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Ganoderic acid Df, a new triterpenoid with aldose reductase inhibitory activity from the fruiting body of *Ganoderma lucidum*

Sri Fatmawati [a,b], Kuniyoshi Shimizu [a], Ryuichiro Kondo [a,*]

[a] Department of Forest and Forest Products Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan, 812-8581
[b] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sepuluh Nopember Institute of Technology, Kampus ITS-Sukolilo, Surabaya, Indonesia, 60111

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ABSTRACT

Ganoderic acid Df, a new lanostane-type triterpenoid, was isolated from the fruiting body of *Ganoderma lucidum*. Its structure was characterized as 7β, 11β-dihydroxy-3, 15, 23-trioxo-5α-lanosta-8-en-26-oic acid by 1D- and 2D-NMR spectra. This compound exhibited potent human aldose reductase inhibitory activity, with an IC_{50} of 22.8 μM *in vitro*. A carboxyl group of this compound's side chain is essential for eliciting inhibitory activity because its methyl ester is much less active.

1. Introduction

Ganoderma lucidum (Leyss;Fr) Karst. (Ganodermataceae) are well-known medicinal woody mushrooms called "Lingzhi" in Chinese, "Reishi" in Japanese, and "Yeongji" in Korean. For hundreds of years, this mushroom has been used to prevent and treat various human diseases. *G. lucidum* has been reported to produce many biologically active compounds such as sterol, polysaccharide, and triterpenoids. More than 100 triterpenoids have been isolated from *G. lucidum* and the genus *Ganoderma* [1]. Triterpenoids that have been isolated from the fruiting body of *G. lucidum* are divided into two groups: ganoderma acids with a carboxyl group in the side chain and ganoderma alcohols with a hydroxyl group in the side chain [2]. The ganoderma acids isolated from this mushroom show anti-androgenic, anti-5α-reductase, anti-inflammatory, anti-tumor and other biological activities [3–5].

Aldose reductase (alditol: $NAD(P)^+$ 1-oxidoreductase, EC 1.1.1.21) is the first enzyme in the polyol pathway. This enzyme catalyzes the reduction of glucose to sorbitol by coupling with the oxidation of NADPH to $NADP^+$. The accumulation of sorbitol then leads to diabetic complications. For this reason, aldose reductase inhibitors have been introduced as a vehicle for the treatment of diabetic complications. In our continuing search for an aldose reductase inhibitory constituent, we have focused on the fruiting body of *G. lucidum*.

In our previous research, we found that the extract of *G. lucidum* had the strongest aldose reductase inhibitory activity among 17 edible and medicinal mushrooms, and significantly alleviated the galactitol accumulation in the eye lenses of galactosemic rats [6]. By using a chloroform extract of *G. lucidum*, we isolated a new compound, 7β,11β-dihydroxy-3,15,23-trioxo-5α-lanosta-8-en-26-oic acid, which we named ganoderic acid Df (**1**). This acid potently inhibited aldose reductase. In the present study, we evaluated the isolated compound (**1**) and its methyl ester (**2**) on aldose reductase inhibition.

2. Experimental

2.1. General experimental procedures

The compound was isolated with *p*-HPLC by using Waters™ 600 Controller, Waters™ 486 Tunable absorbance detector, and Waters 600 Multisolvent Delivery System.

* Corresponding author. Tel./fax: +81 92 642 2811.
E-mail address: ryukondo@agr.kyushu-u.ac.jp (R. Kondo).

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HRESIMS were measured with an AccuTOFCS JMS-T100CS mass spectrometer (JEOL). The NMR spectrum were recorded by JNM-AL400 FT NMR spectrometer (JEOL, 400 MHz). Optical rotations were recorded on a JASCO DIP-370 digital Polarimeter. Column chromatography was carried out on silica-gel (Wakogel C-200 particle size 75-150 μm, Wako). Thin layer chromatography (TLC) was carried out on pre-coated Silica-gel 60 F_{254} plates (0.25 mm, Merck) and spot were detected with I_2 detection and under UV light. The compound was dissolved in chloroform-d_1 (50%) and methanol-d_4 (50%) then chemical shifts were referred to deuterated solvents. The compounds were assigned for ^{1}H, ^{13}C, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and NOESY.

2.2. Fungal material

The fruiting body of *G. lucidum* was obtained from Bisoken Inc. (Oita, Japan). The materials were identified by Mr. Shuhei Kaneko, Fukuoka Prefecture Forest Research & Extension Center (Fukuoka, Japan). The voucher specimen (BMC9049) was deposited at the herbarium of the Department of Forest and Forest Product Sciences, Kyushu University, Fukuoka, Japan.

2.3. Extraction and isolation

The dried and milled fruiting body of *G. lucidum* (3 kg) were extracted with $CHCl_3$ (3 × 8 L) at room temperature for 24 h. The extracts were filtered through ADVANTEC No.2 filter paper, concentrated under vacuum and then freeze-dried to obtain $CHCl_3$ extract (111 g). The concentrated extracts (85.5 g) were suspended in 5% $NaHCO_3$, and $CHCl_3$ was used to extract the neutral fraction (59.7 g). The aqueous layer was acidified with 2 M HCl to pH 3 and then re-extracted with $CHCl_3$ to yield an acidic fraction (24.5 g). A portion of acidic fraction (23.2 g) was applied to a silica gel (550 g of Wakogel C-200, 6 × 36 cm) and eluted with a gradient $CHCl_3$-MeOH solvent system of increasing polarity (1:0 → 0:1) to afford 25 fractions (Fr A1–Fr A25). Fr A10 (850 mg) was subjected to column chromatography over silica gel (36 g of Wakogel C-200, 3 × 11 cm) again and eluted with CH_2Cl_2/MeOH (1:0 100 mL, 70:5 75 mL, 60:5 65 ml, 45:5 50 mL, 4:1 50 mL, 3:2 50 mL and MeOH 150 mL) to give seven fractions (Fr A10-1 to Fr A10-7). Fr A10-3 was applied to *p*-HPLC (column Inertsil ODS-3: 20 mm i.d × 250 mm), the mobile phase was composed of 1% AcOH/H_2O-CH_3CN (0 min, 60:40; 45 min, 58:42), with flow rate: 8 mL/min and the detecting wavelength was set at 252 nm. This fractionation afforded Fr A10-3-7 (**1**, R_T: 21.5 min).

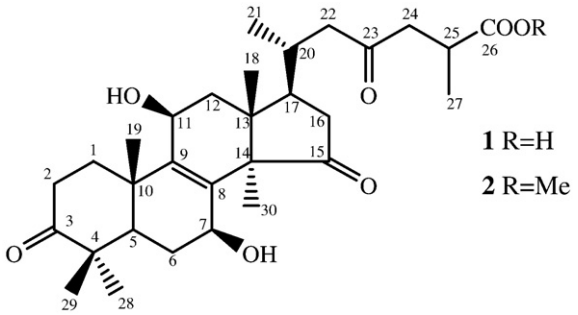

Fig. 1. Ganoderic acid Df (**1**) and its methyl ester (**2**) from *Ganoderma lucidum.*

Table 1
NMR spectroscopic data of **1** [a] (*J* in Hz).

Position	$\delta_{C,\ mult.}$	δ_H	HMBC (H to C)
1	35.42, CH_2	1.74, m(α), 2.88, m(β)	2, 3, 10, 19
2	34.66, CH_2	2.90, m(α), 2.64, m(β)	3
3	220.96, qC		
4	47.22, qC		
5	45.66, CH	2.13, dd (9.0, 3.2)	4, 6, 7, 10, 28, 29
6	28.12, CH_2	1.77, m(α), 1.27, d (11.4) (β)	4, 5, 7, 8, 10
7	67.12, CH	4.62, m	5, 6, 8
8	161.77, qC		
9	140.16, qC		
10	38.39, qC		
11	72.04, CH	4.58, dd (10.5, 8.7)	8, 9
12	52.38, CH_2	2.83, m(α), 2.43, m(β)	9, 11, 13, 14, 18
13	47.63, qC		
14	54.05, qC		
15	200.98, qC		
16	35.33, CH_2	2.78, m(α), 2.41, m(β)	15, 17
17	49.54, CH	2.03, m	18, 20
18	17.75, CH_3	0.92, s	12, 13, 14, 17
19	17.88, CH_3	1.04, s	1, 5, 9, 10
20	33.21, CH	1.99, m	21, 22
21	19.50, CH_3	0.86, d (5.6)	17, 20, 22
22	50.02, CH_2	2.28, m(α), 2.53, dd (4.3, 12.4) (β)	20, 21, 23
23	210.70, qC		
24	47.23, CH_2	2.93, m(α), 2.49, m(β)	23, 25, 27
25	37.40, CH	2.95, m	26, 27
26	179.01, qC		
27	17.37, CH_3	1.20, d (7.0)	25, 26
28	27.84, CH_3	1.17, s	3, 4, 5, 29
29	20.68, CH_3	1.09, s	3, 4, 5, 28
30	21.40, CH_3	1.30, s	8, 13, 14

[a] Spectra were recorded in CD_3OD (50%) and $CDCl_3$ (50%).

2.4. 7β,11β-dihydroxy-3,15,23-trioxo-5α-lanosta-8-en-26-oic acid

White amorphous powder; $[\alpha]_D^{25}$ + 177.0 (*c* 0.11, DMSO); ^{1}H and ^{13}C (CD_3OD (50%) and $CDCl_3$ (50%), 400 MHz) data are reported in Table 1; HRESIMS *m/z* 515.0066 $[M-H]^-$, (calcd for $C_{30}H_{43}O_7$ 515.0008). NOESY cross-peaks H-1β/H_3-19; H-5/H-2α, H-7, H_3-28; H-6α/ H-5; H-7/H-11, H_3-28; H-12β/ H_3-18; H-17α/H_3-30; H_3-18/H_3-19; H_3-30/ H-16α.

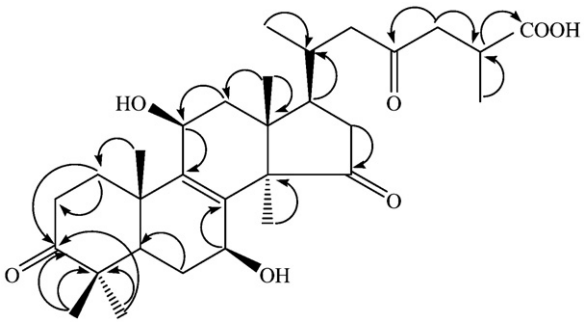

Fig. 2. Selected HMBC (H → C) correlations of **1.**