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Gloeophyllins A–J, Cytotoxic Ergosteroids with Various Skeletons from a Chinese Tibet Fungus *Gloeophyllum abietinum*

Jun-Jie Han,[†] Li Bao,[†] Qiao-Qiao Tao, Yi-Jian Yao, Xing-Zhong Liu, Wen-Bing Yin, and Hong-Wei Liu*

State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing, 100101, China

Supporting Information

ABSTRACT: Ten new ergosteroids, gloeophyllins A–J (**1**–**10**), have been isolated from the solid cultures of *Gloeophyllum abietinum*. The absolute configurations of **1**, **2**, and **9** were determined by X-ray crystallographic analysis. Compound **1** has a rare C-nor-D-homosteroid skeleton. Compound **9** possesses an unusual ergostane skeleton having a 10-oxabicyclo [4.3.1] decane moiety replacing 6/5 fused C/D rings. Compound **10** represents the first ergosteroid featuring the cleavage of a C8–C14 bond. The cytotoxicity of **1**–**10** was tested against the human cancer cell lines K562 and HCT116. The biosynthetic pathway for **1**–**10** is postulated.

Steroids are important biomolecules that are widely distributed in nature and play significant roles in human beings and other organisms. Fungi are known as producers of steroids with novel structures and diverse bioactivities. Examples include antcamphins A–L with cytotoxicity against MDA-MB-231 breast cancer cells and A549 lung cancer cells from the famous medicinal mushroom *Antrodia camphorate*,[1] strophasterols A–D with a novel skeleton and antiendoplasmic reticulum stress activity from the mushroom *Stropharia rugosoannulata*,[2] penicillitone with an unprecedented skeleton and strong anti-inflammatory activity from the fungus of *Penicillium purpurogenum*,[3] and dankasterone with a new ergostane skeleton and strong cytotoxicity from a marine fungus *Gymnascella* sp.[4]

The fungi belonging to the genus *Gloeophyllum* are characterized by the formation of tough, brown, shaggy-topped fruiting bodies and the production of a brown rot of wood. *Gloeophyllum* species have been reported to produce antibiotics, such as oosponol,[5a,b] four rearranged illudalanes, one rearranged protoilludane, and one sterpurane.[6] We separated a strain of *G. abietinum* from its fruiting bodies collected in the Tibet plateau in 2012. In continuation of our ongoing search for new secondary metabolites from higher fungi, ten new ergosteroids with different chemical skeletons, named gloeophyllins A–J (**1**–**10**), were isolated from the solid culture of this fungus (Figure 1). Herein, we describe the isolation, structure elucidation, and cytotoxicity of **1**–**10** and discuss their possible biogenetic pathway in this fungus.

The molecular formula of gloeophyllin A (**1**, $[\alpha]_D^{25}$ +32.2) was assigned as $C_{30}H_{46}O_3$ (eight degree of unsaturation) on the basis of the HRTOFMS data at *m*/*z* 455.3517 $[M + H]^+$. The ^1H, ^{13}C NMR (Table 1) and HSQC spectra of **1** showed the presence of three secondary methyls [δ_H/δ_C 0.89 (d, J = 6.5 Hz)/18.6, 1.02 (d, J = 6.8 Hz)/21.9, 1.02 (d, J = 6.8 Hz)/22.2], two tertiary methyls [δ_H/δ_C 0.96 (s)/20.1, 1.16 (s)/10.6], one oxygenated methine [δ_H/δ_C 4.05 (dd, J = 4.3, 11.7 Hz)/75.7], three pairs of double bonds [δ_H/δ_C 4.66 (br s), 4.82 (br s)/111.7 (C-18), 4.67 (s), 4.72 (s)/106.3 (C-30); δ_C 136.9, 142.4, 151.6 156.6], and one carboxylic moiety [δ_C 183.0] in its structure. The ^1H–^1H COSY correlations of H_2-1-H_2-2-H-3, H-5-H_2-6-H_2-7 and H_2-11-H-12-H-14-H_2-15-H_2-16-H-17 and the HMBC correlations from H_2-18 to C-12, C-13, and C-17; from H_3-19 to C-1, C-5, C-9, and C-10; and from H_3-29 to C-3, C-4, C-5, and C-28 confirmed the presence of a 6/6/5/6 ring system (Figure S1 in the Supporting Information). The structure of **1** was finally confirmed by single-crystal X-ray crystallographic analysis (Figure 2). The Flack parameter [0.00(2)] obtained by Cu Kα radiation is near 0.0, which allows the determination of the absolute configuration as 3*S*,

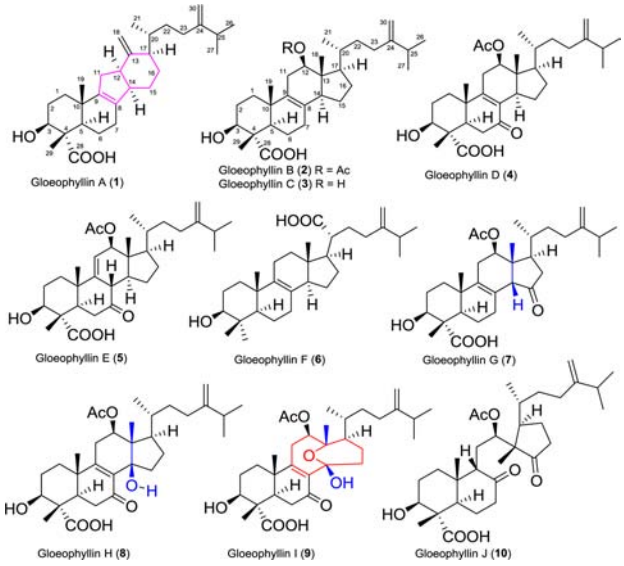

Figure 1. Structures of gloeophyllins A–J (**1**–**10**).

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Table 1. NMR Spectroscopic Data for 1 and 9 in $CDCl_3$[a]

	1		9	
no.	δ_H	δ_C	δ_H	δ_C
1	1.38 m	35.3	1.76 m	34.7
	1.70 m		1.92 m	
2	1.67 m	27.0	1.75 m	26.7
	1.78 m		1.94 m	
3	4.05 dd (4.3, 11.7)	75.7	4.11 d (8.8)	74.1
4		53.5		52.7
5	1.90 br d (11.2)	46.8	2.42 dd (2.0,14.5)	43.7
6	1.40 m	21.6	2.31 m	38.0
	1.65 m		2.59 m	
7	2.00 m	24.7		201.6
8		136.9		136.9
9		142.4		167.4
10		35.0		40.9
11	2.23 m	35.1	2.29 m	28.8
			3.23 dd (10.8, 14.1)	
12	2.84 m	45.4	4.88 br d (10.5)	76.8
13		151.6		77.8
14	2.37 m	48.2		98.7
15	1.11 m	23.9	1.92 m	34.5
	1.52 m		2.04 m	
16	1.45 m	26.3	1.68 m	17.1
	1.62 m		1.81 m	
17	1.98 m	48.5	1.19 m	43.0
18	4.66 br s	111.7	1.27 s	19.9
	4.82 br s			
19	0.96 s	20.1	1.10 s	19.1
20	1.59 m	33.3	1.83 m	33.3
21	0.89 d (6.5)	18.6	0.98 d (6.8)	19.9
22	1.11 m	32.7	1.18 m	33.0
	1.70 m		1.75 m	
23	1.91 m	31.5	1.92 m	33.8
	2.13 m		2.15 m	
24		156.6		156.3
25	2.22 m	33.8	2.25 m	33.6
26	1.02 d (6.7)	21.9	1.01 d (6.7)	21.9
27	1.02 d (6.7)	22.2	1.02 d (6.7)	22.2
28		183.0		180.8
29	1.16 s	10.6	1.23 s	10.4
30	4.67 br s	106.3	4.70 br s	106.9
	4.72 br s		4.73 br s	
$\underline{C}OCH_3$				170.7
$CO\underline{C}H_3$			2.08 s	21.4

[a]"m" means multiplet or overlapped with other signals.

4*S*, 5*R*, 10*S*, 12*R*, 14*R*, 17*R*, and 20*R*. Compound **1** has an interesting C-nor-D-homoergosteroid skeleton. Natural products with such a skeleton are rare in nature, including veramine and neojerminine from *Veratrum album*,[7a] germinine from *V. lobelianum*,[7b] impranine and dihydroimpranine from *Fritillaria imperialis*,[7c] puqienines C–E, puqiedine and 3α-puqiedin-7-ol from *F. puqiensis*,[7d] imperiazine from *Petilium eduardi*,[7e] and nakiterpiosin and nakiterpinosinone from a marine sponge of *Terpios hoshinota*.[7f] It is the first report of this type of ergosteroids from fungi.

The molecular formula of gloeophyllin B (**2**, $[\alpha]_D^{25}$ +41.5) was determined to be $C_{32}H_{50}O_5$ (eight degree of unsaturation) on the basis of HRTOFMS at *m*/*z* 515.3740 $[M + H]^+$. The ^1H, ^{13}C, and HSQC spectra of **2** indicated the presence of three secondary methyls [δ_H/δ_C 0.89 (d, *J* = 6.8 Hz)/20.8, 1.00 (d, *J* = 6.8 Hz)/21.9, 1.01 (d, *J* = 6.8 Hz)/22.1], three tertiary methyls [δ_H/δ_C 0.69 (s)/8.1, 0.98 (s)/19.9, 1.12 (s)/10.6], two oxygenated methines [δ_H/δ_C 3.98 (dd, *J* = 4.1, 11.7 Hz)/75.3, 4.86 (t, *J* = 8.1 Hz)/79.6], two pairs of double bonds [δ_H/δ_C 4.64 (br s), 4.70 (br s)/106.3, δ_C 127.8, 135.3, 156.7], an acetyl group [δ_H/δ_C 2.02 (s)/21.7, 171.0], and one carboxylic moiety [δ_C 182.4] in its structure. A comprehensive analysis of its 2D NMR spectra, including $^1H-^1H$ COSY, HMQC, and HMBC experiments (Figure S1 in the Supporting Information), established the planar structure of **2**. The proposed structure of **2** was finally confirmed by single-crystal X-ray crystallographic analysis (Figure S2 in the Supporting Information). The absolute configuration of **2** was assigned as 3*S*, 4*S*, 5*R*, 10*S*, 12*R*, 13*R*, 14*S*, 17*R*, and 20*R* on the basis of the Flack parameter [−0.15(7)].

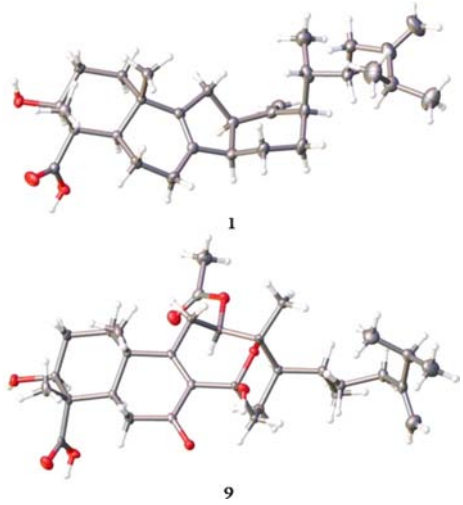

Figure 2. X-ray crystallographic structures of **1** and **9**.

Gloeophyllin C (**3**, $[\alpha]_D^{25}$ +16.9) was assigned the molecular formula of $C_{30}H_{48}O_4$ (seven degree of unsaturation) on the basis of its HRESIMS at *m*/*z* 473.3621 $[M + H]^+$ and NMR data. The NMR data of **3** were quite similar to those of **2** except for the absence of the acetyl group. The structure of **3** was established from HSQC, HMBC, and ROESY spectral analysis (Figures S1 and S3 in the Supporting Information).

Gloeophyllins D (**4**, $[\alpha]_D^{25}$ +12.0) and E (**5**, $[\alpha]_D^{25}$ −31.0) were determined to have the same molecular formula of $C_{32}H_{48}O_6$ on the basis of their HRTOFMS and NMR data. The NMR data of **4** showed much similarity with those of **2**, except for the loss of a methylene group and the presence of an extra ketone moiety in **4**. The HMBC correlation from H-5, H-6, and H-14 to the carbonyl carbon at δ_C 199.8 supported the location of a ketone group at C-7 in **4**. For compound **5**, $^1H-^1H$ COSY correlations of H-8-H-14, H-11-H-12, as well as HMBC correlations from H-12 to C-9, C-11 C-13, and C-14 and from H-8 to C-6, C-7, C-9, C-10, C-11, C-13, C-14, and C-15 confirmed the structural features in the B and C rings. NOE correlations of H-8 with H-18 and H-19, together with the larger coupling constant of 9.8 Hz between H-8 and H-14, indicated the β orientation of H-8 and the α orientation of H-14. A detailed examination of 2D NMR spectroscopic data of **4** and **5** assigned their structures (Figures S1 and S3 in the Supporting Information), respectively.

The formula of gloeophyllin F (**6**, $[\alpha]_D^{25}$ +25.0) was established as $C_{30}H_{48}O_3$ (seven degree of unsaturation) by its HRTOFMS at *m*/*z* 457.3682 $[M + H]^+$. The NMR data of **6**

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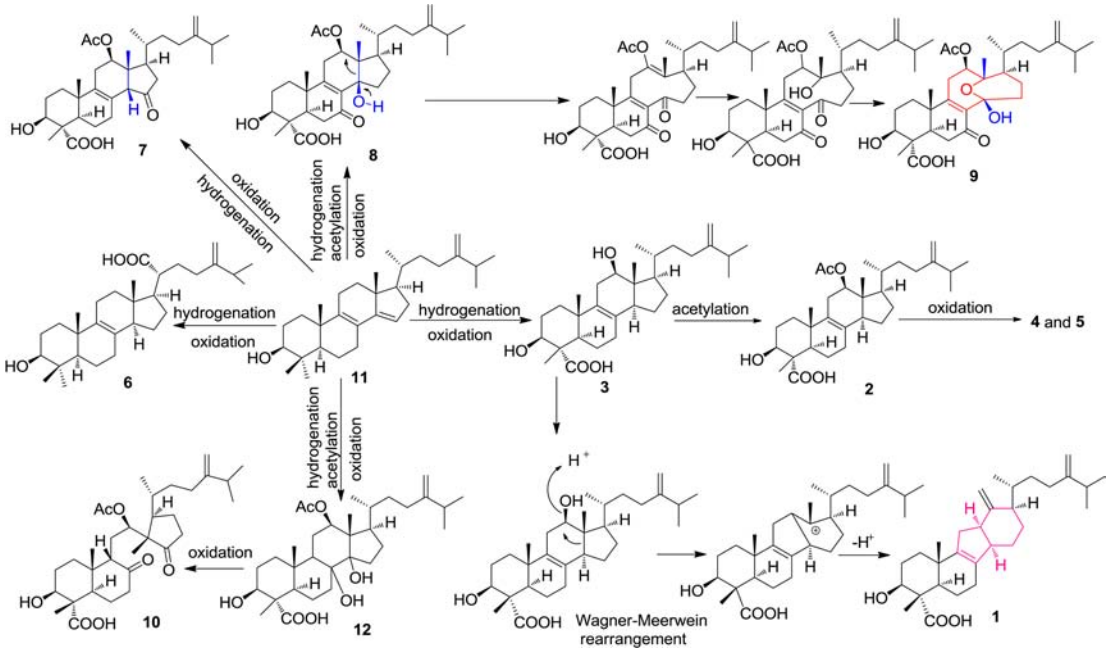
Scheme 1. Hypothetical Biogenetic Pathway of 1−10



resembled with those of eburicoic acid,[8] except for the loss of a tertiary methyl group. The structure of **6** was fully assigned by detailed interpretation of its HSQC, HMBC, and ROESY spectra (Figures S1 and S3 in the Supporting Information).

Gloeophyllins G (**7**, $[\alpha]_D^{25}$ −41.0) and H (**8**, $[\alpha]_D^{25}$ +19.0) were determined to have the molecular formula of $C_{32}H_{48}O_6$ and $C_{32}H_{48}O_7$ by HRTOFMS data [**7**: *m*/*z* 529.3523 $[M + H]^+$; **8**: *m*/*z* 567.3300 $[M + Na]^+$], respectively. The comparison of NMR data between **7** and **2**, as well as HMBC correlations from H-14, H_2-16, and H-17 to C-15 (δ_C 216.5), determined the structure of **7** with a ketone group located at C-15. NOE correlations of H-14 with H_3-18 and H_3-19 confirmed the *cis*-C/D ring junction. A comparison of NMR and MS data between **8** and **4** revealed the presence of an additional hydroxyl group in **8**. The position of this hydroxyl group was assured by HMBC correlations of H-12, H_3-18, and H-17 to the oxygenated quaternary carbon at δ_C 82.7. To determine the relative configuration at C-14, the 1D and 2D NMR spectra of **8** were recorded in DMSO-d_6. A strong NOE correlation was observed between 14-OH (δ_H 4.27 s) and H_3-18 (δ_H 0.86 s), determining the *cis*-C/D ring junction in **8**. Naturally occurring ergosteroids with a *cis*-C/D ring junction are rare in nature. The first ergosteroid with a *cis*-C/D ring junction, named camphoratin J, was isolated from the famous medicinal mushroom *Taiwanofungus camphoratus*.[9] Compounds **7** and **8** represented the second and the third examples for this special group of ergosteroids. The NMR signal assignment for **7** and **8** was achieved by detailed analysis of their 2D spectra (Figures S1 and S3 in the Supporting Information), respectively

Gloeophyllin I (**9**, $[\alpha]_D^{25}$ +11.0) was obtained as white needles. HRTOFMS spectral analysis of **9** revealed an $[M + Na]^+$ ion at *m*/*z* 583.3243, determining a molecular formula of $C_{32}H_{48}O_8$. The molecular weight difference of 16 Da between **9** and **8**, together with an additional oxygenated quaternary carbon at δ_C 98.7 in the ^{13}C spectrum of **9**, predicted the presence of a hemiketol group in **9**. Furthermore, the HMBC correlations from H_3-18 (δ_H 1.27, s) to two oxygenated carbons (δ_C 76.8, C-12; δ_C 77.8, C-13) and one tertiary carbon (δ_C 43.0, C-17) and from H-16 to C-13 and C-14 (δ_C 98.7), in combination with the $^1H-^1H$ COSY correlations of H_2-15-H_2-16-H-17 and H_2-11-H-12, confirmed the formation of an oxo bridge between C-13 and C-14. The structure of **9** was finally determined by single-crystal X-ray crystallographic analysis, as shown in Figure 2. It has an unprecedented ergostane skeleton with the incorporation of a 10-oxabicyclo [4.3.1] decane moiety replacing 6/5-fused C/D rings. The ^1H and ^{13}C signal assignment of **9** was made by detailed analysis of its HSQC, HMBC, and ROESY spectra (Figure S1 in the Supporting Information). The absolute configuration of **9** was determined to be 3*S*, 4*S*, 5*R*, 10*S*, 12*R*, 13*S*, 14*R*, 17*R*, and 20*R* on the basis of the Flack parameter [0.04(13)].

Gloeophyllins J (**10**, $[\alpha]_D^{25}$ −12.0) possessed the molecular formula of $C_{32}H_{50}O_7$ (eight degree of unsaturation), as determined by HRESIMS at *m*/*z* 569.3445 $[M + Na]^+$. The ^1H, ^{13}C, and HSQC spectra of **10** showed the resonances due to three secondary methyls [δ_H/δ_C 1.02 (d, *J* = 6.7 Hz)/21.9, 1.03 (d, *J* = 6.7 Hz)/22.2, 1.05 (d, *J* = 6.8 Hz)/19.1], three tertiary methyls [δ_H/δ_C 0.70 (s)/15.2, 0.97 (s)/13.3, 1.17 (s)/10.8], two oxygenated methines [δ_H/δ_C 4.07 (dd, *J* = 3.6, 11.7 Hz)/75.4, H-3; 4.96 (t, *J* = 10.5 Hz)/75.1, H-12], a pair of double bonds [δ_H/δ_C 4.66 (br s), 4.73 (br s)/106.7, δ_C 156.3], an acetyl group [δ_H/δ_C 2.11 (s)/21.3, 171.9], one carboxylic moiety [δ_C 181.6], and two ketone moieties [δ_C 210.3 (C-8), 221.7 (C-14)] in its structure. A detailed examination of its 2D NMR spectral data revealed an ergostane skeleton with the cleavage of the C8−C14 bond (Figures S1 and S3 in Supporting Information). The $^1H-^1H$ COSY correlations of H-12-H_2-11-H-9 [δ_H/δ_C 1.99(br d, *J* = 10.9 Hz)/55.9] and the HMBC correlations from H-12, H-17, and H_3-18 to C-14 and from H_2-6, H_2-7, H-9, and H_2-11 to C-8 supported the cleavage of the C8−C14 carbon bond and the substitution of two ketone groups at C-8 and C-14. NOE correlations of H_3-19 with H-9 and H_3-29, H-3 with H-5, and H_3-18 with H-20, together with the biosynthetic origin proposed for **1**−**10**, confirmed the β orientation of H-9, H_3-18, H_3-19, H-20, and

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H_3-29 and the α orientation of H-3, H-5, H-12, and H-17. The absolute configuration of **10** was tentatively determined to be 3*S*, 4*S*, 5*R*, 9*S*, 10*S*, 12*R*, 13*S*, 17*R*, and 20*R*.

The hypothetical biosynthesis of **1**−**10** is illustrated in Scheme 1. Compound **11** biosynthesized from lanosterol by 14-α demethylase in eukaryotic cells could be the precursor of **1**−**10**. Beginning with **11**, compound **9** is generated by a sequence of oxidation, retro-aldol reaction, acetylation, and ketol reaction. Compounds **3**, **6**−**8** can be biosynthesized from **11** through the hydrogenation of the C14−C15 double bond, followed by oxidation and acetylation. Compound **3** is subsequently transformed into **1** by a Wagner−Meerwein rearrangement reaction. With compound **12** as a possible intermediate, compound **10** can be produced from **11** by the oxidation cleavage of the C8−C14 bond. The above-mentioned chemical analysis indicates a complicated biosynthetic pathway is involved in the synthesis of **1**−**10** and brings new insight into the biosynthesis of ergosteroids in fungi. The cleavage of the C13−C14 and C8−C14 carbon bonds is the key step for the biosynthesis of **1**, **9**, and **10**, respectively.

In a cytotoxicity assay against K562 and HCT116 cell lines (Table S4 in the Supporting Informaton), compounds **1**, **2**, and **5** showed strong antiproliferative activity against K562 cells with IC_{50} values of 4.73 ± 0.62, 8.72 ± 1.12, and 8.85 ± 1.29 μg/mL, respectively.

In summary, the new skeleton, strong cytotoxicity, and unique biosynthetic pathway of **1**, **7**−**10**, as well as the special origin of the producing fungus, will make them valuable target molecules for total organic synthesis, bioactivity evaluation, and biosynthetic investigation.

■ ASSOCIATED CONTENT

Supporting Information

Full details of microbial cultivation and extraction, isolation and purification of compounds, bioassay, the ^{1}H and ^{13}C NMR spectra of **1**−**10**, and the NMR signal assignment of **2**−**8** and **10** are provided in the Supporting Information. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01080.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: liuhw@im.ac.cn.

Author Contributions

†J.-J.H. and L.B. contributed equally.

Notes

The authors declare no competing financial interest.

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