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NOTE

A novel polycyclic meroterpenoid with aldose reductase inhibitory activity from medicinal mushroom *Ganoderma leucocontextum*

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The Diabetes Control and Complication Trial (DCCT) indicated that hyperglycemia is a key risk factor for the development of diabetic complications.[1,2] Aldose reductase (AR) (alditol: NAD(P)$^+$ 1-oxidoreductase, EC 1.1.1.21) reduces the aldehyde form of glucose to sorbitol in the presence of NADPH as a cofactor. Sorbitol dehydrogenase oxidizes sorbitol to fructose in the polyol pathway, leading to loss of the functional integrity of the lens and subsequent cataract formation.[3] Therefore, AR inhibitors have been developed as drugs to treat long-term diabetic complications.[4]

Ganoderma species (Ganodermataceae, Polyporales) have been used as folk medicines to treat and prevent various diseases for centuries, particularly in China, Japan and Korea. Most of the phytochemical investigations have focused on the *Ganoderma* triterpenoids and polysaccharides. In our continuous research on bioactive components from *Ganoderma leucocontextum*, eight meroterpenes, including three new compounds with inhibitory effects on HMG-CoA reductase and α-glucosidase were isolated.[5] Further chemical investigations on this mushroom led to the isolation of six polycyclic meroterpenes, including one new compound—compound **1**. Herein, we would like to describe the isolation, structure determination and aldose reductase inhibitory activity of compounds **1–6**.

The ethanol extract of *G. leucocontextum* was partitioned between water and ethyl acetate. The ethyl acetate-soluble fraction was subjected to chromatographic separation using silica gel, ODS, Sephadex LH-20 and preparative HPLC to yield six meroterpenoids: **1–6**. Five known compounds (**2–6**) were identified as spiroapplanatumine K (**2**),[6] spiroapplanatumine L (**3**),[6] (±)-spirolingzhine A (**4, 5**)[7] and spirolingzhine D (**6**)[7] by comparison of their spectroscopic data with literature data.

Compound **1** gave a pseudomolecular ion peak at *m/z* 591.1861 [M+H]$^+$ in its high-resolution time-of-flight mass spectrometry (HRTOFMS) spectrum, indicating a molecular formula of $C_{32}H_{30}O_{11}$ (17 degrees of unsaturation). Analysis of its ^{1}H, ^{13}C, and HSQC NMR data (Table 1 and Supplementary Figures S1–S3) revealed the presence of eight methylene (two oxygenated and two olefinic methylenes), ten methine (six olefinic) and fourteen quaternary carbons (two olefinic, two ketones, two carboxylic carbonyls, six substituted benzenoid carbons and a pair of oxygenated aliphatic). The ^{1}H−^{1}H COSY and HMBC spectra analysis confirmed the presence of two isolated polycyclic meroterpenoid moieties which corresponded to **2** (Figure 1a and Supplementary Figure S4). Finally, an ester bond was assigned between C-8 and C-10″ to satisfy the requirement of the molecular weight, which was also supported by the HMBC (Figure 1b)

Table 1 NMR spectroscopic data for 1 in methanol-d_4[a]

No.	δ_C	δ_H *(mult., J in Hz)*	*No.*	δ_C	δ_H *(mult., J in Hz)*
1	204.4		1″	204.3	
2	98.1		2″	97.8	
3	56.0	3.28 overlapped	3″	55.7	3.26 overlapped
4	27.8		4″	27.5	
5	30.5		5″	30.1	
6	54.0	2.90 m	6″	53.7	2.68 m
7	145.5		7″	140.8	
8	67.8	4.22 d (7.4)	8″	65.3	3.78 br.
		4.11 d (7.4)			
9	117.0	4.86 br.	9″	113.5	5.07 d (1.5)
		4.85 br.			4.93 br.
10	174.1		10″	171.8	
1′	167.3		1‴	167.0	
2′	122.9		2‴	122.9	
3′	107.9	6.84 d (2.7)	3‴	107.8	6.82 d (2.7)
4′	153.9		4‴	153.9	
5′	128.6	7.17 dd (8.9, 2.7)	5‴	128.3	7.14 dd (8.9, 2.7)
6′	114.6	6.99 d (8.9)	6‴	114.9	6.92 d (8.9)

[a]Recorded at 500 MHz, δ_H in p.p.m, *J* in Hz.

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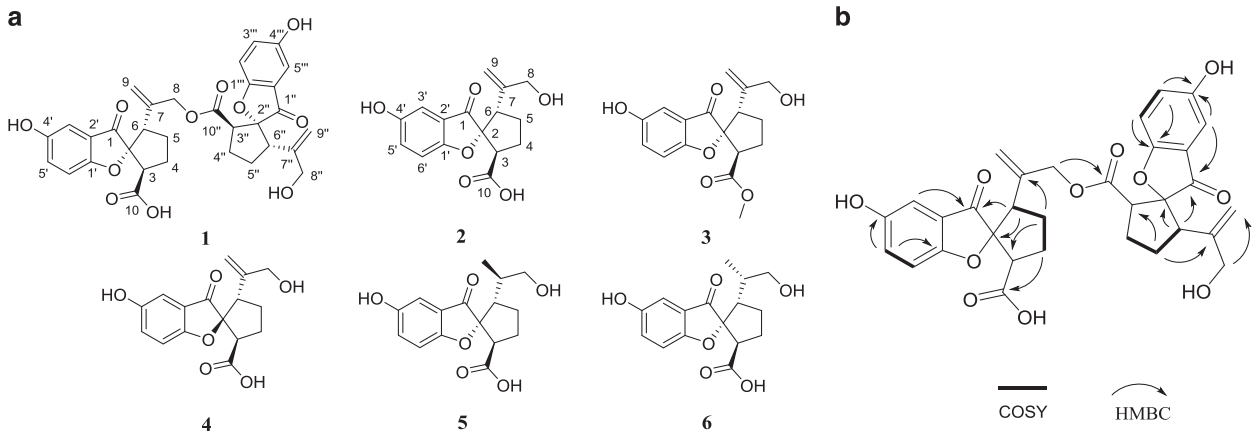

Figure 1 (**a**) Structures of compounds **1**–**6**. (**b**) Key ^{1}H-^{1}H COSY and HMBC correlations of **1**.

Table 2 Biological activities of compounds 1–6 (IC_{50}, μM)

	Enzyme inhibition		*Cytotoxicity*	
Compounds	*Aldose reductase*	*HMG-CoA reductase*	*HCT-116*	*K562*
1	28.9±10.1	27.9±5.2	>100	>100
2	>50	>100	>100	>100
3	17.2±3.2	>100	>100	>100
4	26.4±6.4	72.0±8.3	>100	27.4±3.2
5	19.6±2.3	>100	>100	>100
6	9.4±2.4	>100	>100	>100
Epalrestat	17.5±1.2	—	—	—
Atorvastatin	—	26.3±3.1	—	—
Paclitaxel	—	—	0.98±0.12	0.59±0.05

correlations from H_2-8 [δ_H 4.22 (d, J=7.4 Hz); 4.11 (d, J=7.4 Hz)] to C-10″ (δ_c 171.8). The alkaline hydrolysis of **1** yielded only compound **2**, which further confirmed the absolute configuration. Thus compound **1** was assigned as shown, and named as ganoleucin D.

Meroterpenes are known to have biological activities that can be important for drug discovery. In recent years, a number of new meroterpenes with unusual skeletons and interesting bioactivities were reported from *Ganoderma* species. These *Ganoderma*-derived meroterpenes presented various bioactivities, including renoprotective activity, anti-fibrotic activity,[8] anti-allergic activity,[9] anti-HIV activity and inhibitory activities against monocyte chemotactic protein 1,[10] p-Smad3,[11] acetylcholinesterase[12] and Ca_v3.1 type calcium channel.[13] These secondary metabolites have attracted great interest in the medicinal chemistry community.

All isolates were evaluated *in vitro* for inhibitory activity against AR. As shown in Table 2, compounds **1**, **3–6** showed inhibitory effects against aldose reductase from bovine lens tissue with IC_{50} values in the range 9.4−28.9 μM.

The enzyme inhibition property against aldose reductase of spirolingzhine D (**6**), the most potent inhibitor, was performed by *in silico* docking study with bovine lens aldose reductase to confirm the interaction by DDI-CPI tool, a web-based server that can predict drug−drug interactions via the chemical−protein interactome.[14,15] Figure 2 shows the homology model of the aldose reductase with the ligand docking into the binding site. The molecular docking results showed that the binding pocket involves amino acid residues Trp20,

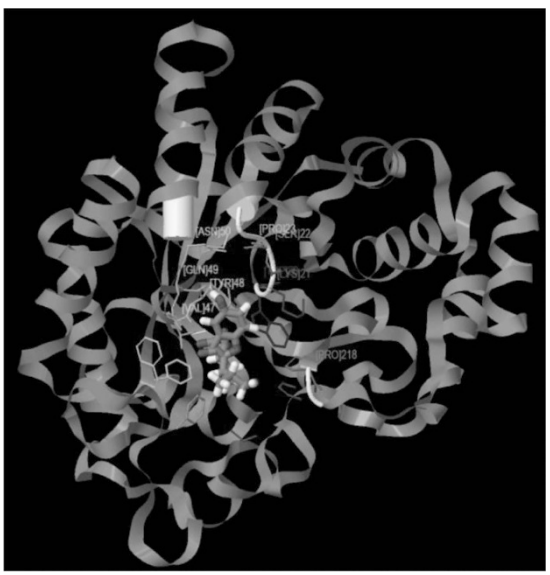

Figure 2 Homology model of the aldose reductase (AR) with the ligand into the binding site. A full colour version of this figure is available at the *Journal of Antibiotics* journal online.

Ser22, Pro23, Val47, Tyr48, Gln49, Asn50 and Pro218. This calculated observation was in good agreement with the enzymatic assay, where spirolingzhine D (**6**) showed strongest inhibition activity with an IC_{50} value of 9.4 μM.

Furthermore, all six compounds were tested for their inhibition effect on HMG-CoA reductase and cytotoxicity against HCT-116 and K562 cell lines. As displayed in Table 2, compound **1** showed moderate inhibitory effect on HMG-CoA reductase, with a IC_{50} value of 27.9 μM. In cytotoxicity assay, only **4** displayed weak activity against K562 cell (IC_{50} 27.4 μM).

In summary, one new meroterpenoid and five known compounds were isolated and identified from the fruiting bodies of *Ganoderma leucocontextum*. Ganoleucin D (**1**), spiroapplanatumine K (**2**), spiroapplanatumine L (**3**), (±)-spirolingzhine A (**4, 5**) and spirolingzhine D (**6**) were found to have inhibitory effects against aldose reductase, and **1** also showed moderate inhibitory activity on HMG-CoA reductase. These results provided evidence for the application of *G. leucocontextum* and its constituents in the prevention and treatment of diabetic complications and other related diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)