Terpenoids Produced by Actinomycetes: Napyradiomycins from *Streptomyces antimycoticus* NT17

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Napyradiomycin SR (**1**), 16-dechloro-16-hydroxynapyradiomycin C2 (**2**), 18-hydroxynapyradiomycin A1 (**3**), 18-oxonapyradiomycin A1 (**4**), 16-oxonapyradiomycin A2 (**5**), 7-demethyl SF2415A3 (**6**), 7-demethyl A80915B (**7**), and (*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**) were isolated from the culture broth of *Streptomyces antimycoticus* NT17. These compounds are derivatives of the napyradiomycins isolated previously from *Chainia rubra* or *Streptomyces aculeolatus.* The structures of the new compounds, some of which exhibit antibacterial activities, were established by comparing their NMR data with data of related known compounds. The unique structure of **1**, containing a highly strained ring, was established by NMR and was confirmed by X-ray analysis. Two of the compounds are C-16 stereoisomers of napyradiomycin A2 and are named napyradiomycins A2a (**9a**) and A2b (**9b**).

Terpenoids are produced by condensation of isopentenyl diphosphate (IPP) and its isomer, dimethylally diphosphate (DMAPP). These starting materials are produced via the mevalonate pathway in eukaryotes, archae, and the cytoplasm of plants, while the methylerythritol phosphate pathway (MEP) is used in prokaryotes and the chloroplasts of plants.[1] Although all Actinomycetes including *Streptomycetes* use the MEP pathway for the formation of IPP, some Actinomycetes strains also use the mevalonate pathway for production of terpenoids as secondary metabolites. Such metabolites include several antibacterial, antitumor, or antioxidative substances.[1]

Our previous experimental results[2] indicated that Actinomycetes using the mevalonate pathway could be a good source of terpenoids. Indeed, we succeeded in isolating several new terpenoids of the oxaloterpins class[3] (Figure 1) from *Streptomyces* sp. KO-3988, a strain that contains two mevalonate pathway gene clusters. In this study we investigated metabolites of *Streptomyces antimycoticus* NT17 that produced the antitumor agent lavanducyanin (unpublished data). Lavanducyanin (Figure 1) is a phenazine derivative substituted with a cyclic monoterpenoid residue produced by *Streptomyces* sp. CL 190.[4] Since the strain NT17 was proven to possess a mevalonate pathway gene cluster by a PCR experiment using HMG-CoA reductase gene as a probe, we expected that the strain would produce other terpenoids in addition to lavanducyanin. We thus aimed to screen for terpenoids from a fermentation broth of NT17 and succeeded in purifying several naphthoquinone terpenoids. The compounds isolated from the EtOAc extract of the filtrate were napyradiomycin SR (**1**), 16-dechloro-16-hydroxynapyradiomycin C2 (**2**), 18-hydroxynapyradiomycin A1 (**3**), 18-oxonapyradiomycin A1 (**4**), 16-oxonapyradiomycin A2 (**5**), 7-demethyl SF2415A3 (**6**), 7-demethyl A80915B (**7**), and (*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**). With the exception of **8**, all compounds were structurally related to a class of antibiotics consisting of napyradiomycins A1, A2, B1-B4, C1, and C2,[5,6] SF2415A1-A3 and B1-B3,[7] and A80915A-D and G[8,9] and diprenylated naphthoquinones isolated recently from a marine sediment-derived *Streptomyces*.[10,11] A recent report analyzing napyradiomycin biosynthetic gene clusters of *S. aculeolatus* NRRL 18422 and *Streptomyces* sp. CNQ-525 revealed 33 open reading frames, three of which putatively encode vanadium-dependent chloroperoxidases.[12]

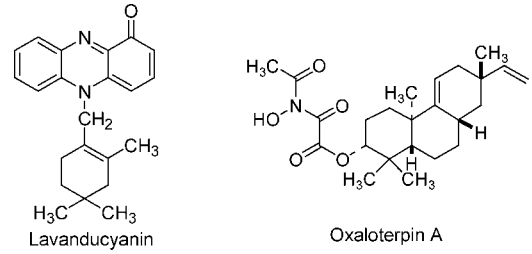

Figure 1. Structures of oxaloterpin A and lavanducyanin biosynthesized via the mevalonate pathway.

Results and Discussion

S. antimycoticus NT17 was cultured at 28 °C for 7 days by rotary shaking in 500 mL baffled Erlenmeyer flasks containing 100 mL of culture medium. The broth filtrate was extracted with EtOAc, and the residue, after removal of the solvent, was analyzed by TLC [*n*-hexane−EtOAc (1:1) or $CHCl_3$−MeOH (20:1)]; compounds were visualized by staining with vanillin−H_2SO_4. Spots that appeared bright purple or violet on the TLC plate were selected as potential candidates for isolation since they are likely terpenoids. Semipreparative purification of these spots was carried out by Si gel column chromatography and C-18 RP-HPLC. The purified samples thus obtained were analyzed by ^{1}H NMR, and fractions showing methyl signals at around δ 1.0 were suspected of containing terpenoids. As a result of this screening, eight fractions showing 2–5 methyl proton singlets were considered as possibly being terpenoids. Further NMR studies including COSY, HSQC, and CT-HMBC[13] (constant time-HMBC) experiments, as well as HR-MS and IR, proved the structures of the following terpenoids: napyradiomycin SR (**1**), 16-dechloro-16-hydroxynapyradiomycin C2 (**2**), 18-hydroxynapyradiomycin A1 (**3**), 18-oxonapyradiomycin A1 (**4**), 16-oxonapyradiomycin A2 (**5**), 7-demethyl SF2415A3 (**6**), 7-demethyl A80915B (**7**), and (*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**).

Napyradiomycin SR (strained ring) (**1**) was isolated as yellow needles. The molecular formula of **1** was established as $C_{25}H_{26}{}^{35}Cl_2O_5$ by HR-MS (*m*/*z* 477.1259 [M + H]$^+$, calcd 477.1230). **1** showed UV absorptions at 359, 319, and 262 nm,

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consistent with a highly conjugated aromatic or phenolic compound. The IR spectrum of **1** showed a conjugated carbonyl group (1710 cm^{-1}). The ^1H and ^{13}C NMR spectra of **1** were analogous to those of napyradiomycin C2[5] (Table 1) and were consistent with a naphthoquinone structure with a cyclic terpenoid moiety. Major NMR chemical shift differences of seven carbon signals, C-15−C-19, C-7, and C-9, however, were observed in the terpenoid residue. Additional structural information was obtained by analyzing HSQC, CT-HMBC, and COSY spectra of **1** (Figure 2). COSY NMR correlations were observed between H-14 and H-16 through H-15. In addition, the olefinic methine proton H-16 showed $^1H-^{13}C$ long-range couplings to C-14, C-15, C-18, and C-19 in the CT-HMBC spectrum. Furthermore, the singlet oxymethylene protons H-19 were coupled to C-8, C-16, C-17, and C-18, and the singlet methylene protons H-18 were coupled to C-6, C-7, C-8, C-17, and C-19. Additionally, the aromatic methine proton H-9 was coupled to C-5a, C-7, C-8, and C-10, and a hydrogen-bonded proton (δ_H 12.22) was coupled to C-5, C-5a, C-6, C-7, and C-8. These collective results indicated that the tetrahydropyrano ring was fused to the naphthoquinone moiety. Furthermore, in the CT-HMBC spectrum of **1**, the methyl singlet 13-CH_3 was coupled to C-12, C-13, and C-14, and the methylene signal H-11 was coupled to C-4a, C-10, C-10a, C-12, and C-13. These correlations suggested the presence of a ring connecting C-10a and C-17.

The geometry of the C-12 and C-13 double bond in **1** was established as *E* based on the ^{13}C chemical shift of the methyl signal appearing at a high field due to the γ-effect [δ_C 14.8, cf. δ_C 13.06 for 3-CH_3 of (*E*)-3,4-dimethyl-2-pentene, and δ_C 18.02 for 3-CH_3 of the *Z*-isomer], suggesting a structure with a highly strained ring. In agreement with this structure, NOESY correlations were observed between the oxymethylene protons H-19 and the methyl singlet 13-CH_3 and between the aromatic proton H-9 and 13-CH_3.

In order to confirm this unusual ring structure of **1**, we performed X-ray diffraction analysis. Compound **1** was obtained as orthorhombic crystals from a solution of $CHCl_3$−MeOH (2:1). The X-ray analysis clearly proved the presence of a ring connecting C-10a and C-17 in **1** (Figure 3). It is noteworthy that C-11 and C-14 are displaced from the double-bond plane formed by C-12 and C-13 due to the ring strain. Biosynthetically **1** can reasonably be assumed to be formed by nucleophilic attack of the C-8 oxygen on C-19 in **2**.

The molecular formula of 16-dechloro-16-hydroxynapyradiomycin C2 (**2**) was established as $C_{25}H_{28}{}^{35}Cl_2O_6$ by HR-MS (*m/z* 495.1301 $[M + H]^+$, calcd 495.1335). Most of the NMR data for **2** were similar to those of napyradiomycin C2[5] (Table1). In the ^{13}C NMR spectrum of **2**, however, the methine carbon signal for C-16 that was observed at δ_C 64.0 in napyradiomycin C2 shifted to lower field at δ_C 75.8. On the basis of the difference in the molecular formulas of **2** ($C_{25}H_{28}Cl_2O_6$) and napyradiomycin C2 ($C_{25}H_{27}Cl_3O_5$), **2** is concluded to be 16-dechloro-16-hydroxynapyradiomycin C2.

18-Hydroxynapyradiomycin A1 (**3**) and 18-oxonapyradiomycin A1 (**4**) were isolated as yellow oils by EtOAc extraction from the broth followed by purification by Si gel chromatography and HPLC. Compound **3** displayed the following UV and IR spectra: UV (MeOH) λ_{max} (ϵ) nm, 360 (5200), 299 (5400, sh), 274 (7300, sh), 244 (8000); IR (KBr) ν_{max} 1700, 1620 cm^{-1}. Compound **4** had the following UV and IR spectra: UV (MeOH) λ_{max} (ϵ) 361 (3200), 300 (4100), 272 (5600, sh), 232 (10 900); IR (KBr) ν_{max} 1700, 1640 cm^{-1}. The molecular formulas of **3** and **4** were established by HR-MS as $C_{25}H_{30}{}^{35}Cl_2O_6$ (*m/z* 497.1465 $[M + H]^+$, calcd 497.1492) and $C_{25}H_{28}{}^{35}Cl_2O_6$ (*m/z* 495.1324 $[M + H]^+$, calcd 495.1335), respectively. Most of the NMR data for **3** and **4** were similar to those of napyradiomycin A1.[5] In the ^1H NMR spectra of **3** and **4**, however, the signal of the C-18 methyl singlet in napyradiomycin A1 was replaced by methylene protons (δ_H 4.15 and 4.07) in **3** and by an aldehyde proton (δ_H 9.36) in **4**. The ^{13}C NMR spectra of **3** and **4** proved the presence of an oxymethylene carbon (δ_C 69.1 for C-18) and an aldehyde carbon (δ_C 196.6 for C-18), respectively. Therefore, **3** and **4** were established as 18-hydroxynapyradiomycin A1 and 18-oxonapyradiomycin A1.

16-Oxonapyradiomycin A2 (**5**) was obtained as a yellow oil [UV (MeOH) λ_{max} (ϵ) nm, 360 (5500), 300 (6000, sh), 270 (9400, sh), 249 (12 100), 223 (12 300); IR (KBr) ν_{max} 1700, 1620 cm^{-1}]. HR-MS established its molecular formula as $C_{25}H_{28}{}^{35}Cl_2O_6$ (*m/z* 495.1360 $[M + H]^+$, calcd 495.1335). While the spectral data for **5** were similar to those of napyradiomycin A2,[6] the ^{13}C NMR spectrum of **5** showed a keto carbonyl carbon (δ_C 202.4 for C-16) replacing the oxymethine carbon at C-16 of napyradiomycin A2. Thus, **5** was established as 16-oxonapyradiomycin A2.

7-Demethyl SF2415A3 (**6**) was obtained as orange needles [UV (MeOH) λ_{max} (ϵ) nm, 428 (2600), 362 (3200), 295 (7500), 248 (9100); IR (KBr) ν_{max} 2140, 1690, 1650 cm^{-1}]. The IR spectrum of **6** showed a characteristic band at 2140 cm^{-1} for a diazo group. The molecular formula of **6** was established as $C_{25}H_{28}{}^{35}Cl_2N_2O_5$ by HR-MS (*m/z* 507.1490 $[M + H]^+$, calcd 507.1454). Most of the NMR data for **6** were similar to those of SF2415A3.[7] In the ^1H NMR spectrum of **6**, however, the singlet signal due to the methyl group at C-7 in SF2415A3 was replaced by an aromatic proton (δ_H 6.40). The ^{13}C NMR spectrum of **6** also showed the disappearance of the methyl signal observed in SF2415A3. Thus, the quaternary carbon in SF2415A3 was replaced by a protonated carbon (δ_C 112.3 for C-7) in **6**. These collective spectroscopic data proved **6** to be 7-demethyl SF2415A3.

7-Demethyl A80915B (**7**) was obtained as an orange powder [UV (MeOH) λ_{max} (ϵ) nm, 416 (2000), 361 (3000), 294 (5200), 252 (7600); IR (KBr) ν_{max} 2150, 1700, 1640 cm^{-1}]. The IR spectrum of **7** also showed a characteristic band at 2150 cm^{-1} for a diazo group. The molecular formula of **7** was determined to be $C_{25}H_{27}{}^{35}Cl_3N_2O_6$ by HR-MS (*m/z* 541.1039 $[M + H]^+$, calcd 541.1058). The ^1H and ^{13}C NMR data of **7** are similar to those of A80915B produced by *S. aculeolatus*.[9] As in the case of **6**, however, the methyl group at C-7 in **7** was absent. Thus, **7** was established as 7-demethyl A80915B.

Unlike other members of this series, (*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**), obtained as orange needles, displayed a UV spectrum [UV (MeOH) λ_{max} (ϵ) nm, 438 (2600), 309 (7200), 262 (13 700)] different from those of the napyradiomycins, suggesting that the chromophore is considerably modified (Figure 4). The molecular formula of **8**, established as $C_{16}H_{15}{}^{35}ClO_5$ by HR-MS (*m/z* 323.0693 $[M + H]^+$, calcd 323.0680), suggested the lack of a side chain that extends from the chromophore unit in other structures. The NMR data for the naphthoquinone skeleton (C-4a to C-10a) and tetrahydropyrano ring (C-2 to C-4) of **8** were similar to those of **3** except for shifts in signals of C-4, C-4a, C-5, C-10, and C-10a and appearance of a new methoxy signal (δ_H 3.87). The ^{13}C NMR chemical shift values of C-5 and C-10 (δ_C 187.9 and 178.4) corresponded to those of typical quinone carbonyl carbons. The methoxy proton signal showed $^1H-^{13}C$ long-range coupling to C-8 in the CT-HMBC spectrum. In the same spectrum the hydrogen-bonded hydroxy proton (δ_H 12.35) was coupled to C-5a, C-6, C-7, and C-8, the aromatic methine proton H-9 was coupled to C-5a, C-7, C-8, and C-10, and the aromatic proton H-7 was coupled to C-6, C-8, and C-9. Thus, the coupling data showed that the methoxy group was attached to C-8. A COSY correlation was observed between H-3 and H-4. Furthermore, in the CT-HMBC spectrum, the methine proton H-3 adjacent to the chlorine atom was coupled to C-2, 2-CH_3, C-4, and C-4a, and the methylene protons H-4 were coupled to C-2, C-3, C-4a, C-5, and C-10a. The results showed that two quaternary olefinic carbons, C-4a and C-10a, were connected by a double bond forming part of a naphthoquinone moiety. The UV spectrum of **8** absorbing at a longer wavelength (438 nm) than the other napyradiomycin members can reasonably be explained by the presence of the naphthoquinone structure. Thus, **8** was determined to be 3-chloro-

Table 1. NMR Data (400 MHz, $CDCl_3$) for **1**−**8** (data of napyradiomycin C2 were taken from ref 5)

	1		napyradiomycin C2		**2**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
2	79.2, qC		79.1 qC		79.1 qC	
2-CH_3	29.3, CH_3	1.51, s	29.1, CH_3	1.53, s	29.0, CH_3	1.51, s
2-CH_3	21.9, CH_3	1.24, s	22.3, CH_3	1.28, s	22.2, CH_3	1.27, s
3	58.6, CH	4.46, m	58.6, CH	4.48, dd (12.0, 4.0)	58.7, CH	4.46, m
4	41.8, CH_2	2.65, dd (14.4, 3.1), 2.47, m	42.1, CH_2	2.60, dd (14.0, 4.0), 2.50, dd (14.0, 12.0)	42.1, CH_2	2.59, 2.50, m
4a	76.2, qC		77.7, qC		77.7, qC	
5	194.4, qC		194.7, qC		194.6, qC	
5a	112.3, qC		111.1, qC		110.9, qC	
6	157.8, qC		162.2, qC		162.5, qC	
7	132.1, qC		120.0, qC		120.5, qC	
8	164.7, qC		162.7, qC		162.8, qC	
9	113.7, CH	6.89, s	107.5, CH	7.11, s	107.8, CH	7.06, s
9a	134.0, qC		133.9, qC		133.8, qC	
10	196.4, qC		195.9, qC		195.9, qC	
10a	85.6, qC		84.8, qC		84.8, qC	
11	43.1, CH_2	2.47, m	41.2, CH_2	2.68, 2.54, m	41.2, CH_2	2.67, 2.51, m
12	116.5, CH	4.46, m	116.6, CH	4.55, m	116.1, CH	4.46, m
13	138.1, qC		141.0, qC		141.6, qC	
13-CH_3	14.8, CH_3	1.14, s	14.5, CH_3	1.10, s	14.6, CH_3	1.10, s
14	39.5, CH_2	2.01, 1.52, m	38.1, CH_2	2.10, 1.30, m	37.2, CH_2	2.03, 1.30, m
15	24.6, CH_2	2.12, 1.86, m	39.8, CH_2	1.60, m	36.9, CH_2	1.33, m
16	131.4, CH	5.13, dd (12.2, 3.4)	64.0, CH	4.07, dd (10.0, 4.0)	75.8, CH	3.97, m
17	133.6, qC		145.4, qC		148.2, qC	
18	31.8, CH_2	3.73, d (11.7), 2.92, d (11.7)	29.5, CH_2	3.98, br d (14.0), 3.57, d (14.0)	28.5, CH_2	3.92, m, 3.48, d (14.5)
19	68.9, CH_2	4.58, s	116.8, CH_2	5.38, 5.33, s	113.6, CH_2	5.19, s
6-OH		12.22, s		12.68, s		12.65, s

	3		**4**		**5**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
2	79.4, qC		78.8, qC		78.6, qC	
2-CH_3	29.0, CH_3	1.49, s	28.9, CH_3	1.48, s	28.5, CH_3	1.48, s
2-CH_3	22.2, CH_3	1.17, s	22.2, CH_3	1.17, s	22.0, CH_3	1.16, s
3	58.8, CH	4.44, dd (11.7, 3.9)	58.7, CH_2	4.42, dd (12.0, 4.2)	58.5, CH	4.40, dd (11.2, 4.4)
4	42.4, CH_2	2.49, 2.38, m	42.6, CH_2	2.46, 2.40, m	42.4, CH_2	2.43, m
4a	78.8, qC		79.3, qC		78.7, qC	
5	193.7, qC		193.7, qC		193.6, qC	
5a	108.9, qC		109.4, qC		109.5, qC	
6	165.1, qC		164.9, qC		164.4, qC	
7	109.5, qC	6.65, d (2.4)	109.4, CH	6.70, d (2.4)	109.2, CH	6.71, d (2.4)
8	165.2, qC		164.5, qC		164.1, qC	
9	108.2, CH	7.06, d (2.4)	107.9, CH	7.07, d (2.4)	107.9, CH	7.19, d (2.4)
9a	134.8, qC		134.9, qC		135.0, qC	
10	194.9, qC		194.5, qC		195.3, qC	
10a	84.5, qC		83.5, qC		83.2, qC	
11	40.5, CH_2	2.71, dd (14.4, 7.3), 2.49, m	40.4, CH_2	2.74, dd (14.2, 7.3), 2.57, dd (14.2, 7.3)	40.8, CH_2	2.66, m
12	116.7, CH	4.82, t (7.3)	117.0, CH	4.84, t (7.3)	115.6, CH	4.70, t (7.6)
13	139.9, qC		139.4, qC		140.8, qC	
13-CH_3	15.8, CH_3	1.23, s	16.2, CH_3	1.34, s	16.3, CH_3	1.32, s
14	38.7, CH_2	1.85, m	37.7, CH_2	1.92, m	33.8, CH_2	1.92, m
15	24.8, CH_2	1.88, m	26.6, CH_2	2.13, m	35.0, CH_2	2.40, m
16	126.6, CH	5.20, t (4.9)	155.5, CH	6.36, t (7.3)	202.4, qC	
17	133.7, qC		139.4, qC		143.8, qC	
18	69.1, CH_2	4.15, d (12.4), 4.07, d (12.4)	196.6, CH	9.36, s	125.5, CH_2	5.89, 7.79, s
19	13.8, CH_3	1.62, s	9.1, CH_3	1.65, s	17.4, CH_3	1.83, s
6-OH		11.90, s		11.85, s		11.82, s

	6		**7**		**8**	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
2	79.3, qC		79.5, qC		80.4, qC	
2-CH_3	28.6, CH_3	1.49, s	28.9, CH_3	1.43, s	25.5, CH_3	1.52, s
2-CH_3	22.3, CH_3	1.17, s	22.4, CH_3	1.17, s	22.3, CH_3	1.49, s
3	57.9, CH	4.38, dd (11.7, 4.4)	57.8, CH	4.38, dd (11.7, 3.9)	57.7, CH	4.07, dd (6.8, 5.4)
4	42.6, CH_2	2.48, m	43.0, CH_2	2.49, 2.07, m	26.9, CH_2	3.09, dd (19.0, 5.4), 2.86, dd (19.0, 6.8)
4a	78.0, qC		79.0, qC		117.5, qC	
5	192.4, qC		192.5, qC		187.9, qC	
5a	113.0, qC		138.5, qC		108.1, qC	

Table 1. Continued

	6		7		8	
position	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)	δ_C, mult.	δ_H (*J* in Hz)
6	164.7, qC		164.8, qC		163.6, qC	
7	112.3, CH	6.40, s	110.1, CH	6.42, s	106.7, CH	6.61, d (2.4)
8	173.4, qC		173.5, qC		165.3, qC	
9	80.8, qC		83.2, qC		108.1, CH	7.18, d (2.4)
9a	137.5, qC		111.4, qC		132.5, qC	
10	193.7, qC		192.6, qC		178.4, qC	
10a	83.8, qC		84.2, qC		153.5, qC	
11	41.6, CH_2	2.71, m	37.6, CH_2	2.51, dd (10.3, 3.9), 2.06, m		
12	114.5, CH	4.75, t (8.1)	46.9, CH	1.73, m		
13	143.7, qC		145.8, qC			
13-CH_3 or 13-CH_2	16.4, CH_3	1.36, s	110.6, CH_2	4.81, 4.55, s		
14	39.7, CH_2	1.74, m	35.6, CH_2	2.14, 2.07, m		
15	26.2, CH_2	1.74, m	34.7, CH_2	1.67, 2.04, m		
16	123.2, CH	4.92, m	69.8, CH	3.74, m		
17	132.2, qC		43.0, qC			
18	25.6, CH_3	1.62, s	26.7, CH_3	0.99, s		
19	17.6, CH_3	1.51, s	15.3, CH_3	0.64, s		
8-OCH_3					56.0, CH_3	3.87, s
6-OH		11.22, s		11.30, s		12.35, s

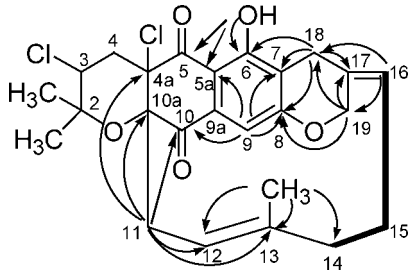


Figure 2. $^1H-^{13}C$ CT-HMBC and $^1H-^1H$ COSY correlations observed for napyradiomycin SR (**1**). Bold lines show COSY correlations.

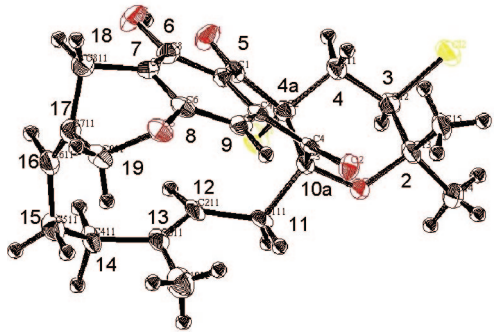


Figure 3. ORTEP representation of napyradiomycin SR (**1**).

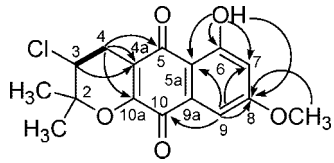


Figure 4. $^1H-^{13}C$ CT-HMBC and $^1H-^1H$ COSY correlations observed for (*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**). Bold lines show COSY correlations.

6-hydroxy-8-methoxy-α-lapachone. It is interesting to note that several derivatives of α-lapachone were previously isolated from plants,[14] but that this is the first instance of **8** being isolated from a microorganism. The configuration at C-3 of **8** was assumed to be the same as in **1**. Lapachone and the related compounds were reported to show antitumor activity.[15]

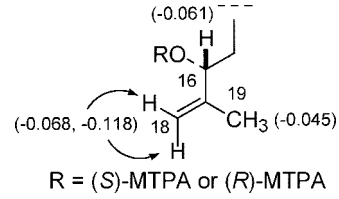


Figure 5. Δ-Values [=δ(*S*)-MTPA − δ(*R*)-MTPA] obtained from the ^1H NMR spectra of the MTPA esters.

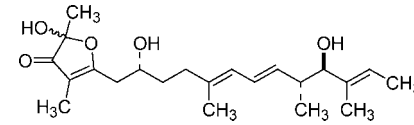
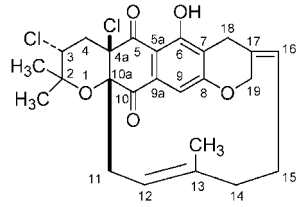


Figure 6. Structure of actinofuranone A produced by marine actinomycetes.

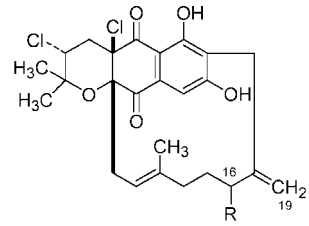
Although **9a** and **9b** were well separated by ODS HPLC (major peak, t_R 15.8 min and minor peak, t_R 14.8 min, in the ratio of ca. 2:1), their ^1H and ^{13}C NMR data were very similar and indistinguishable from the known compound napyradiomycin A2. This result implied that they were C-16 stereoisomers of napyradiomycin A2 with one isomer being napyradiomycin A2. Comparison by HPLC of the isolated compounds with an authentic sample of napyradiomycin A2 provided by the Institute of Microbial Chemistry revealed that the authentic napyradiomycin A2 was also a mixture of two components with retention times identical to **9a** and **9b**. Thus, we renamed the major and minor components of napyradiomycin A2 as A2a and A2b, respectively. Since the C-16 configuration of napyradiomycin A2 was unknown, we defined the absolute configuration of napyradiomycin A2a (**9a**) by the modified Mosher's method.[16] The differences in chemical shift values of the (*R*)-MTPA and (*S*)-MTPA esters [$\delta\Delta = \delta$(*S*)-MTPA − δ(*R*)-MTPA] are shown in Figure 5. On the basis of this result, the C-16 absolute configuration of napyradiomycins A2a (**9a**) and A2b (**9b**) were concluded to be 16*R* and 16*S*, respectively, as shown in Chart 1.

Antibacterial Activity. 7-Demethyl SF2415A3 (**6**) and 7-demethyl A80915B (**7**) showed antibacterial activity against *Staphylococcus aureus* 209P JC-1 (MIC 2.0 and 3.7 nM/mL), *Bacillus subtilis* ATCC6633 (1.0 and 3.7 nM/mL), *Enterococcus faecalis* ATCC19433 (31.6 and 14.8 nM/mL), *Enterococcus faecium* ATCC19434 (15.8 and 14.8 nM/mL), and *Streptococcus pyogenes* ATCC12344 (7.8 nM/mL for **6**) [Mueller Hinton agar medium

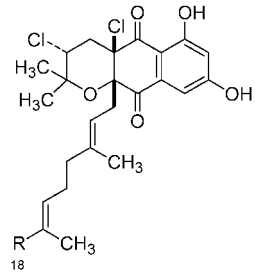
Chart 1



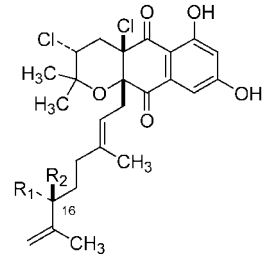
napyradiomycin SR (**1**)



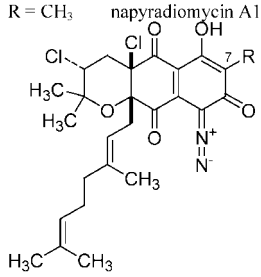
R = OH 16-dechloro-16-hydroxynapyradiomycin C2 (**2**)
R = Cl napyradiomycin C2



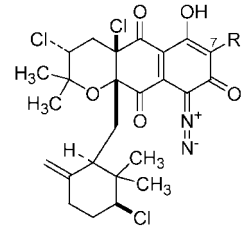
R = CH_2OH 18-hydroxynapyradiomycin A1 (**3**)
R = CHO 18-oxonapyradiomycin A1 (**4**)
R = CH_3 napyradiomycin A1



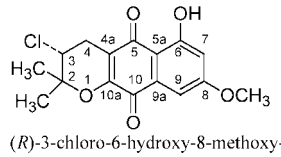
R_1, R_2 = =O 16-oxonapyradiomycin A2 (**5**)
R_1 = OH, R_2 = H napyradiomycin A2a (**9a**)
R_1 = H, R_2 = OH napyradiomycin A2b (**9b**)



R = H 7-demethyl SF2415A3 (**6**)
R = CH_3 SF2415A3



R = H 7-demethyl A80915B (**7**)
R = CH_3 A80195B



(*R*)-3-chloro-6-hydroxy-8-methoxy-α-lapachone (**8**)

(Difco)]. The activity, however, was considerably suppressed in the presence of horse blood serum. The antibacterial activity of **6** was almost the same as that of SF2415A3. Thus, the methyl group at C-7 in **6** did not affect the biological activity. The other compounds were inactive, with MICs of >16 μg/mL.

In conclusion, we isolated eight sesquiterpene compounds from a culture broth of the strain *Streptomyces antimycoticus* NT17 and determined that structures **1**−**8** all possessed the napyradiomycin skeleton. They were derivatives of other napyradiomycins and are formed simply by replacement of chlorine with a hydroxy group, oxygenation, or demethylation. Napyradiomycin SR (**1**) was, however, unique in having a highly strained ring not found in any other member of this class of compounds. (*R*)-3-Chloro-6-hydroxy-8-methoxy-α-lapachone (**8**) was found to be structurally related to α-lapachone previously isolated from plants.

In addition to these napyradiomycins we isolated from strain NT17 actinofuranone A (unpublished data), which is a compound that has been previously reported to be a product of marine actinomycetes.[17] Recently, Fenical's group reported several unique bioactive metabolites from marine actinomycetes.[18] They claimed that marine actinomycetes are attractive sources for screening of new bioactive compounds. In this context, it is interesting to note that actinofuranone A was also isolated from terrestrial Actinomycetes.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Jasco DIP-1000 or a DIP-140 polarimeter. UV spectra were recorded on a Hitachi U-3310 spectrophotometer. IR spectra were obtained with a Shimadzu 8300 FTIR spectrometer. Both 1D ^1H and ^{13}C NMR spectra were recorded on a JEOL Alpha 400 NMR spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C. Two-dimensional $^1H-^1H$ COSY, NOESY, $^1H-^{13}C$ HSQC, and CT-HMBC spectra were recorded on a JEOL alpha 500 or a Varian Inova 500 NMR spectrometer. Samples were dissolved in $CDCl_3$, and the solvent peak was used as an internal standard (δ_H 7.24 and δ_C 77.0). High-resolution FAB and ESI mass spectra were obtained using a JEOL JMS-SX/SX-102A or an Applied Biosystems MDS SCIEX Q-STAR LC-MS. ESI mass spectra were obtained using an Applied Biosystems API 2000 LC-MS.

HPLC purifications were carried out using a Senshu Pak Pegasil ODS column (20 × 250 mm) equipped with a Hitachi High Technologies L-2450 diode array detector. Merck Si gel 60 F_{254} plastic-backed sheets were used for TLC analysis.

Cultivation of Strain NT17. *Streptomyces antimycoticus* NT17 was inoculated into 15 mL test tubes containing 5 mL of a preliminary seed medium consisting of soluble starch 1.0%, polypeptone 1.0%, molasses 1.0%, and meat extract 1.0% (pH 7.2) and was cultured at 28 °C for 2 days on a rotary shaker at 170 rpm. One milliliter aliquot of the seed culture was inoculated in each of 500 mL Erlenmeyer flasks containing 100 mL of medium consisting of glucose 2.0%, soybean meal

1.0%, NaCl 0.3%, and $CaCO_3$ 0.3%, pH 7.0. The microorganism was cultured at 28 °C. After 7 days of productive fermentation the broth was separated into mycelial cake and filtrate by suction filtration. The supernatant was extracted with an equal amount of EtOAc, the organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed.

Purification of Napyradiomycin SR (1), 16-Dechloro-16-hydroxynapyradiomycin C2 (2), 18-Hydroxynapyradiomycin A1 (3), 18-Oxonapyradiomycin A1 (4), 16-Oxonapyradiomycin A2 (5), 7-Demethyl SF2415A3 (6), 7-Demethyl A80915B (7), (*R*)-3-Chloro-6-hydroxy-8-methoxy-α-lapachone (8), Napyradiomycin A2a (9a), and Napyradiomycin A2b (9b). The EtOAc extract from the filtrate of strain NT17 was subjected to Si gel column chromatography (*n*-hexane−EtOAc (1:1)). Napyradiomycin SR (**1**) was eluted first, being separated from fractions containing mixtures of **2**–**8**, **9a**, and **9b**. The compounds were visualized by UV light and reaction with vanillin−H_2SO_4 on Si gel TLC (*n*-hexane−EtOAc, 1:1). Fractions containing **1** (R_f 0.9) were combined and concentrated to dryness under reduced pressure, and the residue was applied to a Si gel column eluted with *n*-hexane−Et_2O (5:1). The main fraction (R_f 0.3, *n*-hexane−Et_2O, 5:1, Si gel TLC) was further purified by ODS HPLC (20 × 250 mm, Senshu Pak Pegasil ODS) with a PDA detector eluted with CH_3CN in H_2O (90%) at a flow rate of 14 mL/min to yield **1** (0.2 mg/L, 16.1 min).

The mixture containing **2**−**8**, **9a**, and **9b** (R_f 0.3–0.8, *n*-hexane−EtOAc, 1:1, Si gel TLC) was applied to a Si gel column eluted with $CHCl_3$−MeOH (20:1) to afford four fractions (A1−A4). Fractions A2−A4 containing **2**, **3**, **9a**, and **9b** (R_f 0.3–0.5, $CHCl_3$−MeOH, 20:1, Si gel TLC) were purified by ODS HPLC using CH_3CN in H_2O (75%) containing 0.1% HCOOH as eluent at a flow rate of 14 mL/min to give **2** (0.3 mg/L, 10.0 min), **3** (0.3 mg/L, 13.8 min), **9a** (0.4 mg/L, 17.0 min), and **9b** (0.2 mg/L, 16.0 min).

Fraction A1 containing **4**–**8** (R_f 0.5–0.8, $CHCl_3$−MeOH, 20:1, Si gel TLC) was further applied to a Si gel column eluted with CH_2Cl_2−Me_2CO (20:1) to afford four fractions (B1−B4). Fraction B2 containing **8** and known napyradiomycins (R_f 0.6–0.9, CH_2Cl_2−Me_2CO, 20:1, Si gel TLC) was purified by ODS HPLC using a gradient of 85–100% CH_3CN in H_2O containing 0.1% HCOOH over 30 min at a flow rate of 14 mL/min to give a pure sample of **8** (0.4 mg/L, 8.0 min), together with known napyradiomycin A1 (20.0 min), napyradiomycin B1 (22.5 min), napyradiomycin C1 (19.0 min), SF2415A3 (25.0 min), and SF2415B3 (24.0 min). Fractions B3 and B4 containing **4**–**7** (R_f 0.3–0.5, CH_2Cl_2−Me_2CO, 20:1, Si gel TLC) were purified by ODS HPLC eluted with CH_3CN in H_2O (85%) containing 0.1% HCOOH at a flow rate of 14 mL/min to give pure samples of **4** (0.3 mg/L, 11.5 min), **5** (0.1 mg/L, 11.0 min), **6** (1.5 mg/L, 23.5 min), and **7** (0.5 mg/L, 23.0 min).

Analysis of Napyradiomycins A2a (9a) and A2b (9b). An authentic sample of napyradiomycin A2 was subjected to ODS HPLC (4.6 × 250 mm, Senshu Pak Pegasil ODS) eluted with CH_3CN in H_2O (70%) containing 0.1% HCOOH at a flow rate of 1.0 mL/min to give **9a** (t_R 15.8 min) and **9b** (t_R 14.8 min).

Preparation of MTPA Esters. To a solution of **9a** containing a catalytic amount of DMAP in CH_2Cl_2−pyridine (1:1) (1 mL) was added (*S*)- or (*R*)-MTPA chloride (40 equiv), and the solution was then stirred at room temperature for 24 h. The reaction mixture was then concentrated to dryness, and the dry residue was extracted with 10 mL of Et_2O. The Et_2O layer was then washed twice with 5 mL of acidic H_2O (pH 3) and then with 5 mL of H_2O (pH 7) and finally washed twice with 5 mL of 5% $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The oily residue was purified by ODS HPLC developed with 100% CH_3CN.

Napyradiomycin SR (1): yellow needles (MeOH−$CHCl_3$); mp 210 °C; $[\alpha]^{25}_D$ −104 (*c* 0.04, $CHCl_3$); UV (*n*-hexane) λ_{max} (ϵ) 359 (3300), 319 (4300), 262 (9100); IR (KBr) 1710, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 477.1259 (calc for $C_{25}H_{27}Cl_2O_5$, $[M + H]^+$ 477.1230).

16-Dechloro-16-hydroxynapyradiomycin C2 (2): yellow powder (MeOH); mp 130–140 °C; $[\alpha]^{25}_D$ +34 (*c* 0.03, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 344 (7600), 275 (12800), 263 (13000); IR (KBr) 1700, 1630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 495.1301 (calc for $C_{25}H_{29}Cl_2O_6$, $[M + H]^+$ 495.1335).

18-Hydroxynapyradiomycin A1 (3): yellow oil; $[\alpha]^{25}_D$ −39 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 360 (5200), 299 (5400, sh), 274 (7300, sh), 244 (8000); IR (KBr) 1700, 1620 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 497.1465 (calc for $C_{25}H_{31}Cl_2O_6$, $[M + H]^+$ 497.1492).

18-Oxonapyradiomycin A1 (4): yellow oil; $[\alpha]^{25}_D$ −31 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 361 (3200), 300 (4100), 272 (5600, sh), 232 (10 900); IR (KBr) 1700, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 495.1324 (calc for $C_{25}H_{29}Cl_2O_6$, $[M + H]$ 495.1335).

16-Oxonapyradiomycin A2 (5): yellow oil; UV (MeOH) λ_{max} (ϵ) 360 (5500), 300 (6000, sh), 270 (9400, sh), 249 (12 100), 223 (12 300); IR (KBr) 1700, 1620 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 495.1360 (calc for $C_{25}H_{29}Cl_2O_6$, $[M + H]^+$ 495.1335).

7-Demethyl SF2415A3 (6): orange needles (MeOH); mp 125 °C; $[\alpha]^{25}_D$ +118 (*c* 0.42, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 428 (2600), 362 (3200), 295 (7500), 248 (9100); IR (KBr) 2140, 1690, 1650 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 507.1490 (calc for $C_{25}H_{29}Cl_2N_2O_5$, $[M + H]^+$ 507.1454).

7-Demethyl A80915B (7): orange powder (MeOH); mp 210 °C; $[\alpha]^{25}_D$ −42 (*c* 0.33, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 416 (2000), 361 (3000), 294 (5200), 252 (7600); IR (KBr) 2150, 1700, 1640 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 541.1039 (calc for $C_{25}H_{28}Cl_3N_2O_6$, $[M + H]^+$ 541.1058).

(*R*)-3-Chloro-6-hydroxy-8-methoxy-α-lapachone (8): orange needles (MeOH); mp 165–170 °C; $[\alpha]^{25}_D$ −8 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 438 (2600), 309 (7200), 262 (13 700); IR (KBr) 1680, 1630 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m*/*z* 323.0693 (calc for $C_{16}H_{16}ClO_5$, $[M + H]^+$ 323.0680).

Napyradiomycin A2a (9a): yellow oil; $[\alpha]^{25}_D$ +16 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 360 (4600), 272 (8100), 253 (9700); IR (KBr) 1700, 1610 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$), δ 196.0 (C, C-10), 193.5 (C, C-5), 165.1 (C, C-6), 165.1 (C, C-8), 146.3 (C, C-17), 141.4 (C, C-13), 134.6 (C, C-9a), 116.3 (CH, C-12), 111.9 (CH_2, C-18), 110.0 (CH, C-7), 109.3 (C, C-5a), 108.3 (CH, C-9), 84.1 (C, C-10a), 78.9 (C, C-4a), 78.9 (C, C-2), 75.3 (CH, C-16), 58.6 (CH, C-3), 42.5 (CH_2, C-4), 40.9 (CH_2, C-11), 35.7 (CH_2, C-14), 31.7 (CH_2, C-15), 28.9 (CH_3, 2-CH_3), 22.2 (CH_3, 2-CH_3), 17.7 (CH_3, C-19), 16.0 (CH_3, 13-CH_3); MS *m*/*z* 497.2 ($C_{25}H_{31}Cl_2O_6$, $[M + H]^+$).

Napyradiomycin A2b (9b): yellow oil; $[\alpha]^{25}_D$ +9 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (ϵ) 360 (4200), 272 (6200), 251 (8100); IR (KBr) 1700, 1620 cm^{-1}; ^{13}C NMR (100 MHz, $CDCl_3$), δ 195.6 (C, C-10), 193.7 (C, C-5), 164.8 (C, C-6), 164.6 (C, C-8), 146.2 (C, C-17), 141.6 (C, C-13), 135.0 (C, C-9a), 116.0 (CH, C-12), 111.9 (CH_2, C-18), 109.6 (CH, C-7), 109.6 (C, C-5a), 108.3 (CH, C-9), 83.7 (C, C-10a), 79.0 (C, C-4a), 78.8 (C, C-2), 76.0 (CH, C-16), 58.7 (CH, C-3), 42.6 (CH_2, C-4), 40.1 (CH_2, C-11), 35.5 (CH_2, C-14), 32.0 (CH_2, C-15), 28.8 (CH_3, 2-CH_3), 22.2 (CH_3, 2-CH_3), 17.4 (CH_3, C-19), 16.2 (CH_3, 13-CH_3); MS *m*/*z* 497.2 ($C_{25}H_{31}Cl_2O_6$, $[M + H]^+$).

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Supporting Information Available: ^1H NMR, ^{13}C NMR, COSY, HSQC, CT-HMBC, HRMS, IR, and UV spectra of napyradiomycins (**1** to **8**, **9a**, and **9b**) are available free of charge via the Internet at http://pubs.acs.org.

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