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Equisetumone, a novel 4-5-olide secocaryophyllane sesquiterpene from *Equisetum palustre*†

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Equisetumone (**1**), an unprecedented sesquiterpenoid, which possesses a novel 4,5-olide tricyclic *trans*-caryophyllane skeleton, was isolated from the *Equisetum palustre* fern. The structure of **1** was established by extensive spectral data analysis. This usual metabolite was probably derived from the caryophyllane class of sesquiterpenes, and a biosynthetic pathway was proposed.

Introduction

Equisetum palustre L. (Equisetaceae, subgenus Equisetum), also called marsh horsetail or humpback, is a pteridophyte widely distributed in wet or boggy areas of the Northern Hemisphere. Its aerial part, as a traditional folk medicine, was reported as a remedy to treat peptic ulcers and hemorrhoids, and to pass kidney stones.[1,2] The decoction of the aerial part proved to possess an effective gastroprotective effect on ethanol-induced gastric ulcer.[3] The hydro-alcoholic extract of *E. palustre* was found to have excellent antioxidant capacity, antimicrobial activity and genotoxicity.[4]

Previous phytochemical investigations of the herbal fern *E. palustre* revealed its richness in several classes of natural products, such as alkaloids,[5,6] flavonoids,[7,8] and essential oils,[9] and exhibited interesting biological activities, *e.g.* anti-ulcerogenic[7,8] and antioxidant[10] activities. However, as of yet, no sesquiterpenoid type compound has been reported from this plant. We report herein the isolation and structural determination of a novel caryophyllane sesquiterpenoid from the aqueous ethanolic extract of *E. palustre*, equisetumone (**1**), possessessing a unique tricyclic ring system.

Results and discussion

Equisetumone (**1**), was isolated as a white powder that gave a pseudomolecular ion $(M + Na)^+$ at *m*/*z* 323.1469 in the HR-ESI-MS, indicating the molecular formula $C_{15}H_{24}O_6$ and four degrees of unsaturation. IR absorptions of **1** were observed at 3434, 1637 cm^{-1}, suggesting the presence hydroxy and carbonyl groups. The ^{13}C NMR and DEPT spectra of **1** (Table 1) suggested the presence of 15 carbons, including four methyls, three sp^3 methylenes, two sp^3 methines (including an oxymethine), and six quaternary carbons (including four oxygenated quaternary carbon and one carbonyl carbons belong to lactone). These data accounted for three degrees of unsaturation suggesting a tricyclic structure for **1**. A signal at δ 178.3 (s, C-5) indicated the presence of a carbonyl group. Additionally, four tertiary methyls (δ 1.31, 3H, s, H_3-12; 1.01, 3H, s, H_3-13; 1.73, 3H, s, H_3-14; 1.24, 3H, s, H_3-15), three pairs of aliphatic methylene protons (δ 2.51, 1H, dd, J = 14.4, 3.6 Hz, δ 1.78, 1H, dd, J = 14.4, 3.6 Hz, H_2-3; δ 2.25, 1H, d, J = 16.8 Hz, δ 3.29, 1H, dd, J = 16.8 Hz, H_2-6; δ 1.96, 1H, t, J = 8.4 Hz, δ 1.64, 1H, t, J = 8.4 Hz, H_2-10), an aliphatic methine proton (δ 3.08, 1H, dd, J = 11.6, 8.4 Hz) and an

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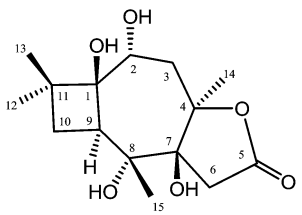
Table 1 ^{1}H and ^{13}C NMR data and HMBC correlations of equisetumone (**1**)

C/H	δ_H[a]	δ_C[b]	HMBC (H→C)
1		84.5 (qC)[d]	
2	3.84 t (3.6)[c]	71.0 (CH)	C-1, -3, -4
3	2.51 dd (14.4, 3.6)	39.7 (CH_2)	C-4
	1.78 dd (14.4, 3.6)		
4		94.8 (qC)	
5		178.3 (qC)	
6	2.25 d (16.8)	40.2 (CH_2)	C-4, -5, -7
	3.29 d (16.8)		
7		87.0 (qC)	
8		74.4 (qC)	
9	3.08 dd (11.6, 8.4)	43.9 (CH)	C-1, -8, -10
10	1.64 t (8.4)	35.6 (CH_2)	C-1, -9, -11
	1.96 t (8.4)		
11		41.8 (qC)	
12	1.31 s	26.7 (CH_3)	C-1, -10, -11, -13
13	1.01 s	24.5 (CH_3)	C-1, -10, -11, -13
14	1.73 s	26.3 (CH_3)	C-3, -4, -7
15	1.24 s	26.4 (CH_3)	C-7, -8, -9

[a] Spectra measured at 400 MHz in CD_3OD at 25 °C. [b] Spectra measured at 100 MHz in CD_3OD at 25 °C. [c] J values (in hertz) in parentheses. [d] Attached protons were deduced by DEPT and HMQC experiments.

oxymethine proton (δ 3.84, 1H, t, $J = 3.6$ Hz) were observed in the ^{1}H NMR spectrum of **1**.

In the ^{1}H–^{1}H COSY experiment of **1** (Fig. 1), it was possible to establish the spin systems that map out the proton sequences from H-9/H_2-10 and H-2/H_2-3. Based on these data and the HMBC correlations observed between H-2/C-1, -3, -4; H_3-14/C-3, -4, -7; H_2-6/C-4, -5, -7; H_3-15/C-7, -8, -9; and H-9/C-1 (Fig. 1 and Table 1), the position of a 4,5-olide γ-lactone group was proposed. Three quaternary carbon signals appeared downfield



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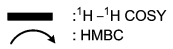

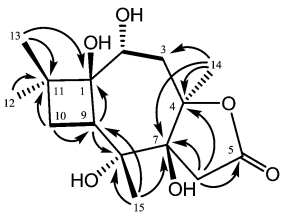

Fig. 1 The structure and its ^{1}H–^{1}H COSY and selective HMBC of **1**.

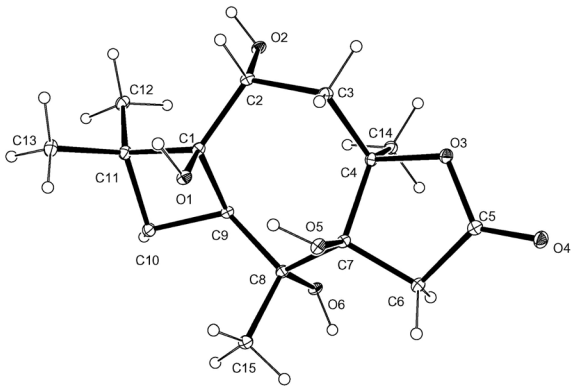

Fig. 2 Molecular projection of **1**.

at δ 94.8 (s, C-4), 87.0 (s, C-7) and 74.4 (s, C-8) suggesting that these carbons were oxygenated C-atoms. Furthermore, the connection of a methyl group and a hydroxyl group to C-8 was confirmed by the HMBC correlations between H_3-15/C-7, -8, -9. Similarly, the connection of a hydroxyl group to C-7 was also confirmed by the HMBC correlations between H_3-12/C-4, -7, H_2-6/C-7. The C-1 oxygenated quaternary carbon (δ_C 84.5) and C-2 oxymethine (δ_H 3.84; δ_C 70.0) of **1** were confirmed by the HMBC correlations between H_3-13/C-1; H-9/C-1 and H_3-14/C-3, -4 (Fig. 1 and Table 1). The cyclobutane ring, fused to the main carbon skeleton at C-1 and C-9, was elucidated by analyzing the HMBC correlations between H-9/C-8; H-9/C-1, -10; H_2-10/C-1, -9, -11; H-12/C-1, -9, -10, -11; and H-13/C-1, -9, -10, -11 (Fig. 1 and Table 1). The *trans*-fused caryophyllane sesquiterpene skeleton of **1** was confirmed by NMR spectral analysis, elucidating the relative stereochemistry as depicted in Fig. 1 and 2.

Compound **1** possesses multiple stereogenic centers and a seven-membered ring, which provide certain degrees of flexibility of **1**. Therefore only a single-crystal X-ray structural analysis is the ultimate solution to confirm the structure and resolve the stereochemistry of this unusual compound. The configuration provided by the X-ray data permitted the unambiguous assignment of the stereochemistry of all stereogenic centres as 1*S*, 2*R*, 4*R*, 7*S*, 8*R*, and 9*S*. Compound **1** was therefore assigned as 1*S*,2*R*,7*S*,8*R*-tatrahydroxy-carophyllan-[4,7]-4,5-olide.

The backbone of compound **1** was speculated to be formed biosynthetically through thermally or photochemically induced sigmatropic rearrangement of caryophyllene. The biosynthesis of compound **1** (Fig. 3) is likely to follow from a caryophyll-4,8(15)-diene precursor **2**. Starting from the intermediate **3**, attack on the ketone carbonyl by the 4,5 double bond electron pair would result in the formation of a hemiketal at C-7 and a [4,7] B/C ring junction architecture. It is less favorable as in the formation of this 4,7 bond; in general, a carbocation could be generated on C-5. When a secondary and less stable carbocationic centre was formed, **3** could undergo a Baeyer–Villiger type oxidation to afford the lactone **4**. Oxidation at positions 1, 2 and 8 may lead to metabolite **1**. In the past, only few seco-caryophyllanes-olide were found, and they will have a cleavage between C-4 and C-5 (*e.g.* rumphellaone A,[11] a marine-derived cytotoxin), between C-6 and C-7 (*e.g.* hebelophyllene E, H[12,13]

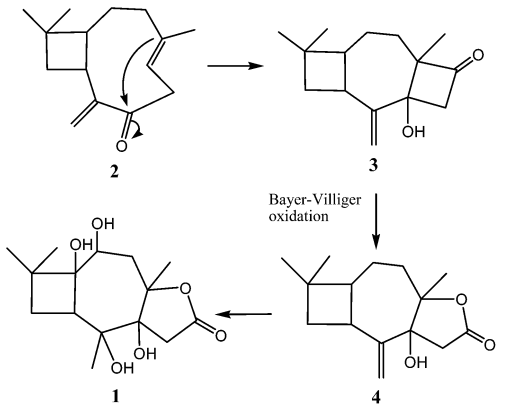

Fig. 3 A possible biogenetic pathway for **1**.

and 2*S*,3*R*-dihydroxy-carophyllan-[5,8]-6,7-olide[14]), and between C-9 and C-10 (*e.g.* 4,5-epoxy-9,10-secocaryophyllen-9,10-olide[15]). Containing a carbon skeleton C-4 and C-5 and forming a novel tricyclic *trans*-caryophyllane-[6,7]4,5-olide, the natural product derived from the herbal fern *Equisetum palustre* was a novel discovery.

Moreover, compound **1** was tested against various microbial pathogens, but showed no antimicrobial activity, except for *Bacillus subtilis* with MIC 250 and MBC 500 μg mL^{-1} (Penicillin: MIC 1.5 and MBC 156 μg mL^{-1}), respectively.

Experimental

Materials and methods

General experimental procedures. Optical rotation was measured on a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). UV spectrum was recorded on a Hitachi U-2800 UV-vis spectrophotometer (Hitachi, Tokyo, Japan). IR spectrum was taken on a Shimadzu IR Prestige-21 FT-IR spectrometer (Shimadzu, Nakagyo-ku, Japan). 1D and 2D NMR spectra were recorded on Bruker 500 AVII NMR spectrometers (Bruker Bio-Spin GmbH, Karlsruhe, Germany). HR-ESI-MS was measured with a Finnigan/Thermo Quest MAT 95XL spectrometer, and ESI-MS/MS was obtained on a Bruker HCT ultra PTM Discovery.

Silica gel 60 (230–400 mesh or 70–230 mesh, Merck, Darmstadt, Germany) was used for column chromatography. Precoated Si gel plates (silica gel 60 F_{254}, Merck, Darmstadt, Germany) were used for analytical TLC. The spots were detected by spraying with 50% H_2SO_4 aqueous solution and then heating on a hot plate.

Single-crystal X-ray structural analysis. 100(2) K, wavelength = 1.54178 Å, crystal system, space group, othorhombic, $P2_12_12_1$ (no. 19), unit cell dimensions: a = 9.4125(4) Å, b = 9.9012(4) Å, c = 16.0619(7) Å, volume = 1496.89(11) $Å^3$, Z = 4, calculated density = 1.413 Mg m^{-3}, absorption coefficient = 0.934 mm^{-1}, $F(000)$ = 688, crystal size = 0.45 × 0.45 × 0.40 mm, theta range for data collection = 5.25 to 66.45 deg. Limiting indices: $-11 \leq h \leq 10$, $-11 \leq k \leq 11$, $-19 \leq l \leq 19$, reflections collected/unique = 9756/2582 [R(int) = 0.0198], completeness to theta = 98.3%, absorption correction semi-empirical from equivalents, max. and min. transmission = 0.9493 and 0.8336, refinement method = full-matrix least-squares on F^2, data/restraints/parameters = 2582/0/207, goodness-of-fit on F^2 = 1.090, final R indices [I > 2sigma(I)]: R_1 = 0.0259, wR_2 = 0.0665, R indices (all data): R_1 = 0.0260, wR_2 = 0.0666, absolute structure parameter x = 0.02(13), largest diff. peak and hole: 0.240 and −0.150 e $Å^{-3}$.

Antimicrobial assay. *Escherichia coli* (ATCC 8739), *Proteus vulgaris* (ATCC 49132), *Pseudomonas aeruginosa* (ATCC 2785), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Staphylococcus epidermidis* (ATCC 12228) and *Candida albicans* (ATCC 10231) were obtained from the Institute of Applied Microbiology, Heilongjiang Academy of Science (China). They were maintained on an agar slant at 4 °C. All strains were incubated on nutrient agar at 37 °C for 24 h.

Bacterial cells (10^5 CFU per mL) were inoculated into a nutrient broth at 0.1 mL per well, in 96-well microtiter plate. MICs were determined by a serial 2-fold dilution following the recommendations of the Clinical and Laboratory Standards Institute (CLSI). After 24 h of incubation at 37 °C, the minimal concentration of equisetumone necessary to prevent growth of given test organisms were determined and defined as MIC. MIC values were determined by a serial 2-fold dilution using microdilution and MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] assays. Fungal cells (10^4 CFU per mL) were inoculated with YPD broth (0.1 mL per well) in microtiter plates. After 24 h of incubation at 25 °C, MIC was defined as the lowest concentration of equisetumone to prevent the growth of given test organisms. MBC was defined as the lowest concentration of equisetumone at which inoculated bacterial and fungal strains were completely killed. Penicillin was used as a positive control. All determinations were performed in duplicate.[16,17]

Plant material. *Equisetum palustre* were collected in autumn 2010 from Inner Mongolia Autonomous Region, China and authenticated by Prof. Shao-Quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, PR China. A voucher specimen was deposited in the herbarium of the Key Laboratory.

Extraction and isolation. Pulverized *E. palustre* (3.0 kg) were extracted with 20 L ethanol–water (80 : 20, v/v) three times (72 h each) by maceration at room temperature. The extraction solution was filtered then evaporated under reduced pressure (45 °C). The resulting extract (406.6 g) was diluted in about 1.5 L distilled water and further extracted with *n*-hexane and ethyl acetate (EA), successively. The EA extract was dried under reduced pressure to yield an EA fraction. The EA fraction (31.5 g) was then separated on a silica gel column (60 × 290 mm) with gradient systems of *n*-hexane–EA (4 : 1–2 : 1, v/v). Compound **1** (18.6 mg) was obtained from *n*-hexane–EA (2 : 1, v/v) as white powder. Recrystalized **1** (10 mg) in 2 mL of the solvent system (MeOH–H_2O 9 : 1) in a glass vial for two weeks, colorless needles of **1** were obtained.

Characterization of compound 1. Equisetumone (**1**): colorless needles; $[\alpha]_D^{25}$ + 84.5 (*c* 0.14, $CHCl_3$); IR ν_{max} (thin film) cm^{-1} = 3434, 1636, 583. ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; HR-ESI-MS m/z 323.1469 [M + Na]$^+$ $C_{15}H_{24}O_6$ requires 323.1471.

Conclusions

Isolated from the fern *Equisetum palustre*, equisetumone (**1**) is a novel sesquiterpenoid with the disconnected carbon skeleton between C-4 and C-5 that forms a tricyclic *trans*-caryophyllane-[6,7]4,5-olide. Equisetumone possesses mild antimicrobial activity. The biosynthetic pathway of this sesquiterpene was also proposed.

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