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Article

Antibacterial Pentacyclic Polyketides from a Soil-Derived *Streptomyces*

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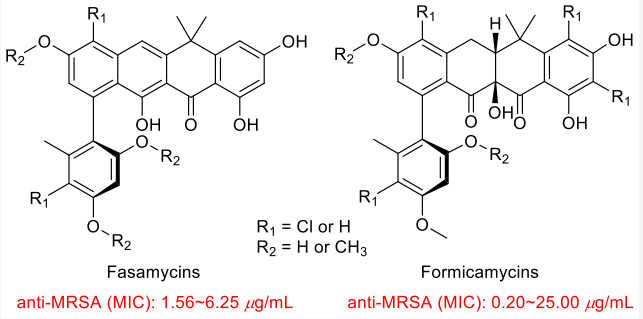
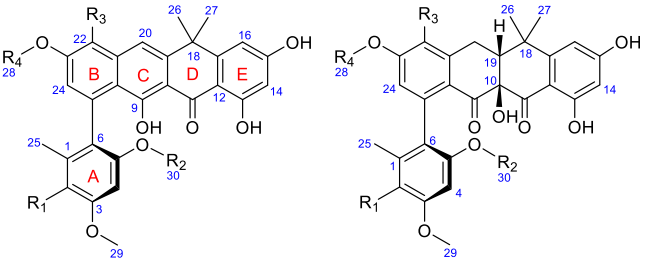
ABSTRACT: Nine new pentacyclic polyketides, fasamycins G−K (**1**−**5**) and formicamycins N−Q (**6**−**9**), along with 10 known analogues (**10**−**19**), were isolated from a rhizospheric soil-derived *Streptomyces* sp. KIB-1414. Their structures and absolute configurations were elucidated by interpretation of NMR and HRMS data and comparisons of CD data. The compounds were active against methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* strains, with MIC values ranging from 0.20 to 50.00 μg/mL.



Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major cause of nosocomial infections since the 1960s.[1,2] It can lead to various human and animal diseases, including skin infections, abscesses, pneumonia, mastitis, osteomyelitis, septicemia, and acute endocarditis.[3,4] Vancomycin was considered as the first-line drug to treat serious MRSA infections, but many clinical isolates of vancomycin-resistant MRSA have been found worldwide.[5,6] Therefore, the exploration of novel and more efficient antibacterial agents against MRSA is urgently needed. Fasamycins and formicamycins are two subtypes of structurally related pentacyclic polyketides with significant antibacterial activity against methicillin-resistant *S. aureus* and vancomycin-resistant *Enterococcus faecalis* (VRE),[7−14] through inhibiting poly(ADP-ribose) polymerase-1 (PARP-1).[15] The synthesis[14,16,17] and biosynthesis[7,18] of fasamycins and formicamycins were recently reported.

As part of our continuing search for new bioactive secondary metabolites from *Streptomyces* isolated from rhizospheric soil,[19,20] we investigated the chemical constituents of *Streptomyces* sp. KIB-1414, a strain from the rhizospheric soil of *Polyalthia cerasoides*, which showed antimicrobial activities against pathogenic bacteria stains including *S. aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, and *Escherichia coli* ATCC 8099. Nine new pentacyclic polyketides (Figure 1), fasamycins G−K (**1**−**5**) and formicamycins N−Q (**6**−**9**), together the known fasamycin C (**10**),[7] fasamycin E (**11**),[7] 7-(2,4-dimethoxy-6-methylphenyl)-2,4,6-trihydroxy-9-methoxy-12,12-dimethyltetracen-5(12*H*)-one (**12**),[14] naphthacemycin B_4 (**13**),[8] formicamycin A (**14**),[7] formicamycin B (**15**),[7] formicamycin C (**16**),[7] formicamycin E (**17**),[7] formicamycin F (**18**),[7] and formicamycin G (**19**),[7] were isolated (Figure S1, Supporting Information). Herein, we report details of the isolation, structure elucidation, and antibacterial activities of these new pentacyclic polyketides.



	R_1	R_2	R_3	R_4
1	H	Me	Cl	Me
2	H	H	H	Me
3	Cl	H	H	Me
4	Cl	H	H	H
5	H	Me	Cl	H

	R_1	R_2	R_3	R_4
6	H	H	Cl	Me
7	Cl	H	H	Me
8	H	H	Cl	H
9	Cl	Me	Cl	Me

Figure 1. Chemical structures of compounds **1**−**9**.

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Table 1. ^{1}H NMR Data for Compounds 1−9 (J in Hz)

no.	1[a,c]	2[b,c]	3[b,c]	4[b,d]	5[b,c]	6[b]	7[b]	8[b]	9[a]
2	6.47 d (2.4)	6.33 br s			6.45 d (2.4)	6.29 d (1.8)		6.27 d (1.8)	
4	6.45 d (2.4)	6.33 br s	6.51 s	6.51 s	6.43 d (2.4)	6.33 d (1.8)	6.50 s	6.31 d (1.8)	6.52 s
9-OH	14.45 s								
13-OH	12.92 s								12.27 s
14	6.27 d (1.8)	6.20 d (1.8)	6.21 d (2.4)	6.20 d (3.0)	6.22 d (2.4)	6.12 d (1.8)	6.10 d (2.4)	6.11 d (2.4)	6.20 br s
16	6.61 d (2.4)	6.65 d (1.8)	6.67 d (2.4)	6.65 d (3.0)	6.68 d (1.8)	6.44 d (1.8)	6.41 d (1.8)	6.43 d (1.2)	6.42 br s
19						2.54 dd (9.0, 6.6)	2.52 dd (10.2, 6.0)	2.51 dd (9.6, 6.7)	2.56 dd (10.2, 6.6)
20	7.89 s	7.50 s	7.53 s	7.37 s	7.88 s	3.51 dd (19.2, 7.2)	3.45 dd (18.0, 6.0)	3.49 dd (18.7, 6.5)	3.53 dd (19.2, 6.6)
						2.76 dd (18.6, 9.0)	2.94 dd (18.0, 10.2)	2.70 dd (18.7, 9.5)	2.75 dd (19.2, 10.8)
22		7.19 d (3.0)	7.22 d (2.4)	7.05 d (3.0)			6.80 d (2.4)		
24	6.93 s	6.68 d (3.0)	6.68 d (2.4)	6.64 d (3.6)	6.77 s	6.71 s	6.49 d (3.0)	6.50 s	6.62 s
25	1.98 s	1.88 s	1.97 s	1.98 s	1.87 s	1.87 s	1.93 s	1.86 s	2.02 s
26[e]	1.74 s	1.72 s	1.73 s	1.71 s	1.75 s	1.39 s	1.40 s	1.42 s	1.47 s
27[e]	1.75 s	1.72 s	1.73 s	1.71 s	1.75 s	1.62 s	1.63 s	1.63 s	1.67 s
28	4.01 s	3.95 s	3.95 s			3.88 s	3.83 s		3.92 s
29	3.89 s	3.58 s	3.58 s	3.59 s	3.85 s	3.61 s	3.61 s	3.61 s	3.73 s
30	3.66 s				3.62 s				3.98 s

[a]Recorded in $CDCl_3$. [b]Recorded in CD_3OD. [c]Recorded at 600 MHz. [d]Recorded at 400 MHz. [e]Exchangeable signals.

Table 2. ^{13}C NMR Data for Compounds 1−9

no.	1[a,c]	2[b,c]	3[b,c]	4[b,d]	5[b,c]	6[b,d]	7[b,c]	8[b,c]	9[a,c]
1	137.2, C	138.0, C	135.7, C	134.1, C	137.9, C	136.5, C	135.7, C	137.9, C	135.4, C
2	106.0, CH	109.3, CH	113.6, C	112.0, C	107.2, CH	108.2, CH	113.8, C	109.6, CH	114.9, C
3	159.7, C	159.0, C	157.2, C	155.6, C	161.1, C	157.8, C	157.4 C	158.2, C	156.1, C
4	96.2, CH	97.5, CH	98.5, CH	97.0, C	96.8, CH	96.0, CH	98.6, CH	97.5, CH	94.2, CH
5	157.6, C	158.1, C	153.7, C	152.0, C	158.9, C	157.0, C	153.8, C	159.3, C	155.2, C
6	125.6, C	125.7, C	126.9, C	125.7, C	126.6, C	121.5, C	124.9, C	123.5, C	123.4, C
7	138.2, C	140.7, C	140.0, C	138.9, C	139.2, C	141.0, C	143.5, C	142.3, C	140.4, C
8	118.5, C	118.9, C	118.6, C	116.4, C	118.8, C	123.6, C	124.0, C	122.9, C[f]	123.1, C
9	166.0, C	166.9, C	166.3, C	165.1, C	166.6, C	193.6, C	195.4, C	194.6, C	193.8, C
10	107.5, C	108.2, C	108.3, C	106.3, C	108.0, C	78.7, C	80.8, C	80.0, C	79.0, C
11	190.5, C	191.8, C	191.9, C	190.1, C	191.8, C	197.0, C	198.7, C	198.6, C	196.6, C
12	108.8, C	108.6, C	108.7, C	107.1, C	108.6, C	107.6, C	109.0, C	109.1, C	109.0, C
13	165.7, C	166.9, C	166.9, C	165.3, C	167.0, C	166.0, C	167.6, C	167.6, C	166.5, C
14	101.8, CH	102.3, CH	102.2, CH	100.7, CH	102.3, CH	100.3, CH	101.8, CH	101.8, CH	101.6, CH
15	162.9, C	167.3, C	166.9, C	165.3, C	167.1, C	166.4, C	168.3, C	168.1, C	163.9, C
16	105.8, CH	107.5, C	107.3, CH	105.7, CH	107.5, CH	106.7, CH	108.3, CH	108.3, CH	106.9, CH
17	154.9, C	155.9, C	156.0, C	154.4, C	155.9, C	154.2, C	155.8, C	155.8, C	154.4, C
18	39.4, C	39.9, C	39.9, C	38.3, C	40.3, C	38.0, C	39.3, C	39.5, C	38.7, C
19	146.6, C	147.0, C	147.2, C	145.3, C	148.2, C	48.5, CH	50.5, CH	48.6, CH	47.7, CH
20	111.5, CH	116.8, CH	116.9, CH	114.7, CH	112.3, CH	28.6, CH_2	34.4, CH_2	30.4, CH_2	29.3, CH_2
21	137.1, C	142.7, C	142.7, C	141.3, C	138.7, C	141.4, C	147.6, C	143.4, C	142.1, C
22	115.7, C	106.8, CH	107.1, CH	108.7, CH	114.0, C	119.9, C	113.1, C	120.3, C	121.1, C
23	155.5, C	162.1, C	162.1, C	158.6, C	155.9, C	158.5, C	165.0, C	155.8, C	159.0, C
24	115.7, CH	122.5, CH	122.6, CH	120.8, CH	121.9, CH	114.1, CH	119.1, CH	120.8, CH	114.2, CH
25	20.9, CH_3	20.7, CH_3	18.4, CH_3	16.8, CH_3	20.9, CH_3	19.0, CH_3	18.1, CH_3	20.5, CH_3	18.0, CH_3
26[e]	34.5, CH_3	34.8, CH_3	34.8, CH_3	33.2, CH_3	35.0, CH	27.6, CH_3	29.6, CH_3	29.3, CH_3	29.1, CH_3
27[e]	34.7, CH_3	34.8, CH_3	34.8, CH_3	33.2, CH_3	35.1, CH_3	32.7, CH_3	31.8, CH_3	34.3, CH_3	34.2, CH_3
28	56.9, CH_3	56.0, CH_3	56.1, CH_3			55.6, CH_3	56.2, CH_3		56.8, CH_3
29	55.5, CH_3	56.1, CH_3	56.2, CH_3	54.7, CH_3	55.9, CH_3	54.6, CH_3	56.2, CH_3	56.1, CH_3	56.3, CH_3
30	56.1, CH_3				56.2, CH_3				56.4, CH_3

[a]Recorded in $CDCl_3$. [b]Recorded in CD_3OD. [c]Recorded at 150 MHz. [d]Recorded at 100 MHz. [e]Exchangeable signals. [f]Signals were not detected in ^{13}C NMR, but were found in an HMBC spectrum.

RESULTS AND DISCUSSION

Fasamycin G (**1**) was isolated as a yellow powder. Its molecular formula was determined to be $C_{30}H_{27}ClO_7$ by HRESIMS analysis, suggesting 17 degrees of unsaturation. The ^{1}H NMR data of **1** (Table 1) showed two active hydrogen signals at δ_H 14.45 and 12.92, two aromatic proton singlets at δ_H 7.89 and 6.93, four *meta*-coupled aromatic protons at δ_H 6.61 (d, 2.4 Hz), 6.47 (d, 2.4 Hz), 6.45 (d, 2.4 Hz), and 6.27

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(d, 1.8 Hz), three methoxyls at δ_H 4.01, 3.89, and 3.66, and three tertiary methyl groups at δ_H 1.98, 1.75, and 1.74. The ^{13}C NMR data of **1** (Table 2) revealed the presence of 30 carbons, including one carbonyl carbon, 16 sp^2 nonprotonated carbons, one sp^3 quaternary carbon, six sp^2 methine carbons, three methoxy carbons, and three tertiary methyl carbons. The HMBC signals (Figure 2) [H-2/(C-3, C-4, C-6); H-4/(C-2,

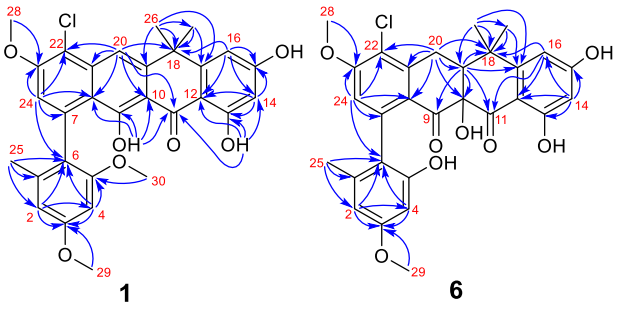

Figure 2. Key HMBC correlations of **1** and **6**.

C-3, C-5, C-6); H_3-25/(C-1, C-2, C-6); H_3-29/C-3; H_3-30/C-5] from **1** demonstrated the presence of a six-membered carbon ring A decorated with a methylation at C-1 and two methoxylations at C-3 and C-5, respectively. Another series of HMBC correlations of **1** [H-20/(C-8, C-10, C-21, C-22); H-24/(C-7, C-8, C-22, C-23); 9-OH/(C-8, C-9, C-10)] verified that ring B was fused with ring C through two shared carbons (C-8 and C-21) (Figure 2). Additionally, it was shown that ring A was linked to ring B through a carbon bond (C-6−C-7) based on the HMBC cross-peak from H-24 to C-6. Likewise, HMBC correlations of H-14/(C-12, C-13, C-15, C-16) and H-16/(C-12, C-14, C-15) supported the subunit of ring E in **1**. In addition, HMBC connections of H_3-26/(C-17, C-18, C-19, C-27); H_3-27/(C-17, C-18, C-19, C-26); (H-16, H-20)/C-18; and (H-20, 13-OH, 9-OH)/C-11 suggested the *gem*-dimethylcyclohexanone subunit (ring D) in **1**. Taking the chemical formula of **1**, as well as the chemical shifts of C-13 (δ_C 165.7, C) and C-22 (δ_C 115.7, C) into consideration, a hydroxylation and a chlorination were deduced to be located at C-13 and C-22, respectively. Thus, the planar structure of **1** was established as an analogue of naphthacemycin B_4 (**13**)[8] with two more methoxyls. The axial chirality at C_6−C_7 in **1** was inferred to be a *P* configuration based on the similar CD spectrum (Figure 3A) to that of fasamycin E (**11**).[7]

Fasamycin H (**2**) displays a molecular formula of $C_{29}H_{26}O_7$ deduced from its HRESIMS data. The ^1H and ^{13}C NMR spectra of **2** (Tables 1 and 2) closely resembled those of fasamycin C (**10**),[7] except an additional methoxyl is present in **2**. This methoxy group was assigned to C-23 based on the correlations from H_3-28 (δ_H 3.95) to C-23 (δ_C 162.1, C) in the HMBC spectrum.

The molecular formula of fasamycin I (**3**) was established as $C_{29}H_{25}ClO_7$ by HRESIMS analysis. Comparing the ^1H and ^{13}C NMR data of **3** with **2** (Tables 1 and 2), indicated two methoxy groups, at C-3 and C-23 in **3**. The chlorine was assigned to C-2 (δ_C 113.6, C) according to the carbon chemical shift and further analysis of HMBC correlations.

Analysis of the 1D NMR data of fasamycin J (**4**) and fasamycin K (**5**) (Tables 1 and 3) implied that they shared the same fasamycin skeleton and that **4** and **5** are demethylation products of **3** and **1**, respectively. The above deduction is in

Table 3. Antimicrobial Activities of Compounds 1−19

	MIC (μg/mL)			
compound	MRSA	*S. aureus*	*B. subtilis*	*E. coli*
1	3.13	3.13	12.50	3.13
2	3.13	3.13	6.25	3.13
3	3.13	6.25	6.25	3.13
4	1.56	3.13	3.13	3.13
5	6.25	6.25	6.25	6.25
6	6.25	6.25	6.25	3.13
7	3.13	3.13	6.25	3.13
8	Not tested	12.50	50.00	25.00
9	25	6.25	25.00	25.00
10	6.25	3.13	6.25	12.50
11	1.56	1.56	3.13	3.13
12	1.56	3.13	3.13	1.56
13	3.13	1.56	6.25	6.25
14	1.56	3.13	3.13	3.13
15	1.56	0.39	1.56	1.56
16	1.56	0.78	3.13	1.56
17	3.13	3.13	1.56	1.56
18	3.13	6.25	3.13	12.50
19	0.20	0.39	0.39	0.78
kanamycin[a]	not tested	1.56	0.78	50.00
vancomycin[a]	1.56	not tested	not tested	not tested
DMSO[b]	not active	not active	not active	not active

[a]Positive control. [b]Negative control.

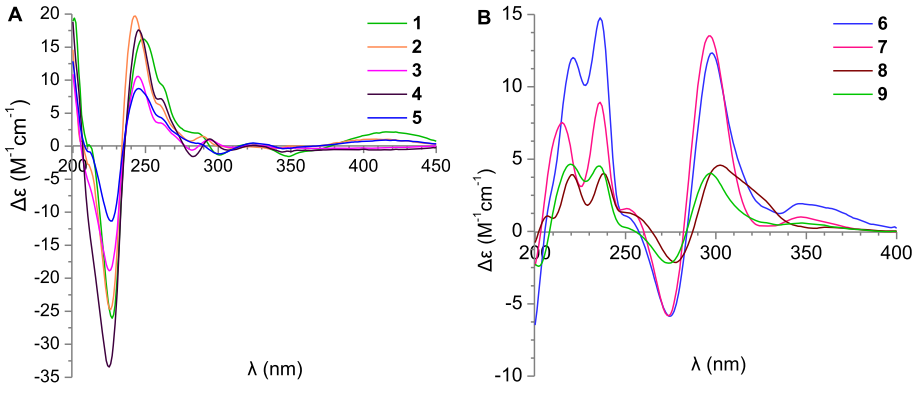

Figure 3. Experimental CD curves of compounds **1**−**9**.

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agreement with their respective chemical formula and was verified by further HMBC analyses. The axial chirality at C_6−C_7 in fasamycins H−K (**2**−**5**) was assigned to be the same as that of **1** on the basis of similar Cotton effects in their CD spectra (Figure 3A).

Formicamycin N (**6**) was isolated as a faint yellow powder. The HRESIMS data of **6** indicated a molecular formula of $C_{29}H_{27}ClO_8$, corresponding to 16 degrees of unsaturation. The NMR data of **6** and formicamycin A (**14**)[7] were similar (Tables 1 and 2) except that one methoxy group (δ_H 3.81, δ_C 54.3) in **14** was absent in **6**. Further HMBC experimentation (Figure 2) was carried out to confirm the formicamycin skeleton and the positions of methoxyls. Key HMBC correlations of H_3-28/C-23 and H_3-29/C-3 in **6** showed the two methoxy groups to be at C-23 and C-3, respectively. As was the case of formicamycin B (**15**),[7] the NOESY spectrum of **6** revealed the same key NOE correlations of H-16/H_3-26, H-16/H_3-27, H-19/H_3-26, H-19/H_3-27, H-20a/H_3-26, H-20b/H_3-26, and H-20a/H-19 (Figure 4). The CD spectra of

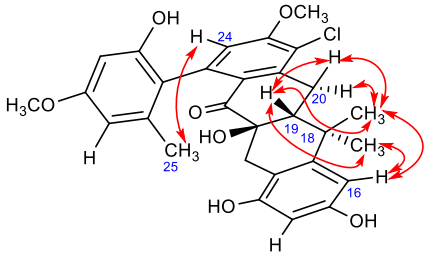

Figure 4. Key NOESY correlations of **6**.

formicamycin B (**15**)[7] and formicamycin N (**6**) (Figure 3B) featured similar Cotton effects. Therefore, as with formicamycin B (**15**),[7] a stereochemistry of (10*R*, 19*R*) was assigned to **6** based on a combination of NOESY NMR and experimental CD data. However, the axial chirality at C_6−C_7 in formicamycin B (**15**) remained unsolved. As reported,[7] neither the observed weak NOE correlation between H-24 and H_3-25 nor the calculated ECDs of relevant *P*,*M*-configurations of the axial chirality can distinguish two possible atropisomers. In view of the shared biosynthesis pathway for fasamycins and formicamycins, we tentatively determined that the axial chirality at C_6−C_7 in formicamycin N (**6**) was the same (*P*-configuration) as that of fasamycins G−K (**1**−**5**).

Formicamycin O (**7**) has the same molecular formula, $C_{29}H_{27}ClO_8$, as formicamycin N (**6**) according to its HRESIMS data. A coupling interaction between H-22 (δ_H 6.80, d, 2.4) and H-24 (δ_H 6.49, d, 3.0) in the ^1H−^1H COSY spectrum of **7** (Figure S48, Supporting Information) suggested that the chlorination was not at C-22 in **7** like that of formicamycin N (**6**). Instead, no aromatic protons coupled with H-4 (δ_H 6.50, s) in the ^1H−^1H COSY spectrum of **7**, and the carbon at C-2 (δ_C 113.8) is nonprotonated rather than olefinic as in **6**, so the chlorine atom should be at C-2. This was further supported by HMBC data.

The molecular formula of formicamycin P (**8**) was indicated as $C_{28}H_{25}ClO_8$ from its HRESIMS data, with one carbon atom and two hydrogen atoms less than that of formicamycin N (**6**). An aromatic methoxyl (δ_H3.61, δ_C 56.1) was indicated by the 1D NMR data of **8** (Tables 1 and 2), and further HMBC correlation from H_3-29 (δ_H 3.61) to C-3 (δ_C 158.2) showed a methoxyl at C-3 (Figure S54, Supporting Information). Thus, the methoxyl in formicamycin N (**6**) at C-23 was replaced by a hydroxy in formicamycin P (**8**).

The quasi-molecular ion peak in the HRESIMS spectrum at *m*/*z* 585.1088 $[M - H]^-$ provided the molecular formula $C_{30}H_{28}Cl_2O_8$ for formicamycin Q (**9**). The NMR data of **9** (Tables 1 and 2) and formicamycin B (**15**)[7] were similar, except for an additional methoxyl (δ_H 3.98, δ_C 56.4) and a downfield shifted (~4 ppm) carbon signal at δ_C 155.2 (C-5). An HMBC correlation from the methoxyl (δ_H 3.98) to C-5 (δ_C 155.2) indicated the additional methoxy group is attached to C-5. Finally, the stereochemistries at C-10 and C-19 were both assigned to be *R* as in formicamycin N (**6**) based on their respective CD spectra (Figure 3B). The axial chiralities at C_6−C_7 in formicamycins O−Q (**7**−**9**) were tentatively assigned to be *P* as in **6**.

Compounds **1**−**19** were evaluated for their antibacterial activities,[21] and their minimum inhibitory concentration (MIC) values are listed in Table 3. All compounds showed antibacterial activities against methicillin-resistant *S. aureus* (MRSA), *S. aureus*, *B. subtilis*, and *E. coli* with MIC values ranging from 0.20 to 50.00 μg/mL. Compounds **4**, **11**, **12**, **14**, **15**, and **16** exhibited strong antibacterial activity against MRSA with an MIC value of 1.56 μg/mL, equal to that of vancomycin. Notably, the antibacterial activity of **19** against MRSA was better than that of vancomycin with an MIC value of 0.20 μg/mL. Compound **6** showed stronger antibacterial activities against all the tested bacterial strains than **8**, which might result from the absence of methoxy modification at C-23 in **8**. Compared with formicamycin Q (**9**), compounds **17** and **18** also showed more potent antibacterial activity. It is possible that the chlorination modification at C-14 or C-16 in ring E played an important role in determining their antibacterial activities. Formicamycin G (**19**), with a *meta*-chlorinated ring, showed the most potent antibacterial activities against all tested strains.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. ECD spectra were obtained using a V100 spectrometer. IR spectra were measured on a Bruker Tensor-27 FTIR spectrometer with KBr disks. NMR spectra were recorded in $CDCl_3$ or CD_3OD using Bruker Avance III 400 or 600 MHz spectrometers with TMS as an internal standard. ESIMS and HRESIMS analysis were carried out on a Shimadzu UPLC-IT-TOF mass spectrometer. Silica gel (200−300 mesh and 300−400 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (18−111 μm, Pharmacia Biotech Ltd., Sweden) were used for the chromatography column (CC). Semipreparative HPLC was conducted on a Hitachi Chromaster system, equipped with a DAD detector and a YMC-Triart C_{18} column (250 × 10.0 mm i.d., 5 μm), using a flow rate of 3.0 mL/min at a column temperature at 28 °C, and 0.1% (v/v) acetic acid was added to each HPLC mobile phase.

Strain Isolation and Cultivation. Strain *Streptomyces* sp. KIB-1414 was isolated from the rhizosphere soil of *Polyalthia cerasoides*, which was collected in Yuxi, Yunnan Province, China, in 2015. It was identified as *Streptomyces* sp. by a 16S rRNA gene sequence (GenBank No. MN065804) showing 99.1% identity to that of strain *Streptomyces sclerogranulatus* JT18 (GenBank No. MG669348.1).

This strain was cultured on MS agar plates (2.0% soybean powder, 2.0% mannitol, and 2.0% agar, pH 7.2) for 5 days at 30 °C. The spores were inoculated into 250 mL baffled Erlenmeyer flasks containing 50 mL of liquid TSB medium (tryptone soy broth, 30 g/L) and shaken on a rotary shaker (200 rpm) at 30 °C for 2 days. Aliquots (12.5 mL) of the cultured seed medium were transferred into 1000 mL baffled Erlenmeyer flasks containing 250 mL of production

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medium (3.0% glucose, 0.1% tryptone, 0.5% beef extract, 0.5% NaCl, 0.25% $CaCO_3$, 0.1% trace element solution consisting of $FeSO_4·7H_2O$ 1.0 g/L, $ZnSO_4·7H_2O$ 1.0 g/L, $CuSO_4·5H_2O$ 0.45 g/L, $MnSO_4·4H_2O$ 0.1 g/L, and K_2MoO_4 0.1 g/L, pH 7.2) and incubated on a rotary shaker (200 rpm) at 30 °C for 5 days. The fermentation scale for strain *Streptomyces* sp. KIB-1414 was 20 L in total.

Extraction and Isolation. The culture was centrifuged at 4000 rpm at 4 °C for 15 min. The supernatant was extracted three times with equal volumes of EtOAc. The mycelia was soaked with acetone and then concentrated under reduced pressure to yield the aqueous concentrate, which was further extracted with equivalent EtOAc three times. Both EtOAc extracts were ultimately combined to get the crude extract due to their similar HPLC metabolite profiles.

The extract (8.6 g) was then subjected to silica gel CC eluting with petroleum ether−EtOAc (1:0, 10:1, 5:1, 3:1, 1:1, and 1:0, v/v) and EtOAc−CH_3OH (1:1, v/v) to give seven fractions (A−G). Main metabolites were distributed in the eluate of petroleum ether−EtOAc (5:1, 3:1, 1:1) by HPLC analysis. Fraction D (petroleum ether−EtOAc 3:1) (2.0 g) was first separated by Sephadex LH-20 CC ($CHCl_3$−CH_3OH, 1:1) to generate six subfractions, which were further purified by semipreparative HPLC to give compounds **2** (9.5 mg), **6** (1.7 mg), **7** (2.7 mg), **11** (2.0 mg), **13** (2.1 mg), **14** (2.5 mg), **15** (9.7 mg), and **19** (3.1 mg). Fraction C (petroleum ether−EtOAc, 5:1) (1.6 g) was sequentially subjected to Sephadex LH-20 CC ($CHCl_3$−CH_3OH, 1:1) and semipreparative HPLC to afford compounds **3** (5.7 mg), **9** (1.3 mg), **16** (17.2 mg), and **17** (1.1 mg). Fraction E (petroleum ether−EtOAc, 1:1) (1.5 g) was separated by Sephadex LH-20 CC ($CHCl_3$−CH_3OH, 1:1) to generate five subfractions, which were further purified by semipreparative HPLC to afford compounds **1** (12.7 mg), **4** (20.0 mg), **5** (4.4 mg), **8** (6.8 mg), **10** (10.2 mg), **12** (6.3 mg), and **18** (0.8 mg).

*Fasamycin G (**1**):* yellow powder; $[\alpha]^{19.0}_D$ +61.7 (*c* 0.09, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 249 (+16.2), 227 (−26.1) nm; IR (ν_{max}) 3450, 2973, 2928, 2851, 1605, 1459, 1346, 1156 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 533.1370 $[M - H]^-$ (calcd for $C_{30}H_{26}ClO_7$, 533.1373).

*Fasamycin H (**2**):* yellow powder; $[\alpha]^{21.0}_D$ +106.7 (*c* 0.10, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 243 (+19.7), 226 (−24.7) nm; IR (ν_{max}) 3431, 2972, 2929, 2853, 1609, 1283, 1201 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 485.1603 $[M - H]^-$ (calcd for $C_{29}H_{25}O_7$, 485.1606).

*Fasamycin I (**3**):* yellow powder; $[\alpha]^{20.0}_D$ −81.6 (*c* 0.08, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 245 (+10.6), 225 (−18.9) nm; IR (ν_{max}) 3428, 2965, 2930, 2852, 1606, 1201, 1151 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 519.1213 $[M - H]^-$ (calcd for $C_{29}H_{24}ClO_7$, 519.1216).

*Fasamycin J (**4**):* yellow powder; $[\alpha]^{20.0}_D$ −73.3 (*c* 0.09, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 245 (+17.6), 225 (−33.5) nm; IR (ν_{max}) 3405, 2974, 2928, 2853, 1605, 1435, 1278, 1177 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 505.1060 $[M - H]^-$ (calcd for $C_{28}H_{22}ClO_7$, 505.1060).

*Fasamycin K (**5**):* yellow powder; $[\alpha]^{22.0}_D$ +15.2 (*c* 0.10, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 245 (+8.7), 226 (−11.3) nm; IR (ν_{max}) 3443, 2964, 2926, 2853, 1606, 1371, 1282, 1154 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 519.1215 $[M - H]^-$ (calcd for $C_{29}H_{24}ClO_7$, 519.1216).

*Formicamycin N (**6**):* yellowish powder; $[\alpha]^{20.0}_D$ +228.8 (*c* 0.10, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 298 (+12.3), 274 (−5.9), 221 (+12.0), 236 (+14.8) nm; IR (ν_{max}) 3416, 2973, 2940, 2848, 1683, 1625, 1315, 1160 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 537.1322 $[M - H]^-$ (calcd for $C_{29}H_{26}ClO_8$, 537.1322).

*Formicamycin O (**7**):* yellowish powder; $[\alpha]^{21.0}_D$ +319.8 (*c* 0.08, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 297 (+13.5), 274 (−5.8), 236 (+8.9), 215 (+7.5) nm; IR (ν_{max}) 3426, 2969, 2853, 1678, 1595, 1262, 1161 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 537.1322 $[M - H]^-$ (calcd for $C_{29}H_{26}ClO_8$, 537.1322).

*Formicamycin P (**8**):* yellowish powder; $[\alpha]^{21.0}_D$ +213.6 (*c* 0.05, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 302 (+4.5), 274 (−2.1), 238 (+4.0), 221 (+3.9) nm; IR (ν_{max}) 3433, 2967, 2923, 2853, 1623, 1261, 1161 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 523.1166 $[M - H]^-$ (calcd for $C_{28}H_{24}ClO_8$, 523.1165).

*Formicamycin Q (**9**):* yellowish powder; $[\alpha]^{21.0}_D$ +276.4 (*c* 0.10, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) 297 (+4.0), 274 (−2.2), 235 (+4.5), 220 (+4.6) nm; IR (ν_{max}) 3454, 3392, 2932, 2852, 1675, 1626, 1319, 1208, 1084 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 585.1088 $[M - H]^-$ (calcd for $C_{30}H_{27}Cl_2O_8$, 585.1088).

Antimicrobial Assay. The antimicrobial activities of compounds **1**−**19** were investigated in 96-well plates using a modified broth microdilution method.[21] Four bacteria are used [methicillin-resistant *Staphylococcus aureus* (shhs A1), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), and *Escherichia coli* (ATCC 8099)]. A 195 μL amount of LB medium (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl, pH 7.4) was dispensed in each well of column 1, while columns 2−12 contained 100 μL of LB medium only. Each tested compound (5 μL, dissolved in DMSO at a concentration of 4 mg/mL), positive controls kanamycin (5 μL, dissolved in water at a concentration of 4 mg/mL) and vancomycin (5 μL, dissolved in water at a concentration of 4 mg/mL), and negative control DMSO (5 μL) were dispensed in wells of column 1, respectively. Then the mixture from columns 1−10 was transferred and mixed, yielding 100 μL of 2-fold serial dilutions per well. Each of the tested bacterial strains was cultured in LB medium on a rotatory (200 rpm) at 37 °C for 12 h and added to columns 1−11, resulting in approximately 5×10^5 CFU/mL per well. Final tested sample concentrations were 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.7813, 0.3906, 0.1953, and 0.0977 μg/mL. Column 12 contained 100 μL of LB medium (as a control to monitor sterility).[22] The microplates were further incubated at 37 °C for 16 h. The minimum inhibitory concentration was determined as the lowest concentration inhibiting visible growth of bacteria. All the experiments were carried out in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00161.

1D and 2D NMR and HRESIMS spectra for compounds **1**−**9** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) National Nosocomial Infections Surveillance (NNIS). *Am. J. Infect. Control* **2004**, *32*, 470−485.
(2) Gosbell, I. B. *Am. J. Clin. Dermatol.* **2004**, *5*, 239−259.
(3) Levy, S. B.; Marshall, B. *Nat. Med.* **2004**, *10*, S122−S129.
(4) (a) Schito, G. C. *Clin. Microbiol. Infect.* **2006**, *12*, 3−8. (b) DeLeo, F. R.; Chambers, H. F. *J. Clin. Invest.* **2009**, *119*, 2464−2474. (c) Morell, E. A.; Balkin, D. M. *Yale J. Biol. Med.* **2010**, *83*, 223−233. (d) Malachowa, N.; DeLeo, F. R. *Cell. Mol. Life Sci.* **2010**, *67*, 3057−3071.
(5) Zheng, Y.; Liu, W.; Chen, Y.; Jiang, H.; Yan, H.; Kosenko, I.; Chekulaeva, L.; Sivaev, I.; Bregadze, V.; Wang, X. *Organometallics* **2017**, *36*, 3484−3490.
(6) Zhang, Y.; Ma, W.; Zhu, Y.; Shi, S.; Li, Q.; Mao, C.; Zhao, D.; Zhan, Y.; Shi, J.; Li, W.; Wang, L.; Fan, C.; Lin, Y. *Nano Lett.* **2018**, *18*, 5652−5659.
(7) Qin, Z.; Munnoch, J. T.; Devine, R.; Holmes, N. A.; Seipke, R. F.; Wilkinson, K. A.; Wilkinson, B.; Hutchings, M. I. *Chem. Sci.* **2017**, *8*, 3218−3227.
(8) Fukumoto, A.; Kim, Y.-P.; Iwatsuki, M.; Hirose, T.; Sunazuka, T.; Hanaki, H.; Ōmura, S.; Shiomi, K. *J. Antibiot.* **2017**, *70*, 568−573.
(9) Fukumoto, A.; Kim, Y.-P.; Matsumoto, A.; Takahashi, Y.; Suzuki, M.; Onodera, H.; Tomoda, H.; Matsui, H.; Hanaki, H.; Iwatsuki, M.; Ōmura, S.; Shiomi, K. *J. Antibiot.* **2017**, *70*, 562−567.
(10) Feng, Z.; Chakraborty, D.; Dewell, S. B.; Reddy, B. V. B.; Brady, S. F. *J. Am. Chem. Soc.* **2012**, *134*, 2981−2987.
(11) Feng, Z.; Kallifidas, D.; Brady, S. F. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 12629−12634.
(12) Wei, X.; Wu, P.; Yang, L.; Xue, J.; Li, H.; Xu, L. C. N. Patent 108503522 A, 2018.
(13) Ōmura, S.; Takahashi, Y.; Kim, Y. H.; Hanaki, H.; Tomoda, H.; Suzuki, M.; Shiomi, K. J.P. Patent 2009046404 A, 2009.
(14) Huang, J. K.; Lauderdale, T.-L. Y.; Lin, C.-C.; Shia, K.-S. *J. Org. Chem.* **2018**, *83*, 6508−6523.
(15) Shen, W.; Lu, X.; Zhu, J.; Mu, Y.; Xu, Y.; Gao, J.; Zhang, X.; Zheng, Z. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 1904−1908.
(16) Wang, S.; Kraus, G. A. *J. Org. Chem.* **2018**, *83*, 15549−15552.
(17) Hirose, T.; Kojima, Y.; Matsui, H.; Hanaki, H.; Iwatsuki, M.; Shiomi, K.; Ōmura, S.; Sunazuka, T. *J. Antibiot.* **2017**, *70*, 574−581.
(18) Qin, Z.; Devine, R.; Hutchings, M. I.; Wilkinson, B. *Nat. Commun.* **2019**, *10*, 3611.
(19) He, X.; Wang, Y.; Luo, R.-H.; Yang, L.-M.; Wang, L.; Guo, D.; Yang, J.; Deng, Y.; Zheng, Y.-T.; Huang, S.-X. *J. Nat. Prod.* **2019**, *82*, 1813−1819.
(20) Yang, F.-X.; Hou, G.-X.; Luo, J.; Yang, J.; Yan, Y.; Huang, S.-X. *J. Antibiot.* **2018**, *71*, 1040−1043.
(21) Tian, H.; Shafi, J.; Ji, M.; Bi, Y.; Yu, Z. *J. Nat. Prod.* **2017**, *80*, 1015−1019.
(22) Elshikh, M.; Ahmed, S.; Funston, S.; Dunlop, P.; McGaw, M.; Marchant, R.; Banat, I. M. *Biotechnol. Lett.* **2016**, *38*, 1015−1019.