Four Pimarane Diterpenes from Marine Fungus: Chloroform Incorporated in Crystal Lattice for Absolute Configuration Analysis by X-ray

Sanae Yoshida,[1] Keijiro Kito,[1] Takashi Ooi,[1] Kaneo Kanoh,[2] Yoshikazu Shizuri,[2] and Takenori Kusumi*[1]
[1]*Institute of Health Biosciences, the University of Tokushima Graduate School, Tokushima 770-8505*
[2]*Marine Biotechnology Institute Co., Ltd., Heita, Kamaishi 026-0001*

(Received August 2, 2007; CL-070827; E-mail: tkusumi@ph.tokushima-u.ac.jp)

Separation of the ethyl acetate extract of marine fungus *Cryptosphaeria eunomia* var. *eunomia* afforded a new pimarane-type diterpene **1** together with known compounds, **2**, **4**, and **5**, whose absolute configurations were unknown. During recrystallization experiments using chloroform, compounds **1**, **2**, and **5** gave crystals suitable for X-ray analyses, which revealed that the crystals incorporated chloroform molecules in the crystal lattices. Based on the intense anomalous scattering by chlorine, the absolute configurations of **1**, **2**, and **5** were unambiguously determined.

Marine natural products continue to draw attention from scientists in industry and academia because of their unique chemical structures and strong bioactivities.[1]

During our study of marine fungus *Cryptosphaeria eunomia* var. *eunomia*, we isolated four pimarane diterpenes: **1** (new; named 11-deoxydiaporthein A),[2] **2** (diaporthein A),[3] **4** (scoparane A),[4] and **5** (diaporthein B).[3] This paper elucidates the structure of **1** and provides X-ray analyses leading to the absolute stereochemistry of the three of the pimaranes (**1**, **2**, and **5**), which was feasible because of the incorporation of chloroform into the crystals.

The filtered cultivation medium of *C. eunomia* var. *eunomia*, separated from an unidentified sponge growing off Pohnpei Island in the South Pacific, was successively extracted with hexane, ethyl acetate, and butanol. The ethyl acetate extract was concentrated and the residue (500 mg) was repeatedly fractionated by flash chromatography followed by HPLC, giving **1** (18.1 mg), **2** (15.4 mg), **4** (1.5 mg), and **5** (3.3 mg) (Figure 1).

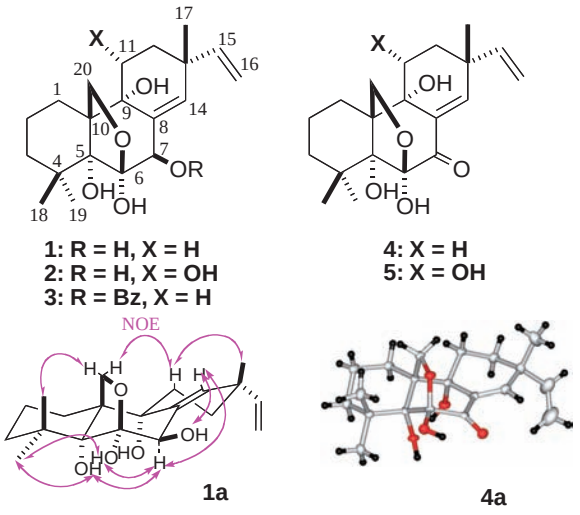

Figure 1. Structures of pimarane diterpenes **1**, **2**, **4**, and **5**, and benzoate **3** and NOESY correlations of **1** (**1a**). **4a**: X-ray structure of **4**.

11-Deoxydiaporthein A (**1**), $[\alpha]_D^{25} = +61.1$ (c 0.46, $CHCl_3$), shows an $[M - H]^-$ ion in the TOFMS-ESI at m/z 349.2008 (Δ −0.7 mDa), indicating its molecular formula is $C_{20}H_{30}O_5$. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) indicates three singlets due to methyls (δ 1.41, 1.23, and 1.07), a methylene connected with an oxygen [δ 3.89 (1H, d, $J = 10$ Hz), 3.40 (1H, d, $J = 10$ Hz)], a vinyl group [δ 5.86 (1H, dd, $J = 18$, 10 Hz), 5.05 (1H, dd, $J = 18$, 1 Hz), 4.99 (1H, dd, $J = 10$, 1 Hz)], and a trisubstituted olefin [δ 5.91 (1H, t, $J = 2$ Hz)], suggesting that this compound is a diterpenoid. In this solvent, the signals of the four hydroxy groups appear separately at δ 4.69 (s), 4.22 (s), 3.93 (s), and 1.89 (d). These characteristics suggest that one of the OH groups is secondary and the others are tertiary.

The ^{13}C NMR (75 MHz, $CDCl_3$) signals support the pres-

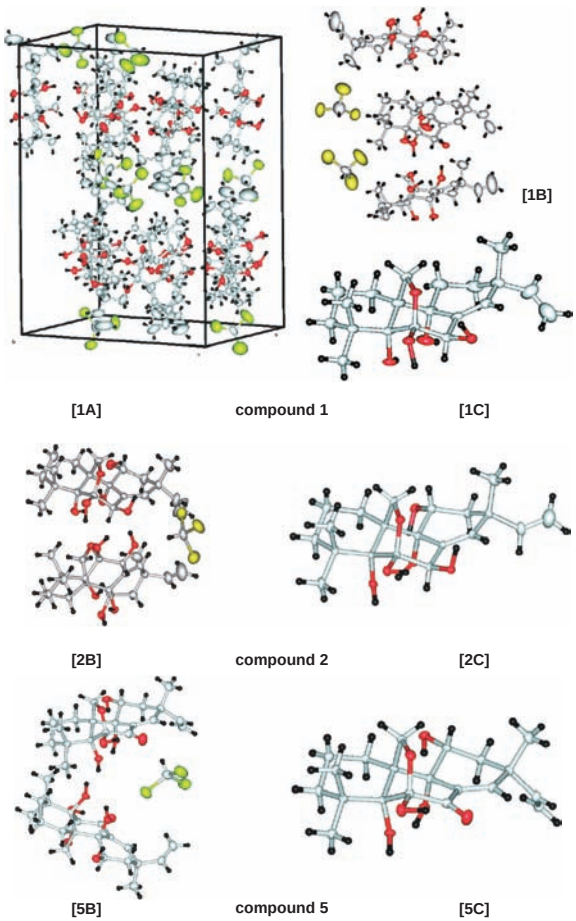

Figure 2. X-ray structures of **1**, **2**, and **5**.

ence of the moieties revealed by the ^{1}H NMR; 3 × Me: δ 27.6, 24.4, 23.9; –CH=CH$_2$: δ 111.3 (t), 147.2 (d); –CR=CHR′: δ 133.9 (d), 137.4 (s); –CH$_2$O: δ 68.2 (t); CH–O: δ 73.2 (d); 3 × quaternary C–O: δ 106.1 (s), 81.0 (s), 76.2 (s). The low-field singlet at δ 106.1 must be an acetal carbon. Additionally, there are five sp^3-methylene signals at δ 37.9, 29.8, 28.4, 21.7, and 17.8.

Eventually, the planar structure of **1** was deduced by the extensive study of its 2D NMR spectra (COSY, HSQC, and HMBC),[9] and the relative stereochemistry (**1a**) was deduced by the ^{1}H coupling constants and the NOESY correlations (Figure 1). The ^{1}H signals of the hydroxy groups were helpful for the analysis.

We then intended to deduce the absolute configurations of **1** by converting it to benzoate **3**[5] (benzoyl chloride/pyridine), which showed a positive split Cotton effect at 230 nm due to the interaction between the benzoyl and the olefin chromophores,[6] implying the R-configuration at C-7 as shown in **1**.

When a chloroform solution of **1** was allowed to stand in a refrigerator, crystals were deposited from the solution. X-ray analysis of the crystal revealed that chloroform was incorporated in the crystal lattice as shown in [1A] of Figure 2. An asymmetric unit incorporating three molecules of **1** and two chloroform molecules is demonstrated in [1B]. Utilizing the strong anomalous scattering by the chlorine atom, the structure including the absolute configuration of **1** was firmly established as in [1C], and this structure is in accord with the structure as deduced by NMR and CD spectra.

The structure of **1** is closely related with that of diaporthein A (**2**), a pimarane that has been obtained from a culture broth of the fungus *Diaporthe* sp. BCC 6140.[3] This compound was also isolated in the present experiment. The absolute configuration of **2** is unknown, and even the relative configuration at C-7 of **2** has been controversial.[3,4] In the present experiment, this compound was initially obtained as an amorphous solid and, inspired by the above finding, we crystallized the solid from chloroform. To our delight, X-ray crystallography revealed that the unit cell was composed of eight molecules of **2** and four chloroform molecules (an asymmetric unit is depicted in [2B]), and the absolute stereochemistry was confirmed as in [2C] by use of X-ray scattering by chlorine atoms of chloroform.

Scopararane A (**4**)[4] and diaporthein B (**5**)[3] were identified by comparing their physical properties including the chiroptical data with those reported in the literature. The latter **5** formed a crystal containing chloroform molecules, which enabled us to elucidate the absolute configuration by X-ray as shown in structure **5** (Figure 2). On the other hand, the crystal of **4** does not include chloroform when recrystallized from chloroform. Recrystallization of **4** from dichloromethane and carbon disulfide was attempted in hopes that the solvent composed of a heavy atom might be contained in the crystal lattice. Although the attempts were fruitless, the crystal from the chloroform solution was good for X-ray analysis, confirming the relative stereochemistry of **4** (Figure 1: **4a**).

There are a few precedent reports[7,8] on the absolute configurational determination by X-ray crystallography utilizing anomalous scattering caused by chloroform in the crystal lattice. Interestingly, diacetylated acrenol, which has the same pimarane skeleton as the present diterpenes, forms a chloroform solvate, and its absolute configuration has been determined by X-ray analysis.[8]

References and Notes

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2 11-Deoxydiaporthein A (**1**): colorless platelet; FT-IR (neat) ν/cm^{-1} 3363, 2929, 1008; ^{1}H NMR (400 MHz, CDCl$_3$) δ 5.91 (1H, t, J = 2 Hz, H-14), 5.86 (1H, dd, J = 18, 10 Hz, H-15), 5.05 (1H, dd, J = 18, 1 Hz, H-16a), 4.99 (1H, dd, J = 10, 1 Hz, H-16b), 4.69 (1H, s, 9-OH), 4.54 (1H, dd, J = 12, 2 Hz, H-7), 4.22 (1H, s, 6-OH), 3.93 (1H, s, 5-OH), 3.89 (1H, d, J = 10 Hz, H-20a), 3.40 (1H, d, J = 10 Hz, H-20b), 1.92–1.84 (2H, overlapped, H-1a, 12a), 1.89 (1H, d, J = 13 Hz, 7-OH), 1.71–1.59 (5H, overlapped, H-11a,b, 2a,b, 3a), 1.55 (1H, overlap, H-2b), 1.40 (1H, overlap, H-1b) 1.41 (3H, s, H-18), 1.23 (3H, s, H-19), 1.27–1.17 (1H, m, H-3b), 1.07 (3H, s, H-17); ^{13}C NMR (75 MHz, CDCl$_3$) δ 147.2 (d, C-15), 137.4 (s, C-8), 133.9 (d, C-14), 111.3 (t, C-16), 106.1 (s, C-6), 81.0 (s, C-5), 76.2 (s, C-9), 73.2 (d, C-7), 68.2 (t, C-20), 48.7 (s, C-10), 37.9 (t, C-3), 37.8 (s, C-4), 37.5 (s, C-13), 29.8 (t, C-12), 28.4 (t, C-11), 27.6 (q, C-19), 24.4 (q, C-18), 23.9 (q, C-17), 21.7 (t, C-1), 17.8 (t, C-2).

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5 11-Deoxydiaporthein A 7-benzoate (**3**): colorless amorphous, $[\alpha]_D^{25}$ +48.9 (*c* 0.08, CHCl$_3$); CD (0.35 mM, MeOH, 2 mm cell), $\Delta\varepsilon_{\lambda max}$ +5.8 (230 nm), −3.4 (208 nm), UV (MeOH) λ_{max} (log ε) 223 nm (4.17); FT-IR (neat) ν/cm^{-1} 3187, 2925, 1716, 1270; ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.14 (2H, d, J = 8 Hz, H-2′), 7.60 (1H, t, J = 7 Hz, H-4′), 7.46 (2H, t, J = 8 Hz, H-3′), 6.23 (1H, d, J = 2 Hz, H-7), 5.76 (1H, dd, J = 17, 10 Hz, H-15), 5.49 (1H, t, J = 2 Hz, H-14), 4.94 (1H, dd, J = 17, 1 Hz, H-16a), 4.87 (1H, dd, J = 10, 1 Hz, H-16b), 3.99 (1H, d, J = 10 Hz, H-20a), 3.58 (1H, d, J = 10 Hz, H-20b), 1.93 (1H, m, H-1a), 1.87 (1H, m, H-12a), 1.71 (2H, m, H-11), 1.70 (1H, m, H-3a), 1.69 (1H, m, H-2a), 1.61 (1H, m, H-2b), 1.50 (1H, m, H-12b), 1.48 (1H, m, H-1b), 1.42 (3H, s, H-19), 1.22 (3H, s, H-18), 1.17 (1H, m, H-3b), 1.06 (3H, s, H-17); ^{13}C NMR (75 MHz, CDCl$_3$) δ 166.2 (s, CO), 147.0 (d, C-15), 133.5 (d, C-4′), 133.3 (d, C-14), 132.4 (s, C-8), 129.9 (d, C-2′), 129.1 (s, C-1′), 128.5 (d, C-3′), 111.2 (t, C-16), 106.1 (s, C-6), 81.9 (s, C-5), 76.3 (s, C-9), 75.4 (s, C-7), 68.7 (t, C-20), 49.2 (s, C-10), 38.0 (s, C-13), 37.9 (s, C-4), 37.7 (t, C-3), 29.9 (t, C-12), 28.5 (t, C-11), 27.7 (q, C-18), 24.0 (q, C-17), 23.9 (q, C-19), 21.6 (t, C-1) 17.8 (t, C-2); TOFMS-ESI m/z: $[M-H]^-$ calcd for $C_{27}H_{33}O_6$ 453.2277; found, 453.2295 (Δ +1.8 mDa).

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