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Chamobtusin A, a Novel Skeleton Diterpenoid Alkaloid from *Chamaecyparis obtusa* cv. *tetragon*

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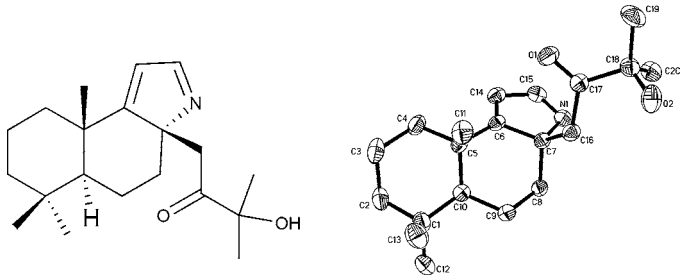
ABSTRACT



The novel diterpenoid alkaloid chamobtusin A (1) was isolated from the branches and leaves of *Chamaecyparis obtusa* cv. *tetragon*. Its structure and relative stereochemistry were mainly determined by MS, 2D NMR, and X-ray methods. The methanol extracts, total alkaloids of *C. obtusa* cv. *tetragon*, and chamobtusin A were tested for their cytotoxicities against A549 and K562 human tumor cell lines.

There are 22 genus and 150 species in the family Cupressaceae, which is spread widely throughout the world. Most species of this family are good wood, and 12 of them are used in folk medicines to treat a wide variety of ailments; in particular, the leaves and seeds of *Platycladus orientalis* are used in traditional Chinese medicine.[1] Plants of Cupressaceae have a rich source of sesquiterpenes, diterpenes, flavones and lignans,[2−7] and some of them are considered to possess antitumor, antimalarial, and antibacterial activities.[8−10] *Chamaecyparis obtusa* cv. *tetragon* belongs to the genus *Chamaecyparis* and is distributed naturally in Japan.[1] According to the literature, no chemical constituent of this plant has been reported until now. As a part of serial investigations on Cupressaceae and in order to seek more novel bioactive compounds, we carried out an extensive chemical study on *C. obtusa* cv. *tetragon*, and a novel skeleton diterpenoid alkaloid named chamobtusin A was

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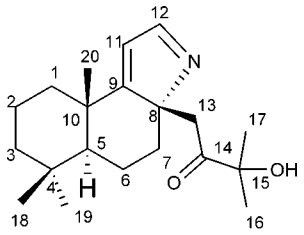
obtained from the $CHCl_3$ extract, which was the first alkaloid observed in the family Cupressaceae. Described herein are the isolation, structure elucidation of chamobtusin A, and biological activities of the methanol extracts, total alkaloids of this plant and chamobtusin A.



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The air-dried and powdered leaves and branches (22.0 kg) were extracted with methanol at room temperature. After evaporation of methanol, the extract was dissolved in hot water and acidified with HCl to PH = 2, then partitioned with a H_2O−EtOAc mixture to give a water-soluble fraction. The water-soluble fraction was basified with NaOH to PH = 10 and then partitioned with a H_2O−$CHCl_3$ mixture to give a $CHCl_3$-soluble fraction (12.5 g). The $CHCl_3$-soluble fraction was subjected to a gel Sephadex LH-20 column using CH_3OH−$CHCl_3$ (1:1) to give five subfractions. Subfraction 3 (226 mg) was further chromatographed on HPLC [YMC-Pack ODS-A, 150 × 10 mm, 5 μm, CH_3OH−H_2O (78:22)] to give chamobtusin A (10 mg).

Chamobtusin A[11] was obtained as a pale yellow crystal. Its molecular formula of $C_{20}H_{31}NO_2$ was established on the basis of positive FAB MS, ^{13}C NMR, and DEPT NMR spectra and confirmed by HR +TOF MS (found 318.2425 $[M + H]^+$, calcd 318.2433 $[M + H]^+$). Its IR (KBr) spectrum showed absorption bands at 3321, 1718, and 1606 cm^{-1} ascribable to hydroxyl, carbonyl, and double bond groups. The UV spectrum revealed the presence of a conjugated system (248 nm). The unsaturated degree of **1** was calculated to be six. The ^1H, ^{13}C, and DEPT NMR spectrum (Table 1) showed the presence of 5 methyls, 6 methylenes, 3 methines (including 2 double bond CH), and 6 quaternary carbons (including 1 carbonyl, 1 double bond quaternary carbon, 1 oxygenated one, and 1 nitrogenous one). Thus, the structure of **1** might be a diterpenoid alkaloid which possesses three rings. The extensive analysis of 1D and 2D NMR spectra (Table 1) first established the structure of **1** (Figure 1 and 2); the still uncertain structure details were established by single-crystal X-ray analysis.

In the H−H COSY spectrum, the proton signals at δ 1.58 (2H, m, H-2) showed correlations with δ 1.82 (1H, m, H-1a), 1.67 (1H, m, H-1b), 1.45 (1H, m, H-3a), and 1.22 (1H, m, H-3b), which suggested the presence of fragment **1a** (Figure 1). The proton signals at δ 1.74 (2H, m, H-6) showed correlations with δ 0.75 (1H, m, H-5), 0.65 (1H, m, H-7a), and 2.61 (1H, m, H-7b), which suggested the presence of fragment **1b** (Figure 1), and δ 7.96 (1H, br s, H-12) showed cross-peaks with δ_H 6.09 (1H, br s, H-11), which gave the fragment of **1c** (Figure 1).

In the HMBC spectrum (Figure 1), δ_H 0.88 (3H, s, H-18), 0.97 (3H, s, H-19), 1.45 (1H, m, H-3a), 1.22 (1H, m, H-3b), and 0.75 (1H, m, H-5) showed cross-peaks with δ_C 34.9 (C-4), δ_H 1.82 (1H, m, H-1a), 1.67 (1H, m, H-1b), 0.75 (1H, m, H-5), 1.15 (3H, s, H-20) correlated with δ_C 41.4 (C-10), which led to the establishment of partial structure **1d** (Figure 1). The cross-peaks between δ_H 1.15 (3H, s, H-20), 0.65 (1H,

Table 1. ^1H and ^{13}C NMR Assignments of **1**[a]

position	δ_H (mult, *J*, Hz)	δ_C (mult)	HMBC (H→C)
1a	1.82 (m)	38.7 t	2, 3, 5, 9, 10, 20
1b	1.67 (m)		
2	1.58 (m)	19.5 t	1, 4, 10
3a	1.45 (m)	43.2 t	1, 2, 4, 5
3b	1.22 (m)		
4		34.9 s	
5	0.75 (m)	59.6 d	3, 4, 6, 9, 10, 20
6	1.74 (m)	20.4 t	4, 5, 7, 8
7a	0.65 (m)	42.1 t	5, 6, 8, 9, 13
7b	2.61 (m)		
8		80.7 s	
9		184.5 s	
10		41.4 s	
11	6.09 (br s)	118.1 d	8, 9, 10, 12
12	7.96 (br s)	165.8 d	8, 9, 11
13a	3.38 (d, 17.8)	42.5 t	7, 8, 9, 14
13b	3.52 (d, 17.8)		
14		213.6 s	
15		77.9 s	
16	1.20 (s)	26.8 q	14, 15
17	1.19 (s)	26.8 q	14, 15
18	0.88 (s)	34.2 q	3, 4, 5, 19
19	0.97 (s)	22.1 q	3, 4, 5, 18
20	1.15 (s)	17.3 q	1, 5, 9, 10

[a] Data were recorded in $CDOD_3$ on Bruker AM-400 MHz and DRX-500 MHz spectrometers.

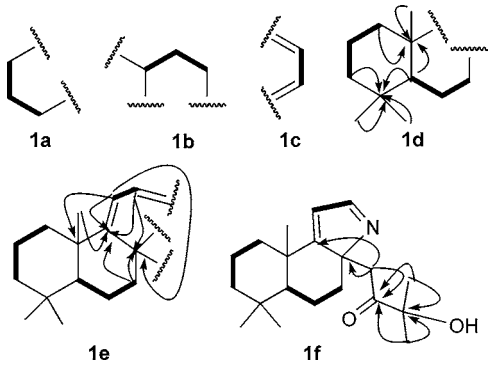

Figure 1. Fragments and key COSY (−) and HMBC (→) correlations of **1**.

(11) Chamobtusin A: $C_{20}H_{31}NO_2$, pale yellow crystals (MeOH−H_2O), mp 148−150 °C; $[\alpha]^{24}_D$ −220.1 (*c* 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 248 (2.48), 322 (1.87) nm; IR (KBr) ν_{max} 3321, 2932, 1718, 1606, 1522, 1465, 1378, 1235, 1194, 1051, 966, 831, 704 cm^{-1}; NMR data can be found in Table 1; FAB^+ MS *m/z* (rel intensity) 318 $[M + H]^+$ (100), 230 (10), 216 (5), 202 (7), 180 (17); HR +TOF MS found 318.2425 $[M + H]^+$, calcd for 318.2433 $[M + H]^+$.

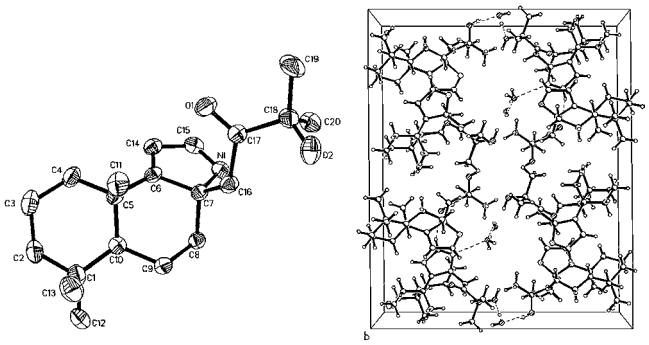

Figure 2. X-ray structure of **1** showing relative configuration.

m, H-7a), 2.61 (1H, m, H-7b), 6.09 (1H, br s, H-11), 7.96 (1H, br s, H-12) and δ_C 184.5 (C-9), δ_H 0.65 (1H, m, H-7a), 2.61 (1H, m, H-7b), 6.09 (1H, br s, H-11) and δ_C 80.7 (C-8), δ_H 6.09 (1H, br s, H-11) and δ_C 41.4 (C-10) suggested the presence of fragment **1e** (Figure 1). δ_H 3.38 (1H, d, J = 17.8 Hz, H-13a), 3.52 (1H, d, J = 17.8 Hz, H-13b) correlated with δ_C 80.7 (C-8), 184.5 (C-9) and 213.6 (C-14), δ_H 1.20 (3H, s, H-16), 1.19 (3H, s, H-17) correlated with δ_C 213.6 (C-14) and 77.9 (C-15), and the correlation between δ_H 7.96 (1H, br s, H-12) and δ_C 80.7 (C-8) suggested that the nitrogen atom should be between C-8 and C-12, and the oxygen atom should be at C-15, thus the whole possible structure of **1** should be as **1f** (Figure 1).

Since the chemical shift of C-9 (184.5) was abnormal downfield, we need further solid evidence such as X-ray diffraction to validate the above deduction. Fortunately, after many attempts with different solvents, a pale yellow single crystal of compound **1** was obtained from MeOH−H_2O (8:2). The analysis of the single-crystal X-ray diffraction[12] of the compound not only confirmed the presence of a δ_C = 184.5 carbon at C-9 but also established the relative stereochemistry of **1** as C-20 and C-13 in β orientation (Figure 2), which was consistant with the NOESY correlation between δ_H 3.38 (1H, d, J = 17.8 Hz, H-13a), 3.52 (1H, d, J = 17.8 Hz, H-13b), and δ_H 1.15 (3H, s, H-20). Thus, the structure of **1** was finally determined as 1-(1,2,3,4,5,6,7,10-octahydro-4,4,10-trimethyl-8*H*-benzo[*e*]indol-2-yl)-3-hydroxy-3-methylbutan-2-one and named chamobtusin A.

According to the literature, chamobtusin A was the first naturally occurring alkaloid in Cupressaceae and even was the first diterpenoid alkaloid from the whole Pinales.[15,16] Because many diterpenoids showed cytotoxic activities on tumor cells,[17,18,19] the methanol extracts, the total alkaloids of *C. obtusa* cv. *tetragon*, and chamobtusin A were tested for in vitro activity on A549 and K562 human tumor cell lines with concentrations of 40, 40, and 10 μg/mL, respectively. The methanol extracts and the total alkaloids showed inhibitory activities with IC_{50} values 32.48 and 5.29 μg/mL against K562, and chamobtusin A showed no cytotoxic activity on both cell lines.

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Supporting Information Available: 1D and 2D NMR spectra and crystallographic data of chamobtusin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) A pale yellow crystal of dimensions 0.50 × 0.80 × 0.04 mm was used for X-ray diffraction on a MAC DIP-2030K diffractometer with Mo Kα radiation and graphite monochromator by maximum 2θ value of 50.0°. The total number of independent reflections was 3714, of which 3306 were observed ([$|F|^2 \geq 2\sigma(|F|^2)$]). Crystal data: molecular formula $(C_{20}H_{31}O_2N_1)\cdot(H_2O)_{2.5}$, M = 317.48 (excluded the solvent), orthorhombic system, space group: $C2$, a = 23.563(1) Å, b = 8.728(1) Å, c = 19.397(1) Å, β = 90.48(1)°, V = 3989.0(5) Å^3, Z = 8, d = 1.132 g/cm^3. The structure was solved by the direct method (SHELXs-97[13]) and expanded using difference Fourier techniques, refined by the full-matrix least-squares method (NOMCSDP[14]). Hydrogen atoms were fixed at calculated positions. The final indices were R_1 = 0.076, wR_2 = 0.1449 ($w = 1/\delta|F|^2$), S = 1.053. Crystallographic data (excluding structure factors) for the structures in this paper, have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 651044. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 (0) 1223-336-033 or e-mail: deposit@ccdc.cam.ac.uk).

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