Studies on Terpenoids Produced by Actinomycetes: Oxaloterpins A, B, C, D, and E, Diterpenes from *Streptomyces* sp. KO-3988

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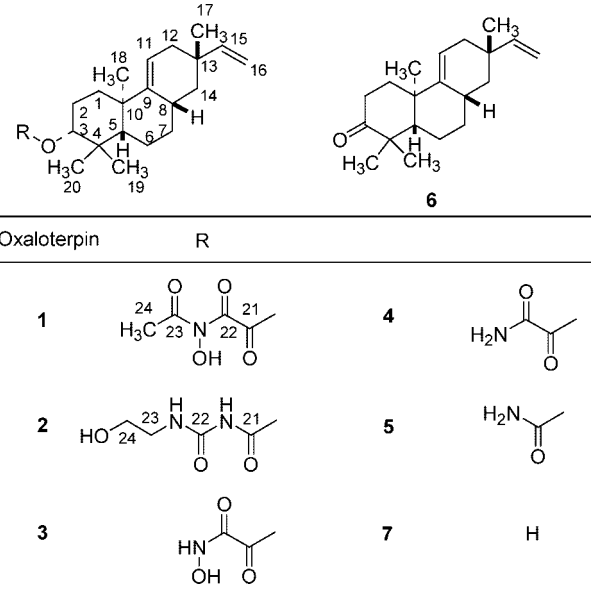
Following screening for terpenoids produced by *Streptomyces* sp. KO-3988, five new diterpenes named oxaloterpins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) together with the known viguiepinone (**6**) were isolated from culture broth, and their structures were established on the basis of extensive NMR and MS analyses. The absolute configuration of oxaloterpin A was determined by the modified Mosher's method as 3*R,* 5*S,* 8*S,* 10*R,* 13*S*.

Terpenoids are the largest family of compounds found in nature, with over 24 000 known examples.[1,2] They are biosynthesized by condensation of two types of C_5 precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). IPP is a product of the mevalonate pathway in eukaryotes and archaebacteria, while it is synthesized through the recently discovered 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway in most bacteria, green algae, and chloroplasts of higher plants.[3] All Actinomycetes including *Streptomycetes* utilize only the MEP pathway for the formation of the primary metabolite IPP. On the other hand, some Actinomycetes strains equipped with the MEP pathway machinery have been shown to use the mevalonate pathway for production of secondary terpenoidal metabolites as illustrated in Figure 1. These include *Kitasatosporia griseola* (terpentecin producer),[4] *Actinoplanes* sp. A40644 (BE-40644 producer),[5] *Streptomyces* sp. CL190 (naphterpin A producer),[6] *Streptomyces* sp. KO-3988 (furaquinocin A producer),[7] and *Chainia rubra* (napyradiomycin A producer).[8] More recently, Heide and co-workers reported that furanonaphthoquinone I and endophenazine A, which are metabolites of *Streptomyces cinnamonensis,* are produced via the mevalonate pathway.[9] Because these terpenoids showed interesting biological activities including antitumor, antibacterial, and antioxidative properties, novel terpenoids produced by Actinomycetes were expected to be promising candidates for drug discovery. Since such terpenoids have not been well exploited, we carried out a screening of terpenoids from Actinomycetes equipped with the mevalonate pathway gene cluster. On the basis of our experimental results,[10] we assumed that such microorganisms possessed the mevalonate pathway gene cluster for the production of secondary terpenoidal metabolites. In order to efficiently select such microorganisms, we first investigated whether the mevalonate pathway could be detected in the strains mentioned above by Southern hybridization using the HMG-CoA reductase gene as a probe. We found that *Streptomyces* sp. KO-3988, the organism producing furaquinocin, possessed "two" corresponding genes.[10] Our later investigations showed that the organism had two mevalonate pathway gene clusters, with one cluster being responsible for production of antitumor agents, the furaquinocins.[11] Subsequent gene analysis of the second mevalonate pathway gene cluster showed that it was flanked by the GGDP (geranylgeranyl diphosphate) synthase gene at its upstream region.[12] Since GGDP is the precursor of diterpenes, the strain was assumed to produce diterpene compounds that had never been isolated from this strain. We thus attempted to isolate diterpenes from the fermentation broth of this strain and succeeded in purifying five new diterpenes as well as the known viguiepinone (**6**).[13]



Results and Discussion

Streptomyces sp. KO-3988 was cultured at 28 °C for 7 days by rotary shaking in 500 mL baffled Erlenmeyer flasks each containing 100 mL of the production medium used for furaquinocin.[14] The mycelial cake prepared from 30 L of fermentation broth was extracted with acetone, and a small portion of the residue obtained after removal of the solvent was analyzed by TLC developed with *n*-hexane–EtOAc (1:1) or $CHCl_3$–MeOH (20:1) and visualized with vanillin–H_2SO_4. Spots that changed to bright purple or violet were selected as candidates for terpenoids, and semipreparative purification of the fractions corresponding to these spots was carried out by Si gel column chromatography, preparative TLC, and C-18 RPHPLC. Samples thus prepared were subjected to ^{1}H NMR analysis, and fractions showing methyl proton singlets at around δ 1.0 in their ^{1}H NMR spectra were assumed to contain terpenoids. As a result of this screening, six fractions showing four methyl

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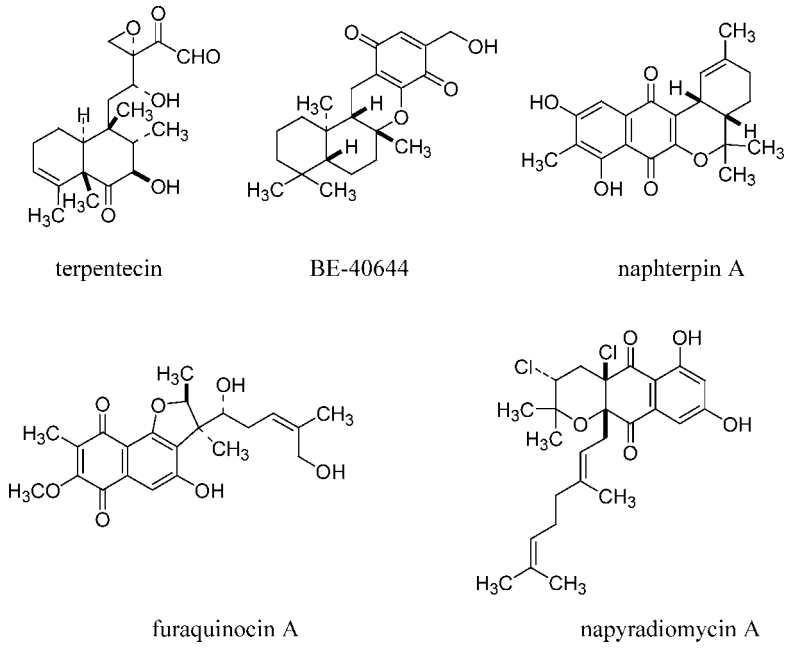


Figure 1. Terpenoids biosynthesized via the mevalonate pathway.

proton singlets at around δ 1.0 in their ^{1}H NMR spectra were selected as plausible terpenoids. Two-dimensional NMR studies on these components by COSY, HSQC, and HMBC as well as HR-MS and IR measurements proved them to be terpenoids as expected, and they were designated as oxaloterpins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**). In addition, a known compound, viguiepinone (**6**),[13] was isolated from this strain.

The HR-MS spectrum of oxaloterpin A (**1**) established its molecular formula as $C_{24}H_{35}NO_5$ (*m/z* 440.2407 $[M + Na]^+$, calcd 440.2425), indicating 8 degrees of unsaturation. The ^{13}C NMR and HSQC spectra confirmed the presence of 24 carbons (Table 1), including three carbonyl carbons [δ_C 167.6 (C-23), 158.0 (C-21), and 152.7 (C-22)], four olefinic carbons [δ_C 150.3 (C-9), 150.1 (C-15), 116.5 (C-11), and 109.2 (C-16)], one oxymethine carbon [δ_C 85.5 (C-3)], two methine carbons [δ_C 45.0 (C-5) and 29.4 (C-8)], six methylene carbons [δ_C 41.5 (C-14), 38.3 (C-1), 37.6 (C-12), 26.6 (C-7), 24.4 (C-2), and 18.2 (C-6)], five singlet methyl groups [δ_C 27.7 (C-20), 25.3 (C-18), 22.5 (C-17), 18.2 (C-24), and 16.4 (C-19)], and three quaternary carbons [δ_C 38.4 (C-4), 37.4 (C-10), and 34.8 (C-13)]. Further structural information on **1** was obtained by analyzing HSQC, HMBC, and COSY spectra (Figure 2).

The singlet methyl protons H-19 and H-20 showed ^{1}H–^{13}C long-range couplings to C-3, C-4, and C-5 in the HMBC spectrum. The singlet methyl protons H-17 were coupled to C-12, C-13, C-14, and C-15, and the singlet methyl protons H-18 were coupled to C-1, C-5, C-9, and C-10. The oxymethine proton H-3 was coupled to C-1, C-2, C-4, C-19, and C-20. Long-range couplings were observed between the methine proton H-5 and the following carbons: C-4, C-6, C-7, C-10, C-18, C-19, and C-20. The olefinic proton H-11 was coupled to C-8, C-9, C-12, and C-13. The exomethylene proton H-16 was coupled to C-15. In addition, the proton spin system from the methine proton H-8 to H-7 and H-14 was confirmed in the COSY spectrum. Furthermore, homoallylic and allylic couplings were observed between H-8 and H-12 and between H-8 and H-11, respectively. These results revealed the presence of a viguiepinol skeleton[15] in **1**.

In addition to these resonances ascribed to the terpenoid moiety, **1** showed the following extra resonances, three carbonyls at δ_C 167.6 (C-23), 158.0 (C-21), and 152.7 (C-22), one methyl at δ_C 18.2 (C-24, δ_H 2.25, 3H, singlet), and one exchangeable OH proton (δ_H 10.08). One of the carbonyls (δ_C 158.0, C-21) was long-range coupled to H-3, and another carbonyl at δ_C 167.6 (C-23) was coupled to a methyl singlet at δ_H 2.25 (H-24) in the HMBC spectrum. Furthermore, the broad singlet hydroxy proton (δ_H 10.08, N-OH) was coupled to C-22 and C-23 in the HMBC spectrum. These couplings could only be observed in the HMBC spectrum taken at 4 °C when the hydroxy proton appeared as a sharp signal. The connection between C-22 and C-23 through a nitrogen atom was thus established, and finally C-21 and C-22 were connected by elimination.

Direct evidence for these linkages was obtained from the EIMS spectrum of **1**; fragment peaks at *m/z* = 343 and 358 were explained by formation of the oxonium ions, as shown in Figure 3. This result clearly established the presence of an oxalyl moiety and a unique *N,N*-diacylhydroxylamine group in **1**. The presence of this functional group was corroborated by the IR spectrum (KBr) that showed an absorption at 1800 cm^{-1} in addition to the expected bands due to an ester carbonyl residue (1760 cm^{-1}) and a hydroxy group (3420 cm^{-1}). The unusual band at 1800 cm^{-1} is compatible with the value reported for *N,N*-diacylhydroxylamine (1799 cm^{-1}).[16] Thus the structure of **1** was established unequivocally. This is the first report of the presence of the *N,N*-diacylhydroxylamine residue in a natural product.

The molecular formula of oxaloterpin B (**2**) was established as $C_{24}H_{38}N_2O_4$ by HR-MS measurement (*m/z* 419.2924 $[M + H]^+$, calcd 419.2904), indicating the presence in **2** of one more nitrogen atom than in **1**. Most of the NMR data of **2** were similar to those of **1** (Table 1); the resonances for the *N*-acetyl group in **1** were, however, replaced by two methylene resonances (δ_C 62.5, δ_H 3.75 for C-24 and δ_C 42.7, δ_H 3.46 for C-23), which were ascribed to an HO–CH_2CH_2–N function. The ^{15}N–^{1}H HSQC spectrum proved the presence of two secondary nitrogens (δ_N 99.5 and 69.1, coupled to protons at δ_H 7.11 and 8.08, respectively) that were ascribed to a substituted urea function (Figure 4) in order to explain ^{1}H–^{13}C long-range couplings between H-23 and C-22 and between H-3 and C-21. This side-chain structure was supported by HR-MS/MS data shown in Figure 5. Among others, the strong product ion at *m/z* = 87 (C_3H_7NO, *m/z* 87.0589 $[M]^+$, calcd 87.0552) derived from the precursor ion at *m/z* = 149 ($C_4H_9N_2O_4$, 149.0597 $[M + H]^+$, calcd 149.0556) via the ion at *m/z* = 131 ($C_4H_7N_2O_3$ 131.0462 $[M + H]^+$, calcd 131.0451) provided evidence for the substituted urea function in **2**. Other relevant ions were assigned to $C_3H_6NO_2$

Table 1. NMR Data (400 MHz, $CDCl_3$) for Oxaloterpins A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**) and Viguiepinone (**6**)

	oxaloterpin A (**1**)		oxaloterpin B (**2**)		oxaloterpin C (**3**)	
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)
1	38.3, CH_2	1.95, 1.42, m	38.3, CH_2	1.91, 1.38, m	38.3, CH_2	1.94, 1.41, m
2	24.4, CH_2	1.81, 1.73, m	24.6, CH_2	1.72, m	24.4, CH_2	1.80, 1.72, m
3	85.5, CH	4.67, dd, (11.6, 4.4)	83.9, CH	4.42, dd, (8.3, 7.8)	85.2, CH	4.67, dd, (11.7, 3.9)
4	38.4, qC		38.1, qC		38.3, qC	
5	45.0, CH	1.51, m	45.0, CH	1.48, m	45.0, CH	1.50, m
6	18.2, CH_2	1.78, 1.58, m	18.1, CH_2	1.79, 1.58, m	18.1, CH_2	1.79, 1.58, m
7	26.6, CH_2	1.68, 1.24, m	26.6, CH_2	1.67, 1.22, m	26.5, CH_2	1.67, 1.24, m
8	29.4, CH	2.26, m	29.3, CH	2.28, m	29.3, CH	2.26, m
9	150.3, qC		150.4, qC		150.1, qC	
10	37.4, qC		37.5, qC		37.5, qC	
11	116.5, CH	5.36, t, (2.4)	116.4, CH	5.34, t, (2.4)	116.4, CH	5.35, t, (2.2)
12	37.6, CH_2	2.02, 1.77, m	37.4, CH_2	2.00, 1.77, m	37.5, CH_2	1.99, 1.77, m
13	34.8, qC		34.8, qC		34.8, qC	
14	41.5, CH_2	1.39, 1.02, m	41.5, CH_2	1.39, 1.01, m	41.4, CH_2	1.38, 1.02, m
15	150.1, CH	5.79, dd, (17.6, 10.7)	150.2, CH	5.80, dd, (17.2, 10.4)	150.1, CH	5.79, dd, (17.6, 10.7)
16	109.2, CH_2	4.85, dd, (10.4, 1.0) 4.91, dd, (17.6, 1.0)	109.2, CH_2	4.87, dd, (10.8, 1.0) 4.90, dd, (16.8, 1.0)	109.2, CH_2	4.88, dd, (10.7, 1.0) 4.92, dd, (17.1, 1.0)
17	22.5, CH_3	0.94, s	22.4, CH_3	0.94, s	22.4, CH_3	0.94, s
18	25.3, CH_3	1.09, s	25.2, CH_3	1.07, s	25.3, CH_3	1.09, s
19	16.4, CH_3	1.02, s	16.2, CH_3	0.93, s	16.4, CH_3	1.07, s
20	27.7, CH_3	0.87, s	27.7, CH_3	0.88, s	27.7, CH_3	0.87, s
21	158.0, qC		153.9, qC		158.2, qC	
22	152.7, qC		154.1, qC		153.9, qC	
23	167.6, qC		42.7, CH_2	3.46, q, (4.9)		
24	18.2, CH_3	2.25, s	62.5, CH_2	3.75, dt, (5.8, 4.9)		
NH				8.08 br, t, (5.8)		
NH				7.11 br, s		
NOH		10.08, s				9.49 br, s

	oxaloterpin D (**4**)		oxaloterpin E (**5**)		viguirpinone (**6**)	
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).
1	38.3, CH_2	1.93, 1.41, m	38.5, CH_2	1.88, 1.43, m	39.7, CH_2	2.11, 1.64, m
2	24.4, CH_2	1.80, 1.71, m	24.8, CH_2	1.70, 1.67, m	35.1, CH_2	2.72, 2.24, m
3	84.9, CH	4.66, dd, (11.9, 4.1)	81.9, CH	4.35, dd, (11.6,; 4.4)	216.9, qC	
4	38.4, qC		38.2, qC		47.9, qC	
5	45.0, CH	1.50, m	45.0, CH	1.50, m	46.7, CH	1.81, m
6	18.1, CH_2	1.77, 1.42, m	18.1, CH_2	1.77, 1.57, m	18.6, CH_2	1.72, 1.67, m
7	26.6, CH_2	1.66, 1.23, m	26.7, CH_2	1.67, 1.24, m	26.7, CH_2	1.73, 1.29, m
8	29.4, CH	2.26, m	29.3, CH	2.27, m	29.3, CH	2.29, m
9	150.4, qC		150.7, qC		149.8, qC	
10	37.5, qC		37.5, qC		37.5, qC	
11	116.4, CH	5.35, t, (2.4)	116.1, CH	5.33, t, (2.4)	117.0, CH	5.42, t, (2.4)
12	37.6, CH_2	2.00, 1.75, m	37.5, CH_2	2.00, 1.75, m	37.5, CH_2	2.02, 1.77, m
13	34.8, qC		34.8, qC		34.9, qC	
14	41.5, CH_2	1.38, 1.01, m	41.5, CH_2	1.39, 1.00, m	41.8, CH_2	1.42, 1.05, m
15	150.2, CH	5.83, dd, (17.5, 10.7)	150.3, CH	5.79, dd, (17.6, 10.4)	150.0, CH	5.80, dd, (17.6, 10.8)
16	109.2, CH_2	4.85, dd, (10.7, 1.0) 4.93, dd, (17.6, 1.0)	109.1, CH_2	4.88, dd, (10.4, 1.2) 4.92, dd, (17.6, 1.2)	109.2, CH_2	4.87, dd, (10.8, 1.2) 4.94, dd, (17.6, 1.2)
17	22.4, CH_3	0.94, s	22.4, CH_3	0.93, s	22.4, CH_3	0.93, s
18	25.3, CH_3	1.09, s	25.3, CH_3	1.06, s	24.1, CH_3	1.26, s
19	16.4, CH_3	1.02, s	16.3, CH_3	0.89, s	24.9, CH_3	1.09, s
20	27.7, CH_3	0.88, s	27.6, CH_3	0.89, s	21.8, CH_3	1.05, s
21	158.4, qC		157.2, qC			
22	159.8, qC					
23						
24						
NH		6.88 br, s		4.65 br, s		
NH		5.79 br, s		4.65 br, s		
NOH						

(*m/z* 88.0854 $[M]^+$, calcd 88.0398), CH_4NO_2 (*m/z* 62.0476 $[M]^+$, calcd 62.0242), and C_3H_4NO (*m/z* 70.0706 $[M]^+$, calcd 70.0293).

Oxaloterpins C (**3**) and D (**4**) were also isolated from mycelium extract as a colorless oil and white needles, respectively. The molecular formulas of **3** and **4** were established as $C_{22}H_{33}NO_4$ (*m/z* 398.2299 $[M + Na]^+$, calcd 398.2307) and $C_{22}H_{33}NO_3$ (*m/z* 382.2355 $[M + Na]^+$, calcd 382.2358), respectively, by HR-MS measurements. The ^{13}C NMR data of **3** and **4** were similar to those of **1** except for the absence of signals corresponding to the acetyl group (C-23 and C-24) in **1** (Table 1). Two broad singlet NH

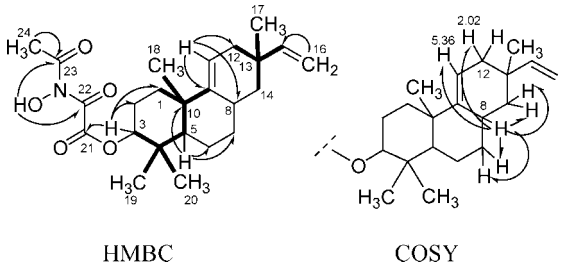


Figure 2. HMBC and COSY correlations observed for oxaloterpin A (**1**). Bold lines show HMBC correlations observed with methyl protons.

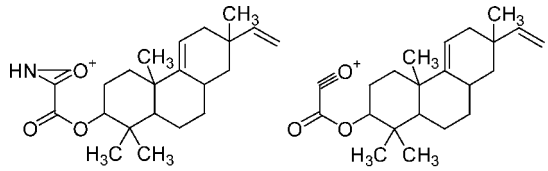


Figure 3. Fragments of oxaloterpin A (**1**) observed by EIMS.

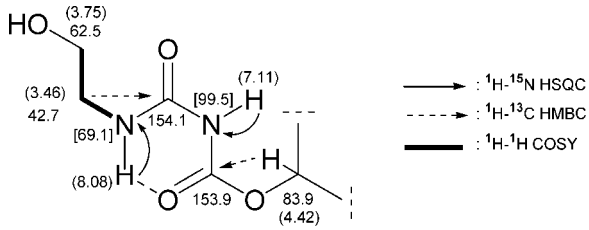


Figure 4. ^{1}H–^{15}N HSQC, ^{1}H–^{13}C HMBC, and ^{1}H–^{1}H COSY correlations observed for oxaloterpin B (**2**). Values represent ^{13}C NMR chemical shifts, and those in brackets and parentheses show ^{15}N and ^{1}H NMR chemical shifts, respectively.

protons observed at δ_H 5.79 and 6.88 in the ^{1}H NMR spectrum of **4** indicated a primary amide function. Thus, the structure of **4** was determined to have an oxalyl amide function. As a consequence, since **3** possessed one more oxygen atom than **4**, it was concluded to have an *N*-hydroxyoxalyl amide residue.

The molecular formula of oxaloterpin E (**5**) was established as $C_{21}H_{33}NO_2$ from HR-MS data (*m/z* 663.5185 [2M + H]$^+$, calcd 663.5095). The ^{13}C NMR data of **5** were similar to those of **4** except for the disappearance of a carbonyl resonance corresponding to C-22 in **4** with an upfield shift of C-3 in **5**. These spectroscopy changes together with the remaining unit ($CONH_2$) were explained by the presence of a carbamoyl ester side chain in **5**. It may be necessary to comment on the unusual MS data of **5**. Although the molecular ion peak (M + H or M + Na) was clearly observed with other members of the oxaloterpins, only the dimeric ion peak was detected with **5** in the complete absence of the corresponding monomeric ion peak. Thus, although very unlikely, there remained the possibility that this compound had a dimeric structure. X-ray diffraction analysis was thus performed to confirm the structure of **5**. The result shown in Figure 6 revealed that the compound had a monomeric form and that the carbamoyl groups formed strong intermolecular hydrogen bonds. The dimeric ion peak observed in MS measurement is considered to be caused by this strong intermolecular hydrogen bonding. The relative configuration of the diterpene moiety was also determined by this analysis as 3*R*,* 5*S*,* 8*S*,* 10*R*,* 13*S**.

Compound **6**, which was obtained as a colorless oil, was shown to have a molecular formula of $C_{20}H_{30}O$ on the basis of HR-MS measurement (*m/z* 287.2372 [M + H]$^+$, calcd 287.2369). Detailed analysis of HSQC and HMBC spectra of **6** revealed the disappearance of the side chain at C-3 with its downfield chemical shift being δ_C 216.9. Thus **6** was proved to be a diterpene with a keto function at C-3 and identified as viguiepinone (**6**).[13]

The absolute configuration of oxaloterpin A was established by application of the modified Mosher's method[17] to a free alcohol prepared by hydrolysis of **4**. This alcohol was assumed as viguiepinol (**7**) by detailed NMR studies and based on the X-ray structure of **5**. The relative configuration of **7** was established by NOESY experiments as summarized in Figure 7. A methine proton H-5 showed NOEs to H-3, H-8, and H-19, and from H-8 to H-17. Singlet methyl proton H-18 showed a NOE to singlet methyl proton H-20.

The absolute configuration of **7** was established by preparation of its MTPA esters that were subjected to analysis by the modified Mosher's method. The differences of chemical shift values obtained by subtracting (*R*)-MTPA ester from (*S*)-MTPA ester [$\delta\Delta = \delta(S)$-MTPA – $\delta(R)$-MTPA] are shown in Figure 8. On the basis of the data obtained, the absolute configuration of **7** was concluded to be 3*R,* 5*S,* 8*S,* 10*R*, and 13*S*. This structure was in agreement with that of viguiepinol (**7**) of plant origin determined by X-ray analysis.

The configurations of **1**, **2**, **3**, **4**, **5**, and **6** were determined on the basis of the absolute configuration of **7**.

Antibacterial Activity. The biological activity of oxaloterpin A (**1**) was investigated using more than 200 assay systems at MDS Pharma services (Taipei, Taiwan, http://www.mdsinc.com). Of the tests conducted so far, **1** showed antibacterial activity against *Bacillus subtilis* ATCC 43223 and *Staphylococcus aureus* ATCC 29213 with IC values of 1.9 and 3.7 nM/mL, respectively. Due to a paucity of material, biological activities of other members of the oxaloterpins were not tested.

In conclusion, six diterpene compounds have been isolated from a culture broth of the strain *Streptomyces* sp. KO-3988. We revealed the structures of oxaloterpins A to E as having an oxalyl residue or its analogue. Among them, the *N*-acetyl-*N*-hydroxyoxalylamide residue present in oxaloterpin A is unique and has never before been observed in a natural product. Furthermore we succeeded in isolating viguiepinone (**6**), an oxidation product of viguiepinol; the production of this alcohol had been suggested by heterologous expression in *E. coli* of genes relevant to the biosynthesis of pimaradiene from geranylgeranyl diphosphate of *Streptomyces* sp. KO-3988.[18]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-1000 polarimeter. IR spectra were obtained with a JASCO 470 plus FTIR or a Shimadzu 8300 FTIR spectrometer. Both 1D ^{1}H and ^{13}C NMR spectra were recorded on a JEOL Alpha 400 NMR spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. Two-dimensional ^{1}H–^{1}H COSY, NOESY, ^{1}H–^{13}C HSQC, HMBC, and ^{1}H–^{15}N HSQC 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). Ammonia (δ_N 0.00) was used as an external standard for ^{15}N NMR spectra. High-resolution FAB, EI, and ESI mass spectra were obtained using a JEOL HX-100 or a JEOL JMS-SX/SX-102A or an Applied Biosystems MDS SCIEX Q-STAR LC-MS.

HPLC purifications were carried out using a Senshu PAK PEGASIL ODS column (20φ × 250 mm, at flow rate of 14.0 mL/min) 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. Preparative TLC was performed using Merck Si gel 60 F_{254} glass-backed sheets. The detection for preparative TLC was carried out using color reactions with vanillin–H_2SO_4.

Cultivation of Strain KO-3988. *Streptomyces* sp. KO-3988 was cultivated in 15 mL test tubes each containing 5 mL of a preliminary seed medium consisting of starch 1.0%, polypeptone 1.0%, molasses 1.0%, and meat extract 1.0% (pH 7.2). The test tubes were shaken on a reciprocal shaker (200 rpm) at 28 °C for 2 days. Aliquots (1 mL) of the broth were transferred to 500 mL baffled Erlenmeyer flasks containing 100 mL of a production medium consisting of starch 2.0%,

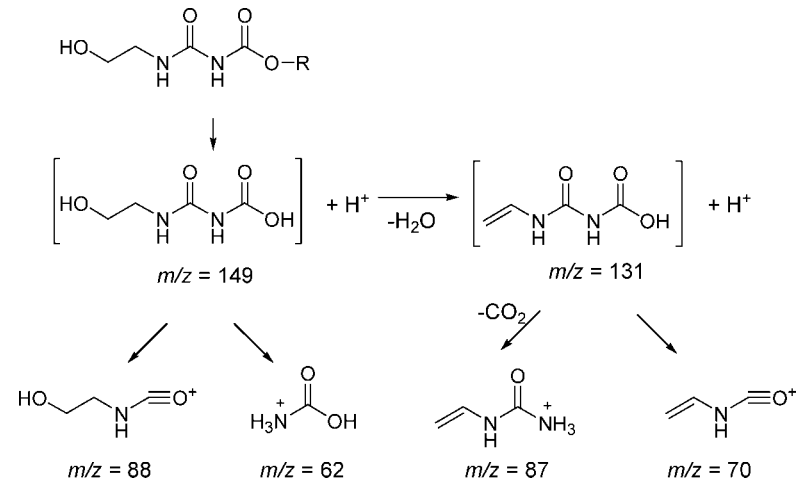


Figure 5. Fragment ions of oxaloterpin B (**2**) observed by LC-MS/MS.

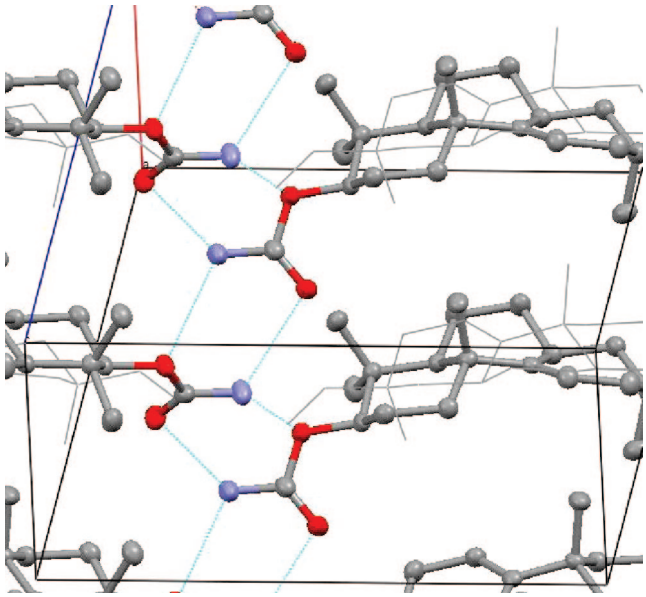


Figure 6. Intermolecular hydrogen bond of oxaloterpin E (**5**) observed by X-ray diffraction analysis.

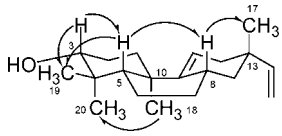
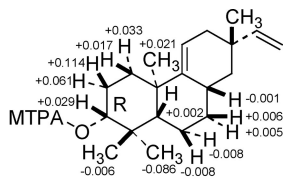


Figure 7. NOESYs observed for viguiepinol (**7**).



δΔ = (S)-MTPA - (R)-MTPA

Figure 8. δΔ values obtained for the MTPA esters of viguiepinol (**7**).

soybean meal 1.0%, NaCl 0.3%, and $CaCO_3$ 0.3% (pH 7.0). The fermentation was carried out at 28 °C and agitation at 170 rpm. After 7 days, the fermentation broth was separated into a mycelial cake and filtrate by suction filtration. The mycelial cake was dipped in 60% acetone for 2 h, and the mycelium residue was removed by filtration. The extract was evaporated *in vacuo* to remove the acetone, and the aqueous residue was extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness.

Purification of Oxaloterpins A (1), B (2), C (3), D (4), and E (5) and Viguiepinone (6). The brown oil (30 L) was subjected to Si gel column chromatography developed with *n*-hexane–EtOAc (1:1) to give fractions containing **3**, **4**, and **6**, and then with EtOAc–MeOH (3:1) to give fractions containing **1**, **2**, and **5**. The combined fraction containing **1**, **2**, and **5** was further purified by preparative TLC ($CHCl_3$–MeOH, 20:1) to give separate bands corresponding to **1**, **2**, and **5**. These fractions were finally purified by HPLC. Detection of the compounds was carried out at UV 200 nm. The column was eluted with MeCN in H_2O (90%) to give **1**, **2**, and **5** in this order. These fractions were separately concentrated *in vacuo* to give pure **1** (white powder, 24.0 mg), **2** (colorless oil, 2.5 mg), and **5** (colorless oil, 2.1 mg). The combined fraction containing **3** and **4** was purified on a Si gel column with $CHCl_3$–MeOH (20:1) as the solvent system. Finally, pure samples of **3** (colorless oil, 4.5 mg) and **4** (white needles, 10.8 mg) were obtained with ODS HPLC eluted with MeCN in H_2O (80%). The fraction containing **6** was purified by Si gel column chromatography using *n*-hexane–ether (5:1) as the solvent system. Fractions containing **6** as a major component were combined and purified by ODS HPLC using MeCN (100%) to yield a pure sample of **6** (colorless oil, 3.2 mg).

Conversion of 4 to Viguiepinol (7). Concentrated ammonia was added dropwise to a MeOH solution of **4** (4.8 mg/ 0.5 mL), and the solution was allowed to stand at room temperature for 2 h. The reaction mixture was then evaporated to give a crude oil, which was purified by preparative TLC with *n*-hexane–EtOAc (1:1) to afford free alcohol **7** as a colorless oil.

Preparation of MTPA Esters. To a solution of **7** 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% MeCN.

X-ray Crystal Data for 6. Crystal data for **6** were as follows: colorless crystal, $C_{21}H_{33}NO_2$, fw 331.50, orthorhombic, crystal size 0.20 × 0.10 × 0.10 mm, space group $P2_12_12_1$, a = 6.2395(7) Å, b = 13.6771(15) Å, c = 22.794(3) Å, V = 1945.2(4) Å^3, Z = 4, D_{calcd} = 1.132 g cm^{-3}, F_{000} = 728.00, 22 210 collected reflections, 3542 unique reflections (R_{int} = 0.027), final R1 = 0.0372 (wR2 = 0.0459) for 22 210 reflections with $I > 2.00\sigma(I)$. The X-ray measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite-monochromated Cu Kα (λ 1.54187 Å) radiation. The structure was solved by direct methods (SIR92) and refined with full-matrix least-squares on F and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model.

Oxaloterpin A (1): white powder; $[\alpha]^{25}_{D}$ –14 (*c* 1.50, $CHCl_3$); IR (KBr) 3420, 1800, 1760, 1720 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 440.2407 (calcd for $C_{24}H_{35}NO_5Na$, $[M + Na]^+$ 440.2425).

Oxaloterpin B (2): colorless oil; $[\alpha]^{25}_{D}$ –19 (*c* 0.03, $CHCl_3$); IR (KBr) 3350, 1730, 1710 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 419.2924 (calcd for $C_{24}H_{39}N_2O_4$, $[M + H]^+$ 419.2904).

Oxaloterpin C (3): colorless oil; $[\alpha]^{25}_{D}$ –14 (*c* 0.23, $CHCl_3$); IR (KBr) 3400, 1740, 1700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 398.2299 (calcd for $C_{22}H_{33}NO_4Na$, $[M + Na]^+$ 398.2307).

Oxaloterpin D (4): white needles (MeOH); mp 205 °C; IR (KBr) 3450, 1740, 1690 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 382.2355 (calcd for $C_{22}H_{33}NO_3Na$, $[M + Na]^+$ 382.2358).

Oxaloterpin E (5): colorless oil; $[\alpha]^{25}_{D}$ –15 (*c* 0.18, $CHCl_3$); IR (KBr) 3430, 1730, 1700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 663.5185 (calcd for $C_{42}H_{67}N_2O_4$, $[2M + H]^+$ 663.5095).

Viguiepinone (6): colorless oil; IR (KBr) 1730, 1710 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$), see Table 1; HR-MS *m/z* 287.2372 (calcd for $C_{20}H_{31}O$, $[M + H]^+$ 287.2369).

Viguiepinol (7): colorless oil; ^1H NMR (400 MHz, $CDCl_3$) δ 5.80 (1H, dd, $J = 17.2$, 10.4 Hz, H-15), 5.34 (1H, t, $J = 2.4$ Hz, H-11), 4.93 (1H, dd, $J = 17.6$, 1.0 Hz, H-16), 4.87 (1H, dd, $J = 10.7$, 1.0 Hz, H-16), 3.21 (1H, dd, $J = 10.5$, 5.0 Hz, H-3), 2.26 (1H, m, H-8), 2.00 (1H, m, H-12), 1.87 (1H, m, H-1), 1.78 (1H, m, H-6), 1.75 (1H, m, H-12), 1.66 (1H, m, H-7), 1.62 (1H, m, H-2), 1.58 (1H, m, H-6), 1.38 (1H, m, H-5), 1.36 (1H, m, H-14), 1.28 (1H, m, H-1), 1.22 (1H, m, H-7), 1.05 (3H, s, H-18), 1.00 (1H, m, H-14), 0.96 (3H, s, H-19), 0.94 (3H, s, H-17), 0.85 (3H, s, H-20); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.0 (C, C-9), 150.3 (CH, C-15), 116.0 (CH, C-11), 109.1 (CH_2, C-16), 79.1 (CH, C-3), 44.8 (CH, C-5), 41.6 (CH_2, C-14), 38.9 (CH_2, C-1), 38.9 (C, C-4), 37.6 (C, C-10), 37.6 (CH_2, C-12), 34.9 (C, C-13), 29.4 (CH, C-8), 28.1 (CH_2, C-2), 27.8 (CH_3, C-20), 26.8 (CH_2, C-7), 25.3 (CH_3, C-18), 22.5 (CH_3, C-17), 18.3 (CH_2, C-6), 15.3 (CH_3, C-19).

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Supporting Information Available: ^1H NMR, ^{13}C NMR, COSY, HSQC, HMBC, HRMS, and IR spectra of oxaloterpins (**1**–**5**) and viguiepinone (**6**) are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Connolly, J. D.; Hill, R. A. *Dictionary of Terpenoids*; Chapman & Hall: New York, 1992.
(2) Dewick, P. M. *Nat. Prod. Rep.* **2002**, *19*, 181–222.
(3) Kuzuyama, T.; Seto, H. *Nat. Prod. Rep.* **2003**, *20*, 171–183.
(4) Isshiki, K.; Tamamura, T.; Sawa, T.; Naganawa, H.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1986**, *39*, 1634–1635.
(5) Seto, H.; Orihara, N.; Furihata, K. *Tetrahedron Lett.* **1998**, *39*, 9497–9500.
(6) Shin-ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *Tetrahedron Lett.* **1990**, *31*, 6025–6026.
(7) Funayama, S.; Ishibashi, M.; Komiyama, K.; Omura, S. *J. Org. Chem.* **1990**, *55*, 1132–1133.
(8) Shiomi, K.; Iinuma, H.; Naganawa, H.; Isshiki, K.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1987**, *40*, 1740–1745.
(9) Bringmann, G.; Haagen, Y.; Gulder, T. A. M.; Gulder, T.; Heide, L. *J. Org. Chem.* **2007**, *72*, 4198–4204.
(10) Kuzuyama, T.; Takahashi, S.; Dairi, T.; Seto, H. *J. Antibiot.* **2002**, *55*, 919–923.
(11) Kawasaki, T.; Hayashi, Y.; Kuzuyama, T.; Furihata, K.; Itoh, N.; Seto, H.; Dairi, T. *J. Bacteriol.* **2006**, *188*, 1236–1244.
(12) Kawasaki, T.; Kuzuyama, T.; Kawamori, Y.; Matsuura, N.; Itoh, N.; Furihata, K.; Seto, H.; Dairi, T. *J. Antibiot.* **2004**, *57*, 739–747.
(13) Guerrero, C.; Nava, A. L.; Quevedo, F.; Toscano, R. A.; Soriano-Garcia, M. *Rev. Latinoam. Quim.* **1986**, *16*, 126–128.
(14) Komiyama, K.; Funayama, S.; Anraku, Y.; Ishibashi, M.; Takahashi, Y.; Omura, S. *J. Antibiot.* **1990**, *43*, 247–252.
(15) Soriano-Garcia, M.; Guerrero, C.; Toscano, R. A. *Acta Crystallogr.* **1986**, *C42*, 729–731.
(16) Ames, D. E.; Grey, T. F. *J. Chem. Soc.* **1955**, 631–636.
(17) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(18) Ikeda, C.; Hayashi, Y.; Itoh, N.; Seto, H.; Dairi, T. *J. Biochem. (Tokyo)* **2006**, *141*, 37–45.

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