New Chemical Constituents from the Endophyte *Streptomyces* Species LR4612 Cultivated on *Maytenus hookeri*

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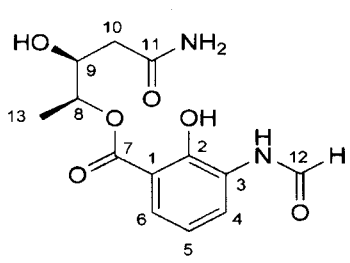
From solid cultures of the biologically important endophyte *Streptomyces* species LR4612, cultivated on *Maytenus hookeri*, four new and two known compounds were isolated. The new compounds were identified as (2*S**,3*S**)-5-amino-3-hydroxy-5-oxopentan-2-yl 3-(formylamino)-2-hydroxybenzoate (**1**), *N*-[(3*R**,4*R**)-3-amino-3,4-dihydro-4-methyl-2,6-dioxo-2*H*,6*H*-1,5-benzodioxocin-10-yl]formamide (**2**), (5β,6α)-6,11-dihydroxyeudesmane (**3**), and 5-(6,7-dihydroxy-6-methyloctyl)furan-2(5*H*)-one (**4**); the known compounds were elucidated as sorbicillin (**5**) and *N*-acetyltyramine (**6**). The structures were established by HR-ESI-MS and in-depth NMR analyses.

Introduction. – Endophytes occurring in higher plants are important sources of natural products with pharmaceutical potential [1][2]. For example, a novel taxol-producing endophytic fungus was discovered some time ago in *Taxus brevifolia* [3]. Further, the anticancer drug maytansine from *Maytenus hookeri* Loes. is currently tested as a lead for the development of new drugs [4]. In the course of our search for new compounds from the endophytic microorganisms of *Maytenus hookeri*, a series of novel compounds were isolated [5–8]. In our ongoing research on metabolites from plant endophytes, we investigated the secondary metabolites produced by the cultured *Streptomyces* species LR4612, a strain isolated from the roots of *Maytenus hookeri*. Herein, we describe the isolation and structure elucidation of four new compounds (**1**–**4**), and of two known constituents, sorbicillin (**5**) and *N*-acetyltyramine (**6**), from the corresponding endophyte $CHCl_3$ extract.

Results and Discussion. – Compound **1** was obtained as yellowish powder. The HR-ESI-MS data indicated a molecular formula of $C_{13}H_{16}N_2O_6$ based on the $[M-H]^-$ ion signal at *m/z* 295.0930 (calc. 295.0930). The ^{13}C-NMR (DEPT) spectrum of **1** (*Table 1*) showed 13 signals: one Me, one CH_2, six CH, and five quaternary C-atoms. The ^{1}H-NMR data of **1** contained aromatic-ring resonances at δ(H) 8.49 (*d*), 7.18 (*d*), and 6.86 (*tdd*), with coupling constants of 7.2–8.0 Hz, indicating three H-atoms in *ortho*-positions, together with a Me *doublet*. The HMBC experiment (*Table 1*) showed correlations between the H-atom at δ(H) 8.49 (H–C(4))[1]) and the C-atoms at δ(C)

[1]) Arbitrary atom numbering. For the systematic names of **1**–**4**, see *Exper. Part.*



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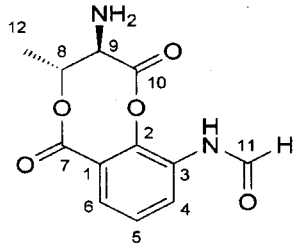

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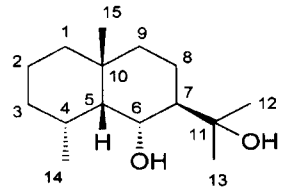

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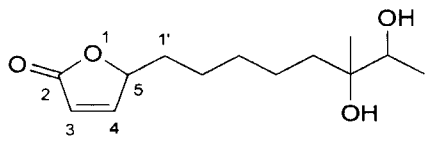

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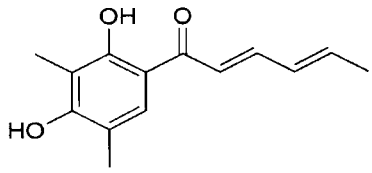

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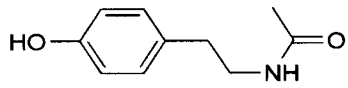

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Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** and **2** (δ in ppm, J in Hz)

Position	**1** (in $CDCl_3$)			**2** (in CD_3OD)		
	δ(H)	δ(C)	HMBC	δ(H)	δ(C)	HMBC
1	–	112.6	–	–	115.7	–
2	–	150.6	–	–	152.3	–
3	–	127.5	–	–	128.0	–
4	8.49 (*d*, J=7.6)	124.8	2, 3, 6	8.27 (*d*, J=7.9)	126.5	2, 3, 6
5	6.86 (*t*, J=8.0)	119.0	1, 2[a]), 3	6.93 (*t*, J=8.0)	119.6	1, 3, 4, 6
6	7.18 (*d*, J=7.2)	120.1	1[a]), 2, 4, 7	7.70 (*d*, J=8.0)	124.1	2, 4, 7
7	–	170.1	–	–	170.9	–
8	4.92 (*m*)	74.9	7, 9, 13	5.03 (*m*)	75.4	7, 10
9	5.04 (*m*)	75.3	8, 10, 11, 13	5.37 (*d*, J=7.5)	57.9	7, 8, 12
10	2.56 (*m*)	31.5	9, 11, 13[a])	–	175.2	–
11	–	175.1	–	8.34 (*s*)	162.2	3
12	8.44 (*s*)	159.0	3	1.34 (*d*, J=6.6)	15.4	8, 9
13	1.15 (*d*, J=6.8)	17.9	8, 9			

[a]) Weak correlation.

150.6 (C(2)), 127.5 (C(3)), and 120.1 (C(6)); between δ(H) 6.86 (H–C(5)) and δ(C) 150.6 (C(2)), 127.5 (C(3)), and 112.6 (C(1)); and between δ(H) 7.18 (H–C(6)) and δ(C) 170.1 (C(7)), 150.6 (C(2)), 124.8 (C(4)), and 112.6 (C(1)), respectively. This further confirmed the *ortho* arrangement of the aromatic H-atoms, in accord with a 2-oxygenated, 3-substituted benzoic acid. The 3-substituent at the aromatic ring was speculated to be an NH group based on the molecular formula and the chemical shift for C(3) (δ(C) 127.5). Further, the long-range correlation between the methine (formyl) H-atom at δ(H) 8.44 (δ(C) 159.0) and δ(C) 127.5 (C(3)) confirmed that this H-atom was linked with the 3-NH group of the aromatic ring through an amide function (formamide). In addition, an ester group was identified by correlations

between the oxygenated methine at δ(H) 4.92 (H–C(8)) and δ(C) 170.1 (C(7)), 75.3 (C(9)), and 17.9 (C(13)); between δ(H) 5.04 (H–C(9)) and δ(C) 175.1 (C(11)), 74.9 (C(8)), 31.5 (C(10)), and 17.9 (C(13)); and between the CH_2 resonance at δ(H) 2.56 (CH_2(10)) and δ(C) 75.3 (C(9)), 175.1 (C(11)), and 17.9 (C(13)), respectively. Moreover, the ^{1}H,^{1}H-COSY spectrum of **1** showed that H–C(8) was correlated with H–C(9), and H–C(9) with H–C(10).

The relative configuration of **1** was established by a NOESY experiment: NOEs were observed between H–C(8) and H–C(9). From these data, compound **1** was determined to correspond to (2*S**,3*S**)-5-amino-3-hydroxy-5-oxopentan-2-yl 3-(formylamino)-2-hydroxybenzoate, and was named *streptomyceamide A*.

Compound **2** was obtained as a yellow, amorphous solid. The HR-ESI-MS data indicated the molecular formula $C_{12}H_{12}N_2O_5$ (*m/z* 265.0821 ($[M+H]^+$; calc. 265.0824)). The NMR data of **2** (*Table 1*) were very similar to those of **1**. Compound **2** also contained a 3-amino-2-hydroxybenzoic acid unit, but the alkyl ester differed from that in **1**. Esterification of the acid had taken place with 2-amino-3-hydroxybutyric acid, and the acid part was connected with the benzene ring through a second (lactone) ester bond, as inferred from 2D-NMR data. An HMBC experiment with **2** (*Table 1*) showed correlations between the H-atom at δ(H) 5.03 (H–C(8)) and δ(C) 170.9 (C(7)) and 175.2 (C(19)); between δ(H) 5.37 (H–C(9)) and δ(C) 15.4 (C(12)), 75.4 (C(8)), and 170.9 (C(7)); and between the Me group at δ(H) 1.34 (Me(12)) and both δ(C) 57.9 (C(9)) and 75.4 (C(8)). At the same time, a NOESY experiment showed NOEs between H–C(12) and H–C(9).

From the above data, the structure of compound **2** was elucidated as *N*-[(3*R**,4*R**)-3-amino-3,4-dihydro-4-methyl-2,6-dioxo-2*H*,6*H*-1,5-benzodioxocin-10-yl]formamide, and the compound was named *streptomyceamide B*.

Compound **3** was obtained as a colorless powder. The HR-ESI-MS data indicated the molecular formula $C_{15}H_{28}O_2$ (*m/z* 241.2171 ($[M+H]^+$; calc. 241.2167)). The ^{1}H-NMR spectrum of **3** (*Table 2*) displayed four Me groups at δ(H) 0.91, 0.99, 1.23, and 1.30. The ^{13}C-NMR (DEPT) spectrum (*Table 2*) indicated that **3** contained a sesquiterpene skeleton [9], with a total of 15 ^{13}C-NMR signals: four Me (δ(C) 14.5, 20.6, 23.9, 30.3), five CH_2 (17.1, 23.0, 33.3, 42.0, 44.0), four CH (26.2, 52.9, 55.0, 70.3), and two quaternary C-atoms (34.6, 75.2). In the HMBC experiment (*Table 2*), the Me(12) and Me(13) groups displayed ^2J correlations with the quaternary C-atom at δ(C) 75.2 (C(11)), and ^3J couplings with the methine C-atom at δ(C) 55.0 (C(7)), which indicated an oxygenated i-Pr group. The Me(14) group was involved in a ^2J coupling with C(10), and in a ^3J coupling with C(1), C(5), and C(9), respectively. Further, Me(14) showed ^2J coupling with C(4), and ^3J coupling with both C(3) and C(5). The oxygenated methine at δ(H) 3.91 (H–C(6)) was correlated with C(4), C(5), C(7), C(10), and C(11). Finally, a NOESY experiment showed NOE interactions between H–C(5) and Me(15), supporting a *cis*-fused perhydronaphthalene ring system. Further NOEs were observed between H–C(6) and both Me(13) and Me(15), as well as between H–C(4) and H–C(5), in accord with a *cis* relationship.

From the above data, the structure of compound **3** was determined as (1*R**,2*R**,4a*R**,8*R**,8a*R**)-decahydro-2-(1-hydroxy-1-methylethyl)-4a,8-dimethylnaphthalen-1-ol, which corresponds to (5β,6α)-6,11-dihydroxyeudesmane (assuming absolute configurations).

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3**. In $CDCl_3$; δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC
1	α: 1.39 (*m*)	42.0	2
	β: 1.09 (*dt*, J = 3.8, 12.6)		9, 10
2	α: 1.66 (*m*)	17.1	1, 3, 4
	β: 1.36 (*m*)		1
3	α: 1.66 (*m*)	33.3	1, 4
	β: 1.49 (*t*, J = 5.3)		1, 2, 4
4	2.27 (*q*, J = 5.7)	26.2	3, 5, 14
5	1.27 (*m*)	52.9	1, 6, 9, 10, 14
6	3.91 (*t*, J = 10)	70.3	4, 5, 7, 10, 11
7	1.57 (*m*)	55.0	6, 8, 9, 11
8	α: 1.55 (*m*)	23.0	6, 7, 9, 10
	β: 1.18 (*m*)		7, 9, 10
9	α: 1.25 (*m*)	44.0	1, 6
	β: 1.18 (*m*)		7, 10
10	–	34.6	–
11	–	75.2	–
12	1.30 (*s*)	23.9	7, 11, 13
13	1.23 (*s*)	30.3	7, 11, 12
14	0.99 (*d*, J = 7.5)	14.5	3, 4, 5
15	0.91 (*s*)	20.6	1, 5, 9, 10

Compound **4** was obtained as a colorless powder. The HR-ESI-MS data indicated the molecular formula $C_{13}H_{22}O_4$ (*m/z* 243.1590 ($[M+H]^+$; calc. 243.1596)). The ^{13}C-NMR (DEPT) spectrum (*Table 3*) showed that **4** contained two Me, five CH_2, four CH, and two quaternary C-atoms, including one C=O group and a C=C bond. The

Table 3. *^{1}H- and ^{13}C-NMR Data of* **4**. In CD_3OD; δ in ppm, J in Hz.

Position	δ (H)	δ (C)	HMBC
2	–	175.8	–
3	6.12 (*dd*, J = 5.7, 1.9)	121.6	2, 4, 5
4	7.71 (*d*, J = 5.6)	159.5	2, 3, 5
5	5.15 (*m*)	85.6	3, 4, 1′, 2′[a])
1′	1.82, 1.64 (2*m*)	34.0	4, 5, 2′, 4′
2′	1.48 (*m*)	26.0	1′, 3′
3′	1.42 (*m*)	24.2	5′, 6′
4′	1.42 (*m*)	31.1	5′, 6′
5′			
	1.48 (*m*)	39.0	1′, 3′
	1.42 (*m*)		6′
6′	–	75.6	–
7′	3.57 (*q*, J = 6.4)	74.1	5′, 6′, 8′, 6′-Me
8′	1.12 (*d*, J = 4.2)	17.7	6′, 7′
6′-Me	1.07 (*s*)	21.6	4′[a]), 5′, 6′ ,7′

[a]) Weak correlation.

HMBC spectrum revealed correlations between the methine at δ(H) 6.12 (H–C(3)) and δ(C) 175.8 (C(2)), 159.5 (C(4)), and 85.6 (C(5)); between the methine at δ(H) 7.71 (H–C(4)) and δ(C) 175.8 (C(2)), 121.6 (C(3)), and 85.6 (C(5); and between H–C(5) at δ(H) 5.15 and δ(C) 159.5 (C(4)), 121.6 (C(3)), 34.0 (C(1′)), and 26.0 (C(2′)), respectively, in agreement with a furan ring. The other HMBC data (*Table 3*) helped to identify the tail of a long alkyl chain: HMBC correlations were observed between δ(H) 3.57 (H–C(7′)) and C(6′), C(5′), C(8′), and Me(6′); and between the Me(6′) H-atoms at δ(H) 1.07 and C(4′), C(5′), C(6′), and C(7′), respectively.

From the above data, the structure of the new compound **4** was elucidated as 5-(6,7-dihydroxy-6-methyloctyl)furan-2(5*H*)-one.

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Experimental Part

General. TLC: precoated *Si-gel-G* plates (*Qingdao Marine Chemical Factory*, Qingdao, P.R. China). Column chromatography (CC): *Sephadex LH-20* (*Pharmacia*); reverse-phase C_{18} (*RP-18*) silica gel (*Merck*); or silica gel *G* (200–300 mesh) and *H* (*Qingdao Marine Chemical Factory*). UV Spectra: *Shimadzu UV-2401PC*; λ_{max} (log ε) in nm. Optical rotation: *Jasco P-1020* polarimeter. ^{1}H- and ^{13}C-NNR (including DEPT, ^{1}H,^{1}H-COSY, NOESY, HMQC, and HMBC) experiments were carried out on *Bruker AM-400* or *DRX-500* spectrometers; chemical shifts δ in ppm rel. to Me_4Si, coupling constants *J* in Hz. ESI- and HR-ESI-MS: *Finnigan LCQ-Advantage* and *VG Auto-Spec-3000* mass spectrometers, resp.; in *m/z*.

Microbial Material. Maytenus hookeri was collected at Xishuangbanna, Yunnan, P. R. China, in August 1998, and was planted in the Kunming Botanical Garden. The roots of the plant were washed with running tap water and then sterilized successively with 75% aq. EtOH (1 min) and 1.2% Na^+ClO^- (8 min), then rinsed with sterile H_2O (5 ×), and cut into small pieces. The pieces were incubated at 25° in *YMG* medium (prepared from 4 g of yeast extract, 10 g of malt extract, 4 g of glucose, and 15 g of agar in 1 l of dist. H_2O), and cultured until colony or mycelium appeared surrounding the segments. After culturing for *ca.* one month, *Streptomyces* (strain number LR4612) was isolated from the sterilized root, as identified and deposited at the Kunming Institute of Botany, Chinese Academy of Science, Kunming, P.R. China. The LR4612 strain was cultured in *YMG* medium (20 l) at 25° for 14 d. Then, the cultures were exhaustively extracted (3 ×) with AcOEt/MeOH/HCOOH 80 : 15 : 5 (*v/v/v*).

Extraction and Isolation. The above microbial extract (20 g) was dissolved in H_2O, and extracted with $CHCl_3$ (5 ×) to afford, after evaporation, 3.68 g of extract, which was purified by MPLC (125 g *RP-18*; H_2O/MeOH 100 : 0 → 70 : 30 → 50 : 50 → 30 : 70): ten fractions (*Fr. 1–10*). *Fr. 1* was subjected to CC (30 g *Sephadex LH-20*; MeOH): *Fr. 1.1–1.4*. Then, *Fr. 1.3* was further purified by CC (2 g silica gel *G*; petroleum ether/acetone 4 : 1): compound **1** (5 mg). *Fr. 6* was subjected to CC (15 g silica gel *G*; petroleum ether/acetone 10 : 1 → 8 : 2): *Fr. 6.1–6.4*. Then, *Fr. 6.3* was subjected to CC (2 g silica gel *H*; petroleum ether/AcOEt 4 : 1): compound **2** (10 mg). *Fr. 2* was isolated by CC (30 g *Sephadex LH-20*; MeOH): *Fr. 2.1–2.3*. *Fr. 2.1* was further purified by MPLC (24 g *RP-18*; MeOH/H_2O 2 : 3): compound **4** (10 mg). *Fr. 7* was purified by CC (1. 30 g *Sephadex LH-20*, MeOH; 2. 1.5 g silica gel *H*, petroleum ether/AcOEt 10 : 1): compound **5** (6 mg). *Fr. 9* was purified by CC (1. 8 g silica gel *G*, petroleum ether/acetone 10 : 1; 2. 30 g *Sephadex LH-20*, acetone): compound **3** (5 mg). *Fr. 3* was further purified by CC (1. 30 g *Sephadex LH-20*, MeOH; 2. 2 g silica gel *G*; petroleum ether/AcOEt 4 : 1): compound **6** (4 mg).

Streptomyceamide A (=*(2S*,3S*)-5-Amino-3-hydroxy-5-oxopentan-2-yl 3-(Formylamino)-2-hydroxybenzoate*; **1**). Yellowish powder. UV ($CHCl_3$): 241.6 (4.07), 321.8 (3.52), 377.6 (2.40). $[\alpha]_D^{26}$ =

+77 ($c = 1.12$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 295 ($[M-H]^+$). HR-ESI-MS: 295.0930 ($[M-H]^-$, $C_{13}H_{15}N_2O_6^-$; calc. 295.0930).

Streptomyceamide B (= N-*[(3*R*,4*R*)-3-Amino-3,4-dihydro-4-methyl-2,6-dioxo-2*H*,6*H*-1,5-benzodioxocin-10-yl]formamide*; **2**). Yellow, amorphous solid. UV (MeOH): 223.8 (4.13), 333.0 (3.38). $[\alpha]_D^{25} = +45$ ($c = 0.20$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 265 ($[M+H]^+$). HR-ESI-MS: 265.0821 ($[M+H]^+$, $C_{12}H_{13}N_2O_5^+$; calc. 265.0825).

(5β,6α-)-6,11-Dihydroxyeudesmane (=*(1*R,2*R,4a*R,8*R,8a*R)-Decahydro-2-(1-hydroxy-1-methylethyl)-4a,8-dimethylnaphthalen-1-ol*; **3**). Colorless powder. $[\alpha]_D^{25} = +23$ ($c = 1.03$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 241 ($[M+H]^+$). HR-ESI-MS: 241.2171 ($[M+H]^+$, $C_{15}H_{29}O_2^+$; calc. 241.2168).

*5-(6,7-Dihydroxy-6-methyloctyl)furan-2(5*H*)-one* (**4**). Colorless powder. UV (MeOH): 208.0 (3.91), 306.0 (1.60). $[\alpha]_D^{25} = +46$ ($c = 0.94$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 3*. ESI-MS: 243 ($[M+H]^+$). HR-ESI-MS: 243.1590 ($[M+H]^+$, $C_{13}H_{23}O_4^+$; calc. 243.1596).

Sorbicillin (**5**). Identified by comparison with the data given in [10]. Yellow powder. ^{1}H-NMR (500 MHz, $CDCl_3$): 7.42 (*m*, H–(6), H–C(3′)); 6.89 (*d*, $J = 14.8$, H–(2′)); 6.32 (*m*, H–C(4′)); 6.23 (*m*, H–C(5′)); 2.15 (*s*, 5-Me); 2.08 (*s*, 3-Me); 1.85 (*d*, $J = 6.2$, Me(6′)). ^{13}C-NMR (125 MHz, $CDCl_3$): 192.5 (C(1′)); 162.6 (C(2)); 158.7 (C(4)); 144.5 (C(3′)); 141.3 (C(5′)); 130.6 (C(4′)); 128.8 (C(6)); 121.9 (C(2′)); 114.5 (C(5)); 113.5 (C(3)); 110.4 (C(1)); 18.9 (C(6′)); 15.6 (5-Me); 7.5 (3-Me). ESI-MS: 233 ($[M+H]^+$).

N-*Acetyltyramine* (**6**). Identified by comparison with the data given in [11]. Colorless powder. ^{1}H-NMR (400 MHz, CD_3OD): 7.02 (*d*, $J = 9.2$, H–C(2,6)); 6.71 (*d*, $J = 8.5$, H–C(3,5)); 3.33 (*t*, $J = 7.5$, CH_2(7)); 2.69 (*t*, $J = 7.5$, CH_2(8)); 1.89 (*s*, Me). ^{13}C-NMR (100 MHz, CD_3OD): 173.2 (C=O); 156.9 (C(4)); 131.2 (C(1)); 130.7 (C(2,6)); 116.2 (C(3,5)); 42.4 (C(7)); 35.7 (C(8)); 22.5 (Me). ESI-MS: 180 ($[M+H]^+$).

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