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Conosilane A, an Unprecedented Sesquiterpene from the Cultures of Basidiomycete *Conocybe siliginea*

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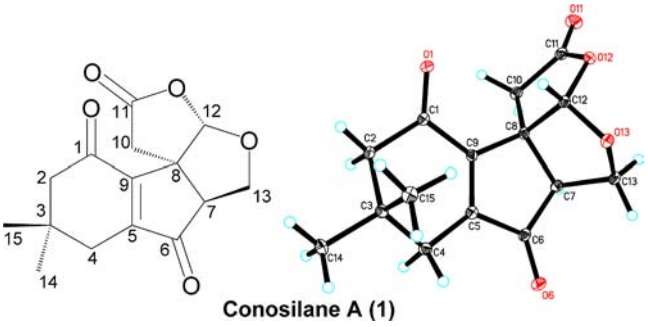
ABSTRACT



Conosilane A (1), a novel sesquiterpene with an unprecedented carbon skeleton, was isolated from the cultures of the basidiomycete *Conocybe siliginea*. Its structure was elucidated by extensive spectroscopic methods, and the absolute configuration was determined by single crystal X-ray diffraction analysis. Conosilane A was found to inhibit 11β-hydroxysteroid dehydrogenase significantly.

The genus *Conocybe* belongs to the order Agaricales and family Bolbitiaceae, which comprises more than 240 species all over the world. Previous investigations of the genus *Conocybe* have reported a number of active compounds, such as psilocybin,[1] psilocin,[2] α-amanitin,[3] and cyclopeptides,[4] which show some toxic and hallucinogenic properties.[5] The mushroom *C. siliginea* has been proven to be a rich resource of sesquiterpenes, from which we have reported a series of tremulane-type sesquiterpenes.[6] Some of them were found to possess vascular-relaxing and 11β-hydroxysteroid dehydrogenase inhibitory activity (11β-HSD).[6] In our continuous search for new and bioactive compounds, a further investigation on the scale-up fermentation of *C. siliginea*[7] resulted in the isolation of a novel sesquiterpene, conosilane A (**1**). Its structure was elucidated on the basis of spectroscopic

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(7) The fungus *C. siliginea* was collected from Linglang County, Yunnan Province, China, in July 2003, and was identified by Prof. Mu Zang, Kunming Institute of Botany, Chinese Academy of Sciences (voucher No. KIB03071801). The fungus was grown in shake cultures (250 rpm) using a medium (glucose 5%, peptone 0.15%, yeast powder 0.5%, KH_2PO_4 0.05%, and $MgSO_4$ 0.05%). Fermentation was carried out on a shaker at 250 rpm for 20 days.

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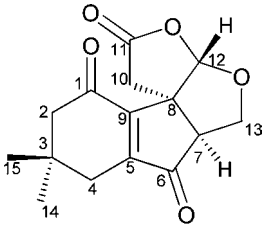
methods, and its absolute configuration was determined by single crystal X-ray diffraction analysis.

The culture broth of *C. siliginea* (80 L) was filtered, and the filtrate was extracted four times with EtOAc. The EtOAc extract was concentrated under reduced pressure to give an oily residue (40 g), which was subjected to column chromatography (CC) over silica gel (200−300 mesh) eluting with $CHCl_3$/MeOH (from 1:0 to 0:1) to afford fractions A−E. Fraction B was separated further by CC over RP-18, eluting with H_2O/MeOH (from 1:0 to 0:1) to give fractions B_1−B_4. Fraction B_2 was purified by CC over silica gel (petroleum ether/Me_2CO, 5:1) and then applied to Sephadex LH-20 (Me_2CO) to yield **1** (8.2 mg).

Conosilane A (**1**)[8] was obtained as colorless cubic crystals (Me_2CO). Its molecular formula $C_{15}H_{16}O_5$ was determined on the basis of the HRESIMS at *m*/*z* 299.0892 $[M + Na]^+$ (calcd 299.0895), corresponding to 8 degrees of unsaturation. The IR spectrum indicated the presence of a lactone group (1792 cm^{-1}) and an α,β-unsaturated diketone moiety (1723, 1676 cm^{-1}), while the UV absorption bands at 262 nm revealed the conjugated moiety in the structure. The ^1H and ^{13}C NMR spectra showed 15 carbon resonances (Table 1). Among them, two ketone carbons (δ_C 198.8 and 206.6), one lactone carbonyl carbon (δ_C 172.2), and two olefinic carbons (δ_C 155.1 and 153.1) occupied four degrees of unsaturation. These data suggested that compound **1** is a sesquiterpenoid possessing a tetracyclic ring system.



Conosilane A (1)

The gross structure of **1** was established initially by analysis of 2D NMR spectra, in particular with HMBC data. In the HMBC spectrum (Figure 1), two singlets at δ_H 1.14 and 1.04 (each 3H, s), assigned to two methyls of Me-14 and Me-15, together with signals of two methylenes of C-2 and C-4, showed significant correlations to an sp^3 quaternary carbon at δ_C 35.2 (s, C-3). H-2 also showed a significant HMBC correlation to a keto carbon at δ_C 198.8 (s, C-1) and a weak correlation to an olefinic carbon at δ_C 153.1 (s, C-9), while H-4 also showed a significant correlation to an olefinic carbon at δ_C 155.1 (s, C-5) and a weak correlation to another keto carbon at δ_C 206.6 (s, C-6). These HMBC correlations establish a six-membered ring A, as shown in Figure 1.

Further, a signal at δ_H 2.86 (1H, dd, *J* = 7.6, 1.6 Hz, H-7) showed HMBC correlations to C-6, C-9, and another sp^3 quaternary carbon at δ_C 57.8 (s, C-8), which revealed a

(8) Conosilane A (**1**): mp 177−180 °C; $[\alpha]^{19}_D$ −52.4 (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ε): 262 (3.19) nm, 201 (2.89) nm; IR (KBr) λ_{max}: 1792, 1723, 1676, 1214, 1109, 962 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Table 1; ESIMS (positive) *m*/*z* 299 $[M + Na]^+$; HRESIMS (positive) *m*/*z* 299.0892 $[M + Na]^+$ ($C_{15}H_{16}O_5Na$, calcd 299.0895).

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data of **1**[a] in $CDCl_3$ (δ in ppm, *J* in Hz)

entry	δ_H	δ_C
1		198.8, s
2a	2.52 (1H, d, 16.3)	53.1, t
2b	2.45 (1H, d, 16.3)	
3		35.2, s
4	2.38 (2H, overlap)	34.4, t
5		155.1, s
6		206.6, s
7	2.86 (1H, dd, 7.6, 1.6)	57.8, d
8		57.8, s
9		153.1, s
10a	3.60 (1H, d, 17.8)	36.5, t
10b	2.76 (1H, d, 17.8)	
11		172.2, s
12	5.91 (1H, s)	107.4, d
13a	4.29 (1H, dd, 9.8, 1.6)	68.4, t
13b	4.21 (1H, dd, 9.8, 7.8)	
14	1.14 (3H, s)	28.4, q
15	1.04 (3H, s)	27.5, q

[a] Data were assigned by the HSQC, HMBC, ^1H−^1H COSY, and ROESY spectra.

five-membered ring B (Figure 1). In the ^1H−^1H COSY spectrum, the cross peaks between H-7 and δ_H 4.29 (1H, dd, *J* = 9.8, 1.6 Hz, H-13a) and 4.21 (1H, dd, *J* = 9.8, 7.8 Hz, H-13b) suggested the linkage from C-13 to C-7, while the HMBC correlation from δ_H 5.91 (1H, s, H-12) to C-8 indicated the linkage from C-12 to C-8. The above HMBC correlations, as well as the key HMBC correlation from H-13 to δ_C 107.4 (d, C-12), constructed a five-membered ring C. Similarly, protons of a methylene at δ_H 3.60 (1H, d, *J* = 17.8 Hz, H-10a) and 2.76 (1H, d, *J* = 17.8 Hz, H-10b) showed HMBC correlations to C-8 and the quaternary carbon at δ_C 172.2 (s, C-11), while H-12 also showed an HMBC correlation to C-11; these HMBC data, as well as taking the degrees of unsaturation into consideration, suggested that C-11, C-10, C-8, and C-12 comprised a lactone ring D, as shown in Figure 1.

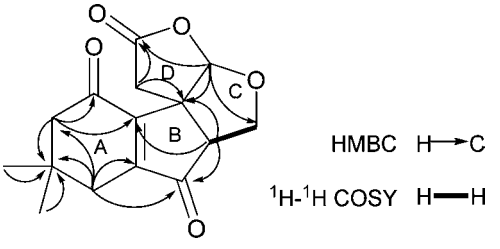


Figure 1. Key 2D NMR correlations of **1**.

The relative configuration of **1** could not be elucidated due to multiquaternary carbons affording limited cross peaks in the ROESY spectrum. Fortunately, the single crystal X-ray diffraction experiment not only confirmed

the structure of **1** as deduced above but also determined the absolute configuration as 7*S*,8*S*,12*S* (Figure 2).[9] Thus, the structure of **1** was established and named as conosilane A.

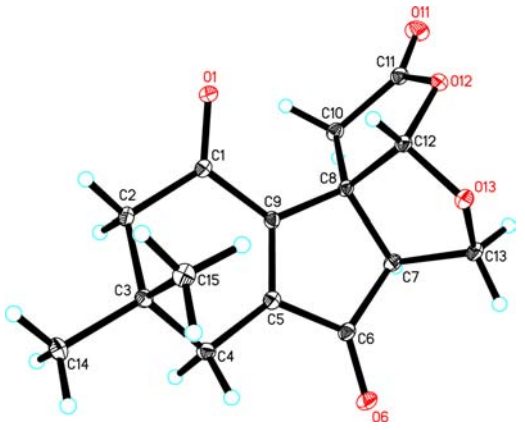


Figure 2. X-ray structure of **1**.

Humulene is formed from farnesyl pyrophosphate by an enzymatic cyclization reaction. Biogenetically, it is suggested that most sesquiterpenoids derived from higher fungi, subdivision Basidiomycotina, were started from humulene with three pathways.[10] One route leads to caryophyllane, and another pathway ends in the irregular sesquiterpenes of the tremulane type. The third pathway is the most important one. It produces the tricyclic sesquiterpene protoilludane which is at the biosynthetical crossroad for many sesquiterpene classes. Recently, we reported that trefolane A, a sesquiterpenoid with a new skeleton from the basidimycete *Tremella foliacea*, started from humulene with the fourth pathway.[11]

(9) Crystallographic data of conosilane A (**1**): $C_{15}H_{16}O_5$, MW = 276.28; monoclinic, space group $P2_12_12_1$; a = 5.75720 (10) Å, b = 11.6775 (2) Å, c = 19.3379 (4) Å, $\alpha = \beta = \gamma = 90°$, V = 1300.08 (4) Å^3, Z = 4, d = 1.412 g/cm^3, crystal dimensions 0.29 × 0.29 × 0.69 mm^3 were used for measurement on a Bruker APEX DUO with a graphite monochromater, Cu Kα radiation. The total number of reflections measured was 13947, of which 4976 were observed, $I > 2\sigma(I)$. Final indices: R_1 = 0.0357, wR_2 = 0.1106. The crystal structure of compound **1** was solved by direct method SHELXS-97 and expanded using the difference Fourier techniques, refined by the program SHLXL-97 and the full-matrix least-squares calculations. Crystallographic data for the structure of compound **1** have been deposited with the Cambridge Crystallographic Data Centre (deposition no. CCDC 894182). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.htm (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, U.K.; fax (+44) 1223-336-033; or desposit@ccdc.cam.ac.uk).

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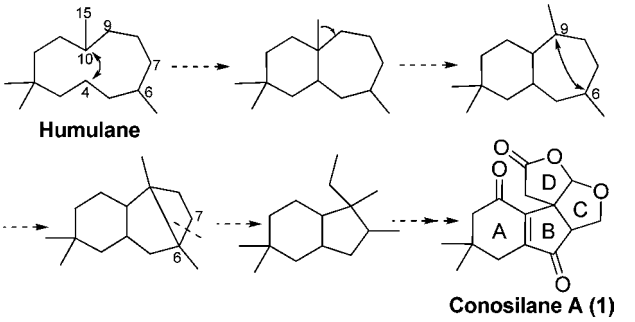
Scheme 1. Plausible Biogenetic Pathway to **1**



We suggest that conosilane A (**1**) starts from the humulane skeleton with a new biogenetic pathway, in which two new carbon bonds are formed between C-4 and C-10, C-6 and C-9, via methyl migration and cleavage of bond C-6/C-7 to construct a 6/5 carbon ring system. Then after an accompanying oxidation and dehydration, a tetrahydrofuran ring C and a five-membered lactone ring D are built (Scheme 1). Conosilane A (**1**) is a nonisoprenoid sesquiterpenoid that represents a new skeleton type in the family of sesquiterpenoids.

Conosilane A (**1**) was evaluated for its cytotoxicity against five human cancer cell lines using the MTT method reported previously[12] with minor modification (method: see Supporting Information). Unfortunately, no activity was detected (IC_{50} > 40 μM). Conosilane A (**1**) was further tested against human and mouse 11β-HSD1 (hydroxysteroid dehydrogenase); it was found to exhibit moderate inhibitory activities against both human and mouse 11β-HSD1 at a concentration of 10 μg/mL, with inhibitory rates of 53.3% and 70.0%, respectively (method: see Supporting Information).

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Supporting Information Available. NMR, MS, and IR spectra and the X-ray crystallographic data (CIF file) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.