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Three New Polyketide–Terpenoid Hybrids from *Penicillium* sp.

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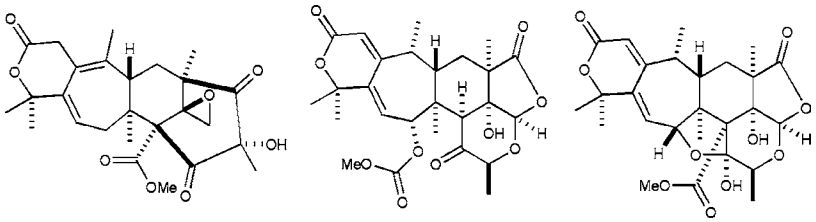
ABSTRACT



Three novel hybrid polyketide–terpenoid metabolites were isolated from a *Penicillium minioluteum* strain. Their structures were determined by NMR spectroscopic analyses and X-ray crystallography. The proposed biosynthetic pathway including a unique *retro*-Claisen migration of methyl carbonate correlates the three compounds with berkeleydione and berkeleytrione.

Marine microorganisms have been attracting increasing attention as important sources of biologically and pharmaceutically active substances.[1] We have been screening marine-derived microorganisms for their antimicroalgal, antibacterial, and antitumor activities to find novel bioactive substances. In the course of screening for antitumor substances, we detected cytotoxic activity against A549 cells (IC_{50} 500 μg/mL) in an acetone extract of the marine-derived fungus, *Penicillium minioluteum* 03HE3-1, which had been isolated from sea mud of Heita Bay, Kamaishi, Japan. The fungus was cultured in a 1/2PD (potato-dextrose) medium containing 50% seawater. After the mycelial cake was removed from the cultivation medium (7.5 L) by filtration, the filtrate was concentrated and separated by normal- and reversed-phase column chromatography and HPLC to yield three new compounds, 22-epoxyberkeleydione (**1**) (1.1 mg), miniolutelide A (**3**) (3.2 mg), and miniolutelide B (**4**) (0.8 mg) (Figure 1).

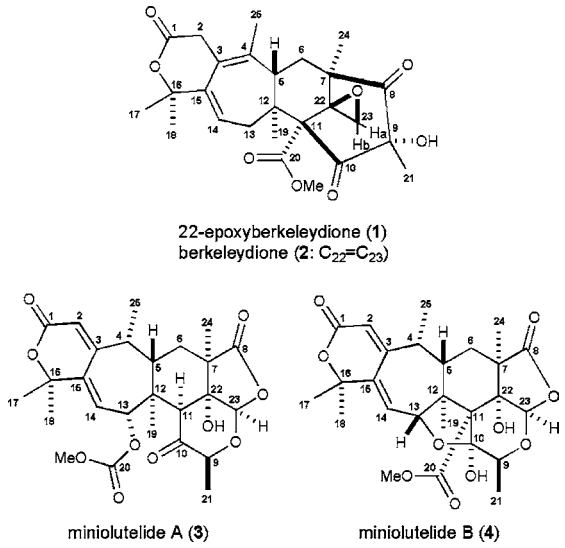


Figure 1. Structures of 22-epoxyberkeleydione (**1**), berkeleydione (**2**), miniolutelide A (**3**), and B (**4**).

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(1) Blunt, J. W.; Copp, B. R.; Murano, M. H. G.; Northcote, P. T.; Prisep, M. R. *Nat. Prod. Rep.* **2004**, *21*, 1−49.

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22-Epoxyberkeleydione[2] (**1**) was isolated as a colorless amorphous solid, and its molecular formula was determined to be $C_{26}H_{32}O_8$ by HRESIMS. The IR spectrum showed absorptions at 3508 (hydroxyl), 1732 (ester), and 1714 cm^{-1} (ketone). The ^{13}C NMR spectrum (Table 1) suggested the presence of two ketone (δ_C 207.6, 203.5) and two ester (δ_C 168.3, 167.7) groups. These groups account for seven oxygens, leaving one oxygen unassigned. A methylene carbon resonating at δ_C 50.3 was ascribable to an epoxyethyl as indicated by its large $^1J_{CH}$ coupling constant (178 Hz). The chemical shifts of the proton signals at δ_H 2.54 (d, J = 3.4 Hz) and 2.50 (d, J = 3.4 Hz), showing HSQC correlations with the carbon signal at δ_C 50.3, agree well with those of epoxyethyl protons. There are two olefinic groups evident from the carbon signals at δ_C 140.4, 133.8, 129.1, and 127.5. On the basis of detailed analyses of COSY and HMBC spectra (Figure 2), structure **1** (planar) was tentatively determined for this compound, although the connectivity of C-11 with C-10, C-20, and C-22 was uncertain. The most puzzling spectral feature of this compound was that the chemical shifts of two quaternary carbons at δ_C 67.8 (C-11) and 68.5 (C-12) were too far downfield for carbons that were not joined with oxygen, yet all eight oxygens had been designated as composing other necessary moieties. At this stage, by surveying the literature, we encountered berkeleydione[3] (**2**), whose structure had been established by X-ray analysis. The chemical shifts of C-11 and C-12 of **2** were reported to be δ_C 71.2 and 67.0, respectively, close to those of **1**. The unusually low-field chemical shifts of these carbons had been also noted by the authors, and they had verified the shifts by computer calculation. The ^1H and ^{13}C chemical shifts of **1** are practically superimposable with those of **2** except for the signals at positions 7, 11, 22, and 23, which enabled us to propose structure (planar) **1** for 22-epoxyberkeleydione. The relative stereochemistry of **1** was established by NOESY and NOE difference spectroscopy (Figure 3).

Table 1. NMR Data (C_6D_6) of 22-Epoxyberkeleydione (**1**) and Miniolutelide B (**4**)

	δ_C **1**	δ_H **1** (J in Hz)	δ_C **4**	δ_H **4** (J in Hz)
1	167.7 s		162.1 s	
2	34.5 t	β −3.08 br d (20.7)	121.8 d	5.40 s
		α −2.94 d quint (20.7, 1.7)		
3	127.5 s		155.4 s	
4	133.8 s		42.9 d	2.22 m
5	43.9 d	1.94 br d (14.1)	41.8 d	1.85 dd (12.7, 5.5)
6	39.7 t	β −1.73 dd (13.2, 3.6)	38.4 t	β −1.81 d (13.4)
		α −1.50 dd (14.1, 13.2)		α −1.68 dd (13.4, 12.7)
7	49.3 s		52.0 s	
8	207.6 s		178.9 s	
9	78.7 s		78.4 d	3.87 q (6.5)
10	203.5 s		103.9 s	
11	67.8 s		68.2 s	
12	68.5 s		50.5 s	
13	37.9 t	β −2.98 dd (14.3, 8.3)	80.7 d	4.72 d (2.8)
		α −1.67 dd (14.3, 5.2)		
14	129.1 d	5.94 dd (8.3, 5.2)	128.9 d	5.97 br s
15	140.4 s		135.4 s	
16	81.0 s		81.0 s	
17	29.3 q	1.06 s	26.0 q	1.24 s
18	26.7 q	1.32 s	27.2 q	1.06 s
19	20.9 q	1.20 s	17.8 q	0.81 s
20	168.3 s		174.3 s	
21	16.1 q	1.67 s	13.7 q	1.48 d (6.5)
22	59.8 s		79.7 s	
23	50.3 t	Hb −2.54 d (3.4)	108.8 d	5.05 s
		Ha −2.50 d (3.4)		
24	17.9 q	0.95 s	19.4 q	1.15 s
25	14.9 q	1.08 br s	18.6 q	0.61 d (7.7)
OMe	52.2 q	3.24 s	51.2 q	2.98 s
OH	(C9)	4.56 br s	(C10)	7.64 s
			(C22)	2.01 s

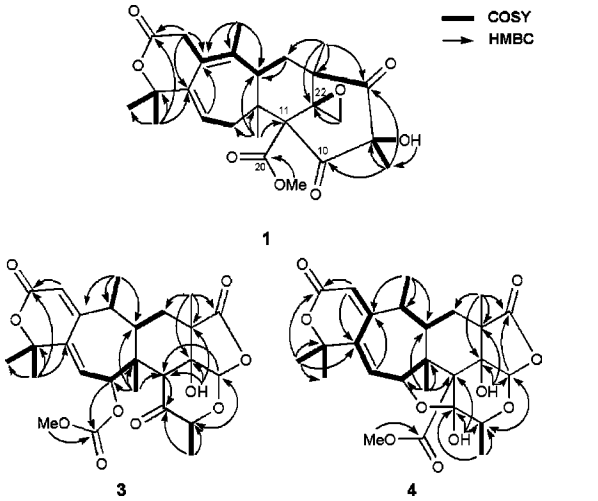

Figure 2. Key COSY and HMBC correlations of **1**, **3**, and **4**.

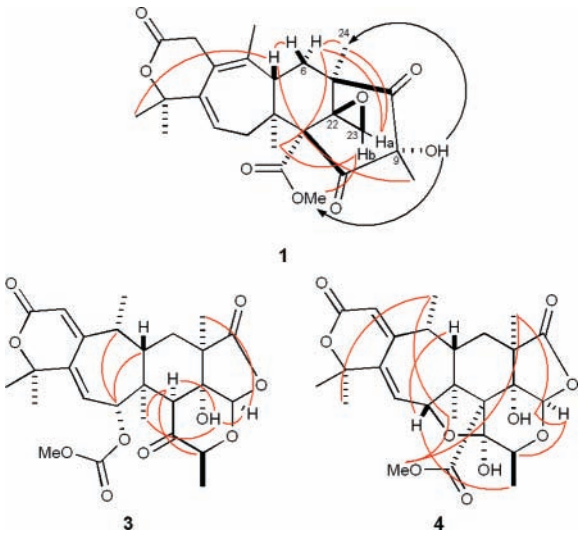

Figure 3. Selected NOE correlations of **1**, **3**, and **4**.

NOEs between H-23a/H-6α and H-23b/CO_2Me and those between 9-OH/H_3-24 and 9-OH/CO_2Me were essential for

(2) 22-Epoxyberkeleydione (**1**): colorless amorphous; $[\alpha]^{25}_D$ +80.3 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 211 (3.42) nm; CD (MeOH) $\Delta\epsilon_{231}$ +7.27, $\Delta\epsilon_{268}$ −1.67, $\Delta\epsilon_{311}$ +1.47; IR (neat) ν_{max} 3508, 2987, 2951, 1732, 1714, 1240, 1122 cm^{-1}; (+)-HRESIMS m/z [M + Na]$^+$ 495.1989 (calcd for $C_{26}H_{32}O_8Na$, 495.1995).

elucidating the stereochemistry of the 22- and 9-positions, respectively.

The absolute configuration of berkeleydione (**2**) has not been determined. We noticed that **1** had a *cis*-homodiene in its B-ring, which could be used for deducing the absolute configuration based on the helicity rule[4] of CD spectroscopy. In this method, the torsion angle of the *cis*-diene moiety is crucial. The angle was estimated to be 45° (absolute value) on the basis of the conformation deduced by the NOEs (Figure 3) and a MM2 calculation.[5]

This value seems to be reasonable because the corresponding angle found for **2** by X-ray analysis was 45°. Compound **1** showed a negative Cotton effect ($\Delta\epsilon = -1.7$) at 268 nm, which elucidated the absolute configuration of **1** as shown in Figure 1.

Miniolutelide A[6] (**3**), $C_{26}H_{32}O_{10}$ (HRESIMS), was isolated as a colorless prism. Fortunately, it yielded a single crystal that was submitted for X-ray analysis (Figure 4). The IR

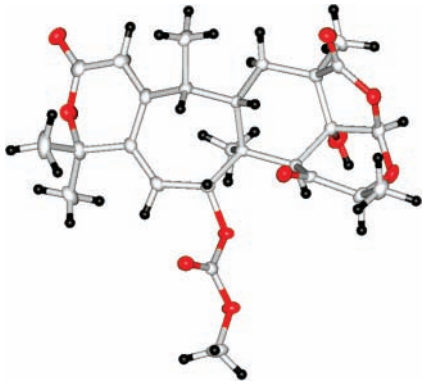

Figure 4. X-ray structure of miniolutelide A (**3**).

absorptions at 3419 (hydroxyl), 1790 (γ-lactone), 1755 (carbonate), and 1695 cm^{-1} (conjugated ester) are consistent with the X-ray structure. On the basis of the structure together with COSY, NOESY, HSQC, and HMBC (Figures 2 and 3) spectra, all the proton and carbon signals were assignable. It should be remarked that the methoxycarbonyl group (C-20: δ_C 168.3) of **1** is seemingly converted to a methyl carbonate (C-20: δ_C 155.5) in **3**. This bizarre functional migration was interpreted by discovering its possible precursor, miniolutelide B (**4**).

The torsion angle of the *trans*-diene of **3** determined by X-ray analysis is 165° (absolute value). The CD spectrum of **3** exhibited a positive Cotton effect ($\Delta\epsilon = +18.6$) at 268 nm, confirming the absolute stereostructure of **3** as shown in Figure 1.

Miniolutelide B[7] (**4**), a colorless amorphous solid, has the same molecular formula, $C_{26}H_{32}O_{10}$ (HRESIMS), as **3**. The ^{13}C NMR spectrum of **4** is very similar to that of **3** except that (i) a new acetal carbon signal appears at δ_C 103.9 (C-10) and (ii) a methoxycarbonyl group (δ_C 174.3) is present in place of a methyl carbonate (δ_C 155.5). The ^1H NMR (C_6D_6, 400 MHz) of **4** showed excellently separated signals, and by detailed analyses of COSY and HMBC spectra (Figure 2), its planar structure was constructed. The relative stereochemistry of **4** was determined by the NOESY spectrum (Figure 3). Of the two hydroxyl groups of **4**, 10-OH shows the proton signal at δ_H 7.64, lower than the signal (δ_H 2.01) of 22-OH (Table 1). When the NMR solvent was changed to acetone-d_6, the latter signal shifted down to δ_H 5.24, while the former signal remained unchanged. This phenomenon can be interpreted by assuming an intramolecular hydrogen bond between 10-OH and the carbonyl oxygen of the methoxycarbonyl group on C-11. The relatively low-frequency IR absorption of the ester carbonyl (1705 cm^{-1}) of **4** may be due to the hydrogen bonding, and these facts support the *cis* relationship of 10-OH with 11-CO_2Me groups.

The molecular models of **4** showed that, owing to the presence of a tetrahydrofuran ring, miniolutelide B had a rigid conformation, in which the *trans*-diene skews at −146° when it has the absolute configuration shown in Figure 1. In fact, the CD spectrum of **4** exhibits a negative Cotton effect ($\Delta\epsilon = -21.9$) at 267 nm, which verifies the absolute configuration.

Studies on the biosynthesis of meroterpenes produced by *Aspergillus* and *Penicillium* have suggested that this group of metabolites is derived from farnesyl pyrophosphate and 3,5-dimethylorsellinic acid (Figure 5).[8] An epoxide-initiated cyclization of **a** generates a tetracyclic intermediate (**b**), which is known as a key precursor of austin[9] and related compounds.[10−12] Oxidative transformations of **b** give rise to berkeleytrione[3] (**5**), recently isolated from an acid mine

(3) Stierle, D. B.; Stierle, A. A.; Hobbs, J. D.; Stokken, J.; Clardy, J. *Org. Lett.* **2004**, *6*, 1049−1052.

(4) Moscowitz, A.; Charney, E.; Weiss, U.; Ziffer, H. *J. Am. Chem. Soc.* **1961**, *83*, 4661−4663.

(5) MM2 calculation was performed using CS Chem3D Pro 9.0.1.

(6) Miniolutelide A (**3**): colorless prism (MeOH/H_2O), mp 186.5−187.2 °C; $[\alpha]^{23}_D$ +77.3 (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 268 (3.95) nm; CD (MeOH) $\Delta\epsilon_{268}$ +18.62; IR (neat) ν_{max} 3419, 2985, 1790, 1755, 1695, 1261, 1165, 1120, 974 cm^{-1}; ^1H NMR (C_6D_6, 400 MHz) δ_H 6.47 (1H, d, J = 1.3 Hz, H-13), 5.78 (1H, t, J = 1.7 Hz, H-14), 5.76 (1H, br s, H-2), 5.15 (1H, s, H-23), 4.10 (1H, q, J = 6.6 Hz, H-9), 3.36 (3H, s, OMe), 2.61 (1H, s, H-11), 2.23 (1H, m, H-5), 2.18 (1H, q, J = 6.8 Hz, H-4), 1.93 (1H, dd, J = 13.5, 2.2 Hz, H-6β), 1.45 (3H, d, J = 6.6 Hz, H-21), 1.33 (3H, s, H-18), 1.29 (1H, s, 22-OH), 1.15 (3H, s, H-17), 0.90 (1H, t, J = 13.5 Hz, H-6α), 0.85 (3H, s, H-24), 0.82 (3H, s, H-19), 0.68 (3H, d, J = 6.8 Hz, H-25); ^{13}C NMR (C_6D_6, 75 MHz) δ_C 207.3 (s, C-10), 174.1 (s, C-8), 162.8 (s, C-1), 155.5 (s, C-20), 154.4 (s, C-3), 141.3 (s, C-15), 130.4 (d, C-14), 116.7 (d, C-2), 103.4 (d, C-23), 83.0 (s, C-22), 81.8 (s, C-16), 79.9 (d, C-13), 76.7 (d, C-9), 58.2 (d, C-11), 54.7 (q, OMe), 50.3 (s, C-7), 43.0 (s, C-12), 39.9 (d, C-5), 37.4 (d, C-4), 28.1 (q, C-17), 26.3 (q, C-18), 26.1 (t, C-6), 19.9 (q, C-24), 18.7 (q, C-21), 15.7 (q, C-19), 15.4 (q, C-25); (+)-HRESIMS *m/z* $[M + Na]^+$ 527.1893 (calcd for $C_{26}H_{32}O_{10}Na$, 527.1893).

(7) Miniolutelide B (**4**): colorless amorphous; $[\alpha]^{23}_D$ −252.5 (*c* 0.08, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 268 (3.92) nm; CD (MeOH) $\Delta\epsilon_{267}$ −21.91; IR (neat) ν_{max} 3354, 2991, 2943, 1788, 1705 cm^{-1}; (+)-HRESIMS *m/z* $[M + Na]^+$ 527.1893 (calcd for $C_{26}H_{32}O_{10}Na$, 527.1893).

(8) Simpson, T. J.; Ahmed, S. A.; McIntyre, C. R.; Scott, F. E.; Sadler, I. H. *Tetrahedron* **1997**, *53*, 4013−4034.

(9) Chexal, K. K.; Springer, J. P.; Clardy, J.; Cole, R. J.; Kirksey, J. W.; Dorner, J. W.; Cutler, H. G.; Strawter, W. J. J. *J. Am. Chem. Soc.* **1976**, *98*, 6748−6750.

(10) Okuyama, E.; Yamazaki, M.; Kobayashi, K.; Sakurai, T. *Tetrahedron Lett.* **1983**, *24*, 3113−3114.

(11) Geris dos Santos, R. M.; Rodrigues-Fo, E. *Phytochemistry* **2002**, *61*, 907−912.

(12) Kosemura, S. *Tetrahedron* **2003**, *59*, 5055−5072.

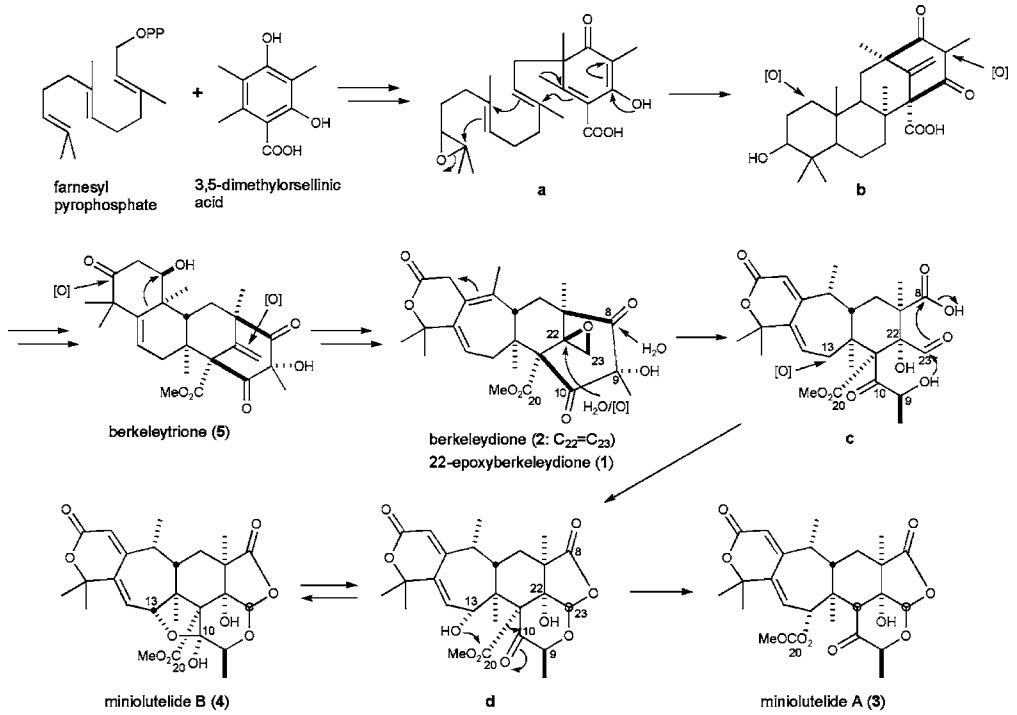

Figure 5. Biosynthetic pathway of compounds **1**−**5** through the presumed intermediates **a**−**d**.

organism together with berkeleydione (**2**). Berkeleydione is supposed to be produced from the trione (**5**) by (i) a Baeyer−Villiger type oxidation of the A-ring ketone and (ii) a 1,2-alkyl shift to form a cycloheptadiene B-ring. Epoxidation of the *exo*-methylene produces 22-epoxyberkeleydione (**1**), which is converted to **c** via hydrolytic cleavage of C-8/C-9 and hydrolysis of the epoxide followed by oxidation of the resulting primary alcohol to an aldehyde. Acetal lactonization and introduction of a hydroxy group at C-13 yields **d**, which forms another acetal between 13-OH and 10-ketone, affording miniolutelide B (**4**). In intermediate **d**, migration of the methoxycarbonyl group (C-20) to 9-OH by intramolecular *retro*-Claisen condensation results in miniolutelide A (**3**).

Due to a limited amount of new compounds, **1**, **3**, and **4**, MRSA inhibitory activity was tested only for **3**, which gave a negative result (50 μg/disk). A larger scale cultivation of the *Penicillium minioluteum* strain to investigate these compounds' cytotoxic activities is now in progress.

Supporting Information Available: Experimental procedures, 1D/2D NMR spectra of compounds **1**, **3**, and **4**, Chem3D files of **1** and **4**; X-ray crystallographic file in CIF format. This material is free of charge via the Internet at http://pubs.acs.org.

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