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Six New Antimicrobial and Nematicidal Bisabolanes from the Basidiomycete *Cheimonophyllum candidissimum*

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Abstract: The structures of six new bisabolane sesquiterpenes, cheimonophyllon A - E (**1** - **5**) and cheimonophyllal (**6**), have been determined by spectroscopic methods. The compounds are fungal metabolites isolated from cultures of the Basidiomycete *Cheimonophyllum candidissium*, and possess antibiotic and nematicidal activities.

Six sesquiterpenes (shown in Figure 1) that are toxic towards the nematode *Caenorhabditis elegans* were recently isolated from extracts of submerged cultures of the Basidiomycete *Cheimonophyllum candidissimum*.[1]

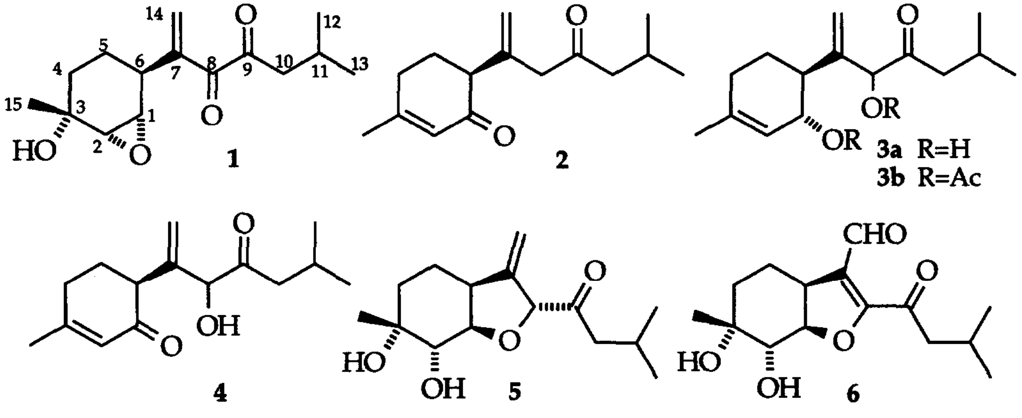

Figure 1. Cheimonophyllon A-E (1-5) and cheimonophyllal (6). These structures represent the relative and not necessarily the absolute stereochemistry.

Cheimonophyllon A (**1**), cheimonophyllon C (**3a**) and cheimonophyllon E (**5**) are the major metabolites, and cheimonophyllon A (**1**) is the most active against nematodes and microorganisms.[1] The structures of the compounds were determined by a combination of high resolution mass spectrometry which indicated the elemental composition of the compounds, and NMR spectroscopy (the NMR data are summarized in Tables 1 and 2 as well as in Figures 2 and 3). Cheimonophyllon C (**3a**) exists in solution as a 1:1 mixture between the open form **3a** and the bicyclic acetal **3c** (see Figure 4). In order to facilitate the structure determination of the compound it was acetylated and isolated as its diacetyl derivative **3b**. ^{1}H-^{1}H as well as ^{1}H-^{13}C long-range correlation spectroscopy revealed that the compounds possess a bisabolane skeleton, and the significant ^{1}H-^{13}C long-range correlations observed for the compounds are shown in Figure 2. The ^1J(C,H) coupling constants observed for C-1 (176.6 Hz) and C-2 (176.7 Hz) in the coupled ^{13}C NMR spectrum of cheimonophyllon A (**1**) confirmed[2] the presence of an epoxide functionality in this compound.

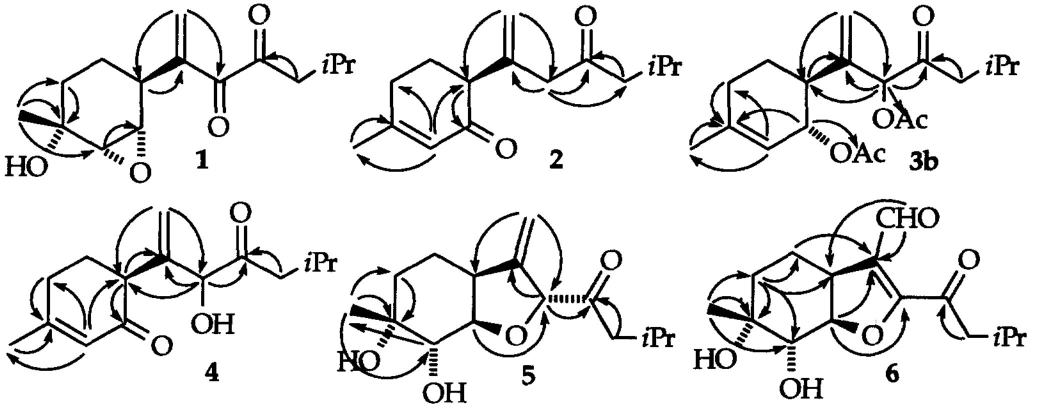

Figure 2. Significant ^{1}H-^{13}C long-range correlations observed for cheimonophyllon A (**1**), B (**2**), D (**4**), E (**5**), diacetylcheimonophyllon C (**3b**) and cheimonophyllal (**6**).

The relative stereochemistry of the compounds was studied by NMR, and especially NOESY experiments (see Figure 3) gave valuable information (the structures are arbitrarily displayed in the (*S*) C-6 configuration). With cheimonophyllon A (**1**), which contains an epoxide and an α-diketo functionality, NOESY correlations (recorded in CD_3OD in which the signals for 2-H and 6-H are separated) were observed between 14-Ha and 1-H, and between 6-H and 4-Ha as well as 5-Ha. $J_{4a\text{-}5b}$ and $J_{5b\text{-}6}$ are large (10.8 and 8.8 Hz) suggesting that H-4a, 5-Hb and 6-H all are axial and α, β and α, respectively, in the preferred conformation of cheimonophyllon A (**1**). Examination of a Dreiding model of this conformation show that the dihedral angle H-1/C-1/C-6/H-6 is close to 90° which is in agreement with the small $J_{1\text{-}6}$ (< 0.5 Hz) observed. Due to signal overlapping, NOESY correlations between 15-H_3 and any of the C-4 or C-5 protons are difficult to observe, but a strong correlation between 15-H_3 and 2-H and a weak correlation between 15-H_3 and 14-Ha support the suggested stereostructure. 6-H is axial also in diacetylcheimonophyllon C (**3b**), and NOESY correlations were observed between H-4a (axial and α) and 6-H, between 1-H (axial and β) and 5-Hb (axial and β), and between 14-Hb and 1-H as well as 5-Hb. $J_{1\text{-}6}$, $J_{4a\text{-}5b}$ and $J_{5b\text{-}6}$ are all 8 Hz or larger, supporting the suggestion that 1-H, 4-Ha, 5-Hb and 6-H are axial. In both diacetylcheimonophyllon C (**3b**) and cheimonophyllon D (**4**) it was impossible to assign the relative configuration of C-8, and insufficient amounts of the compounds were available for the preparation of suitable derivatives. The trans-diaxial relationship between 1-H and 2-H of cheimonophyllon E (**5**) is indicated by the large coupling constant (8 Hz), and confirmed by the NOESY correlation between 2-H and 8-H. Further NOESY correlations between 1-H and 6-H as well as between 6-H and 10-H_2 places 6-H on the same side as 1-H, which is consistent with the observed coupling constant between the two protons. 15-H_3 gives a NOESY correlation to 2-H, but not to 1-H, while 4-Ha correlates to 2-H and 14-Hb, and this determines the relative stereostructure of cheimonophyllol E (**5**) unequivocally. In cheimonophyllal (**6**) the similar NOESY correlation between 15-H_3 and 2-H, but not to 1-H, is observed, while a strong correlation is observed between 1-H and 6-H, showing that compounds **5** and **6** have the same relative stereochemistry.

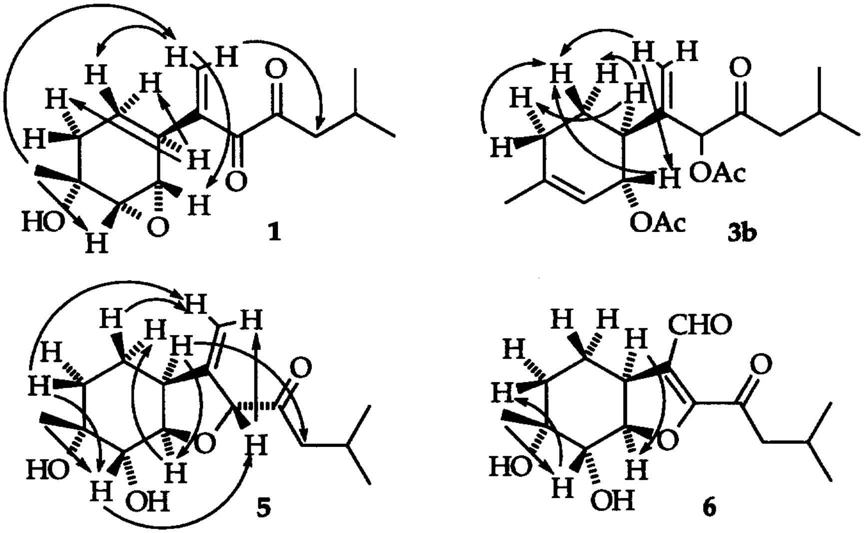

Figure 3. Significant NOESY correlations observed for cheimonophyllon A (**1**), diacetylcheimonophyllon C (**3b**), cheimonophyllon E (**5**) and cheimonophyllal (**6**).

Cheimonophyllon C (**3a**) and cheimonophyllon E (**5**) are formed predominantly in the end of the fermentation, while cheimonophyllon A (**1**) is formed in the beginning, and it may therefore be that cheimonophyllon A (**1**) is the precursor to the other bisabolanes isolated from *C. candidissimum*. The bisabolanes are a large group of sesquiterpenes mainly found as constituents of higher plants, and for many of them various biological activities have been reported.[3] Bisabolanes containing a C-14 exomethylene group[4] or the bicyclic structure of cheimonophyllon E (**5**) and cheimonophyllal (**6**) have previously been reported,[5,6] although no bisabolanes with a 1,2 epoxide or an α-diketo functionality are listed in reference 3. Interestingly, few bisabolanes have been

Table 1. ^1H (500 MHz) NMR Data (δ; Multiplicity; *J*) for Cheimonophyllon A (**1**), B (**2**), D (**4**), E (**5**), Diacetylcheimonophyllon C (**3a**) and Cheimonophyllal (**6**). The Spectra were Recorded in $CDCl_3$, and the Solvent Signal (7.26 ppm) was Used as Reference. The Coupling Constants *J* are Given in Hz.

	1	2	3b	4	5	6
1	3.18; d; 3.9	-	5.34; dm; 8	-	4.35; dd; 8, 8	4.74; dd; 6.7, 9.7
2	3.09; d; 3.9	5.86; brs	5.37; m	5.90; brs	3.14; d; 8.1	3.57; d; 6.7
4a	1.52; ddd; 2.9, 10.8, 13.4	2.35; m	2.07 m	2.39; ddd; 4.5, 12, 18	1.58; ddd; 3.2, 4, 14	1.73; ddd; 4.4, 8.6, 14.1
4b	1.41; ddd; 3.0, 7.5, 13.4	2.33; m	1.92; ddd; 5, 5, 17.8	2.32; ddd; 4, 4, 18	1.43; ddd; 4.5, 13, 14	1.53; ddd; 4.5, 8.2, 14.1
5a	1.66; dddd; 2.9, 6.3, 7.5, 13.7	2.10; m	1.85; dddd; 3.2, 4, 5, 13.3	2.21; m	2.12; m	2.10; m
5b	1.29; dddd; 3.0, 8.8, 10.8, 13.7	2.06; m	1.60; dddd; 5.4, 9.4, 10.5, 13.3	2.02; dddd; 3.6, 5, 5, 13.2	1.76; dddd; 3, 3, 4.3, 14.5	1.85; m
6	3.09; dd; 6.3, 8.8	3.02; dd; 4.9, 11.2	2.49; ddd; 3.2, 7.9, 10.5	3.08; dd; 4.6, 12.5	2.91; m	3.53; ddd; 7, 7, 9.7
8a	-	3.31; d; 5.8	5.38; s	4.63; d; 4.0	4.69; ddd; 2, 2, 2	-
8b	-	3.18; d; 5.8	-	-	-	-
10a	2.60; d; 6	2.37; m	2.40; dd; 8.7, 16.8	2.57; dd; 7.0, 16.7	2.50; dd; 6.7; 17.4	2.65; dd; 6.9, 16.8
10b	2.60; d; 6	2.37; m	2.29; dd; 6.7, 16.8	2.50; dd; 6.7, 16.7	2.33; dd; 7.0, 17.4	2.62; dd; 6.7, 16.8
11	2.18; m	2.12; m	2.16; m	2.21; m	2.91; m	2.21, m
12	0.96; d; 6.7	0.92; d; 6.8	0.92; d; 7	0.95; d; 6.6	0.89; d; 6.7	0.97; d; 6.7
13	0.96; d; 6.7	0.92; d; 6.8	0.91; d; 7	0.95; d; 6.6	0.87; d; 6.7	0.97; d; 6.7
14a	6.19; s	5.04; brs	5.25; s	5.25; brs	5.22; dd; 2, 3	10.21; s
14b	6.10; d; 0.7	5.01; brs	5.21; s	5.16; brs	5.02; dd; 2, 3	-
15	1.37; s	1.95; brs	1.69; brs	1.98; brs	1.23; s	1.30; s
1-OAc	-	-	2.00; s	-	-	-
8-OAc	-	-	2.14; s	-	-	-
8-OH	-	-	-	4.25; d; 4.0	-	-

reported from Basidiomycetes, examples are α-bisabolol, a sesquiterpene possessing antiinflammatory activity which can be obtained from the essential oil of *Matricaria chamomilla* (chamomille oil) and which previously has been isolated from *Merulius tremellosus*,[7] and lipistirone (7) (see Figure 4) isolated from *Lepista irina*.[6]

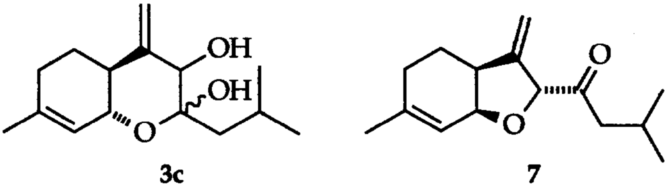

Figure 4 **3c** **7**

Table 2. ^{13}C (125 MHz) NMR Data (δ; Multiplicity) for Cheimonophyllon A (**1**), B (**2**), D (**4**), E (**5**), Diacetylcheimonophyllon C (**3b**) and Cheimonophyllal (**6**). The Spectra were Recorded in $CDCl_3$, and the Solvent Signal (77.0 ppm) was Used as Reference.

	1	2	3b	4	5	6
1	58.8; d	199.0; s	72.2; d	199.7; s	83.9; d	88.1; d
2	59.7; d	126.6; d	120.6; d	126.4; d	74.8; d	74.8; d
3	68.6; s	161.8; s	139.5; s	163.1; s	72.2; s	70.8; s
4	32.7; t	30.7; t	29.3; t	31.1; t	31.8; t	33.8; t
5	24.2; t	27.6; t	26.7; t	28.8; t	18.8; t	20.9; t
6	35.4; d	52.2; d	41.9; d	49.3; d	41.4; d	40.1; d
7	144.6; s	141.1; s	143.0; s	145.9; s	146.1; s	125.5; s
8	194.2; s	50.2; t	80.4; d	81.0; d	85.2; d	161.7; s
9	202.7; s	208.8; s	203.7; s	209.6; s	208.3; s	195.1; s
10	47.6; t	51.3; t	47.1; t	47.0; t	46.6; t	49.3; t
11	24.0; d	24.4; d	24.0; d	24.4; d	23.5; d	24.1; d
12	22.5; q	22.5; q	22.6; q	22.6; q	22.6; q	22.5; q
13	22.5; q	22.5; q	22.4; q	22.5; q	22.4; q	22.5; q
14	132.8; t	116.1; t	116.8; t	117.5; t	106.8; t	188.8; d
15	24.5; q	24.1; q	23.0; q	24.2; q	26.7; q	26.8; q
1-OAc			21.2; q/170.6; s			
8-OAc			20.7; q/170.0; s			

EXPERIMENTAL

The fermentation of the producing organism (*Cheimonophyllum candidissimum* (Berk. & Curt.) Sing.), as well as the biological activities of the six bisabolanes **1-6** is reported separately.[1] The compounds were isolated from an ethyl acetate extract of the culture fluids, by chromatography on silica gel columns eluted by cyclohexane:ethyl acetate 3:1. The extracts of 20 l culture fluids yielded 54 mg of **1**, 9 mg of **2**, 30 mg of **3**, 2 mg of **4**, 16 mg of **5**, and 7 mg of **6**. The NMR spectra (see Tables 1 and 2) were recorded with a Bruker ARX500 spectrometer, the UV spectra with a Perkin Elmer λ16, the IR spectra with a Bruker IFS48, and the mass spectra with a Jeol SX102 spectrometer.

Cheimonophyllon A (**1**) was obtained as a yellow oil. $[\alpha]_D$ -38 ° (c 3.0 in methanol). UV (methanol) λ_{max} (ε): 224 nm (5,200). IR (KBr): 3380, 2920, 1690, 1610, 1160 and 770 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 267.1582 (M^+ + H, 15 %, $C_{15}H_{23}O_4$ requires 267.1596), 249 (63 %), 181 (100 %), 164 (85 %), 149 (92 %), 135 (97 %), 121 (94 %), 107 (97 %).

Cheimonophyllon B (**2**) was obtained as a colourless oil. $[\alpha]_D$ -40 ° (c 0.1 in chloroform). UV (methanol) λ_{max} (ε): 234 nm (11,700). IR (KBr): 3425, 2960, 1710, 1670, 1465, 1370, 1135 and 945 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 234.1633 (M^+, 100 %, $C_{15}H_{22}O_2$ requires 234.1620), 219 (19 %), 217 (25 %), 177 (82 %), 159 (36 %), 151 (81 %), 149 (83 %), 135 (75 %), 122 (59 %), 107 (56 %).

Diacetylcheimonophyllon C (**3b**) was obtained as a colourless oil after acetylation of cheimonophyllon C (**3a**) with acetic anhydride in pyridine at room temperature over-night. $[\alpha]_D$ +139 ° (c 1.6 in chloroform). UV (methanol): no maximum above 210 nm. IR (KBr): 2935, 1730, 1370, 1240, 1025, 910 and 605 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 336 (M+, 1 %), 293.1772 (M^+ - Ac, 6 %, $C_{17}H_{25}O_4$ requires 293.1753), 276 (4 %), 234 (19 %), 217 (25 %), 192 (22 %), 164 (46 %), 149 (90 %), 132 (60 %), 122 (59 %).

Cheimonophyllon D (**4**) was obtained as a colourless oil. $[\alpha]_D$ +65 ° (c 0.1 in chloroform). UV (methanol) λ_{max} (ε): 235 nm (8,600). IR (KBr): 3435, 2955, 1715, 1665, 1435, 1380, 1210 and 1035 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 250.1551 (M^+, 3 %, $C_{15}H_{22}O_3$ requires 250.1569), 232 (13 %), 165 (100 %), 148 (11 %), 137 (20 %), 119 (18 %).

Cheimonophyllon E (**5**) was obtained as a colourless oil. $[\alpha]_D$ +125 ° (c 2.2 in chloroform). UV (methanol): no maximum above 210 nm. IR (KBr): 3465, 3380, 2955, 1710, 1665, 1450, 1400, 1370, 1280, 1120, 1060, 1035, 940, 900, 860 and 815 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 268.1670 (M^+, 8 %, $C_{15}H_{24}O_4$ requires 268.1674), 250 (18 %), 232 (15 %), 183 (63 %), 165 (100 %), 147 (97 %), 137 (41 %), 119 (54 %).

Cheimonophyllal (**6**) was obtained as a yellow oil. $[\alpha]_D$ +107 ° (c 0.3 in chloroform). UV (methanol) λ_{max} (ε): 314 nm (2000). IR (KBr): 3420, 2960, 1700, 1650, 1370, 1200, 1125, 1060 and 930 cm^{-1}. For NMR data, see Tables 1 and 2. MS (EI, 70 eV), m/z: 282.1476 (M^+, 51 %, $C_{15}H_{22}O_5$ requires 282.1467), 239 (71 %), 203 (16 %), 175 (28 %), 151 (35 %), 109 (43 %), 43 (100 %).

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