ +112 (*c* 0.25, MeOH), was isolated as a colorless powder. ESI-MS gave molecular ions at *m*/*z* 742.2 and 744.2 $[M+Na]^+$ in the natural abundance isotope ratio of ^{35}Cl and ^{37}Cl, indicating the presence of chloride in the structure. The ^{13}C-NMR and DEPT spectra showed forty carbon signals for seven methyl, two methylene, sixteen methine, and fifteen quaternary carbon atoms. The HMQC, HMBC and ^{1}H-^{1}H COSY experiments revealed a naphthomycin-type structure and determined the NMR assignments for **3**. The carbons at δ 76.3 (C-2), 75.4/75.5 (C-28) and δ 52.5 (C-5) (Table 2) showed distinct differences compared to those of **1**, whereas all other signals are almost the same in both spectra with minor differences of chemical shift. The ketone atoms at δ 178.9 (C-28), the olefinic carbons at δ 131.5 (C-2) and 132.0 (C-5) in **1** was replaced by oxygenated carbon at δ 75.4/75.5 (C-28), 76.3 (C-2) and a quaternary carbon at δ 52.5 (C-5) in **3**, respectively. The chemical shifts of these three carbons suggested a direct connection between carbons C-5 and C-28, and a connection between C-2 and C-28 *via* an oxygen atom. This was further supported by the molecular formula $C_{40}H_{46}ClNO_9Na$ derived by HRESI-MS (*m*/*z* 742.2774, calcd.: 742.2758) and an NOE effect between δ_H 5.03 (H-6) and 2.30 (H-26a). ESI-MS gave the molecular ion of the fully acetylated **3** as *m*/*z* 910 $[M+Na]^+$, which suggested the presence of four free hydroxyl groups in the structure, and further confirmed the connection between C-2 and C-28 *via* an oxygen. Therefore, **3** was determined to be a new ansamycin named naphthomycin K (Fig. 1). The stereochemistry of **3** at C-8, C-9, C-15, C-18, C-19 and C-20 is suggested to be the same as **1** based on NMR data comparison. The relative configurations of C-2, C-5 and C-28 were resolved by analyzing the results of ROESY experiments, which showed NOE effects between the protons at δ_H 1.68 (H-2a) and 5.91 (H-3), and 2.30 (H-26a) and 7.30 (H-27), 0.57 (H-8a), and 2.84 (H-5) and 5.99 (H-4), 5.35 (H-7), 5.03 (H-6), and 7.30 (H-27) and 5.03 (H-6) (Fig. 1).

Ansamycin antibiotics of the naphthalenic subgroup are characterised by the presence of a bicyclic aromatic or quinonoid nucleus across which a polyketide chain is linked to form a macrocyclic lactam [8], as exemplified by **1** [2, 3], naphthomycins B and C [9]. Members of this subgroup display a range of potent antibacterial and antifungal activities [5]. In our experiments, using disk diffusion testing [10], **1** isolated as the main component showed evident inhibitory activity against *P. avellaneum* UC-4376, *Staphylococcus aureus* and *Mycobacterium tuberculosis* (obtained from Key Laboratory for Conservation and Utilization of Bioresources, Yunnnan University) in dose-dependent manner, respectively (data not shown). But **3** showed no inhibitory zones against the tested microorganisms at 200 μg/disc.

Previous studies showed that naphthomycin has antineoplastic activity and further investigated its mode of action [11]. In our experiments, **1** showed cytotoxic activity against P388 and A-549 cells by sulforhodamine B and microculture tetrozolium methods at IC_{50} 0.07 and 3.17 μM measured at 48 hours after exposure, respectively.

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