Arch Pharm Res Vol 29, No 6, 479-483, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Constituents from the Fruiting Bodies of *Ganoderma applanatum* and Their Aldose Reductase Inhibitory Activity

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(Received February 7, 2006)

Eight compounds were isolated from the fruiting bodies of *Ganoderma applanatum*, and were identified as 2-methoxyfatty acids (**1**), 5-dihydroergosterol (**2**), ergosterol peroxide (**3**) 3β,7β,20,23ξ-tetrahydroxy-11,15-dioxolanosta-8-en-26-oic acid (**4**), 7β,20,23ξ-trihydroxy-3,11,15-trioxolanosta-8-en-26-oic acid (**5**), cerevisterol (**6**), 7β,23ξ-dihydroxy-3,11,15-trioxolanosta-8,20*E*(22)-dien-26-oic acid (**7**), and 7β-hydroxy-3,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester (**8**) by spectral analysis. All compounds were isolated for the first time from this fruiting bodies, and their effect on rat lens aldose reductase (RLAR) activity was tested. Among these eight compounds, ergosterol peroxide (**3**) was found to exhibit potent RLAR inhibition, its IC_{50} value being 15.4 μg/mL.

Key words: *Ganoderma applanatum*, Polyporaceae, Ergosterol peroxide, Aldose reductase activity

INTRODUCTION

The fruiting bodies of *Ganoderma lucidum* are a well-known Chinese medicine that has been used clinically in China, Japan and Korea for a long time. More than 130 highly oxygenated lanostane-type triterpenoids have been isolated from the fruiting bodies, mycelia and spores of *G. lucidum*, including common fungal steroids derived from ergosterol, some of them exhibiting a bitter taste or useful biological activities (Kim and Kim, 1999). Other *Ganoderma* spp. have also been used in traditional medicine for the treatment of cancer, hypertension and chronic bronchitis, and as a tonic or sedative in China, Japan and Korea (Ming *et al.*, 2002; Yoshikawa *et al.*, 2002).

In a previous paper, we reported on the isolation of compounds from *G. applanatum* and their rat lens aldose reductase (RLAR) activity (Lee *et al.*, 2005). In our ongoing search for the bioactive principles contained in crude drugs, eight compounds were isolated from the fruiting bodies of *G. applanatum*. Herein, the isolation of compounds from *G. applanatum* and their effects on RLAR activity *in vitro* are described.

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MATERIALS AND METHODS

Plant material

The fruiting bodies of *G. applanatum* (Leyss. ex Fr.) Karst. (Polyporaceae) were provided by St. Clair Milk and Grocery (Niagara Falls, Canada) in March 2002. The botanical identification was made by Mr. Gregory J. Belmore (Ministry of Natural Resources, Ontario, Canada). A voucher specimen (No. 2002-02) was deposited at the Herbarium of Natural Products Research Institute, Seoul National University, Korea.

Instruments and reagents

Melting points were recorded with a Mitamura-Riken melting point apparatus and are uncorrected. EIMS spectra were measured with a Hewlett Packard model 5989B GC/MS system. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian Gemini-2000 (300 MHz) spectrometer using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. TLC analysis was performed on Kieselgel 60 F_{254} precoated plates (Merck,

No. 5715), with compounds visualized by spraying with 20% H_2SO_4 followed by heating. Silica gel (Merck, 70-230 mesh) was used for column chromatography. All other chemicals and reagents were of analytical grade.

Extraction and isolation

The grounded fruiting bodies of *G. applanatum* were extracted five times with MeOH under reflux. The MeOH extract was suspended in water and successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH.

A portion of the CH_2Cl_2 fraction was separated by silica gel column chromatography with $CHCl_3$-containing increasing amounts of MeOH (1, 2, 3, 5, 10, 50 and 100%) as the eluent to give 16 fractions (MC01-MC16). Fractions MC06 and MC05 were separately crystallized from MeOH to afford compounds **1** and **2**, respectively. Fraction MC09 was subjected to silica gel column chromatography, with increasing amounts of *n*-hexane-acetone (5, 10, 15, 20, 30, and 50%) as the eluent to yield 13 fractions (MC09-01-MC09-13). Fraction MC09-04 was purified by silica gel column chromatography, with increasing amounts of $CHCl_3$-MeOH (0.5, 1 and 3%) as the eluent to yield compound **3**. Fraction MC09-08 was further purified by crystallization from MeOH to yield compound **7**. Fractions MC09-07, MC10, MC11 and MC12 were further purified on a silica gel column with *n*-hexane-EtOAc (= 10:1, 8:5, 5:8, 1:10) as the eluent to yield compounds **8**, **4**, **5** and **6**, respectively.

2-Methoxy fatty acid (1)

m.p. 64-65°C; EIMS (%) : *m/z* 412 $[M]^+$ (2-methoxypentacosanoic acid, 0.8), 398 $[M]^+$ (2-methoxylignoceric acid, 5.8), 384 $[M]^+$ (2-methoxytricosanoic acid, 1.5), 370 $[M]^+$ (2-methoxybehenic acid, 2.1), 90 $[C_3H_6O_3]^+$ (23.1); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.86 (3H, d, *J* = 6.6 Hz, CH_3), 1.25, 1.30 [each s, $(CH_2)_n$], 3.71 (3H, s, 2-OCH_3), 4.55 (1H, dd, *J* = 5.1, 7.4 Hz, H-2); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 175.9 (C-1), 71.2 (C-2), 51.5 (OCH_3), 35.2, 32.1, 30.0, 29.9, 29.7, 29.6, 25.7, 22.9 (all CH_2), 14.3 (CH_3).

5-Dehydroergosterol (2)

m.p. 170-173°C; EIMS (%): *m/z* 398 $[M]^+$ (16.5), 383 $[M - CH_3]^+$ (7.4), 355 $[M - C_3H_7]^+$ (2.5), 300 (8.3), 285 $[300 - CH_3]^+$ (3.3), 273 $[M - SC(C_9H_{17})]^+$ (17.4), 271 $[M - (SC + 2H)]^+$ (54.5), 255 $[M - (SC + H_2O)]^+$ (18.2), 229 (9.9), 213 $[M - (ring\ D + H_2O)]^+$ (7.4), 55 (100); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.54 (3H, s, 18-CH_3), 0.80 (3H, s, 19-CH_3), 0.82 (3H, d, *J* = 6.9 Hz, 26-CH_3), 0.84 (3H, d, *J* = 6.6 Hz, 27-CH_3), 0.91 (3H, d, *J* = 6.9 Hz, 28-CH_3), 1.02 (3H, d, *J* = 6.9 Hz, 21-CH_3), 3.60 (1H, m, H-3), 5.19 (2H, m, H-22,23); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 37.1 (C-1), 31.5 (C-2), 71.1 (C-3), 38.0 (C-4), 40.3 (C-5), 29.6 (C-6), 117.5 (C-7), 139.5 (C-8), 49.5 (C-9), 34.2 (C-10), 21.5 (C-11), 39.5 (C-12), 43.3 (C-13), 43.3 (C-14), 55.1 (C-15), 22.9 (C-16), 56.0 (C-17), 12.1 (C-18), 13.0 (C-19), 40.5 (C-20), 21.1 (C-21), 135.7 (C-22), 131.9 (C-23), 42.8 (C-24), 33.1 (C-25), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28).

Ergosterol peroxide (3)

m.p. 182-184°C; EIMS (%): *m/z* 428 $[M]^+$ (0.8), 410 $[M - H_2O]^+$ (1.6), 396 $[M - O_2]^+$ (6.5), 363 (3.3), 285 $[M - H_2O - SC(C_9H_{17})]^+$ (3.0), 253 $[M - O_2 - H_2O - SC]^+$ (4.9), 213 (6.5), 149 (18.7), 105 (58.5), 83 (44.7), 69 (89.4), 55 (100); ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.81 (3H, s, 18-CH_3), 0.81 (3H, d, *J* = 6.6 Hz, 27-CH_3), 0.83 (3H, d, *J* = 6.9 Hz, 26-CH_3), 0.88 (3H, s, 19-CH_3), 0.91 (3H, d, *J* = 6.9 Hz, 28-CH_3), 1.00 (3H, d, *J* = 6.9 Hz, 21-CH_3), 3.97 (1H, m, H-3), 5.13 (1H, dd, *J* = 8.1, 15.3 Hz, H-22), 5.23 (1H, dd, *J* = 6.9, 15.3 Hz, H-23), 6.24 (1H, d, *J* = 8.6 Hz, H-6), 6.50 (1H, d, *J* = 8.6 Hz, H-7); ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 34.9 (C-1), 30.4 (C-2), 66.7 (C-3), 37.2 (C-4), 82.4 (C-5), 135.7 (C-6), 131.0 (C-7), 79.7 (C-8), 51.4 (C-9), 37.2 (C-10), 23.6 (C-11), 39.6 (C-12), 44.8 (C-13), 51.9 (C-14), 20.9 (C-15), 28.9 (C-16), 56.5 (C-17), 13.1 (C-18), 18.4 (C-19), 40.0 (C-20), 21.1 (C-21), 135.4 (C-22), 132.6 (C-23), 43.0 (C-24), 33.3 (C-25), 19.9 (C-26), 20.2 (C-27), 17.8 (C-28).

3β,7β,20,23ξ-Tetrahydroxy-11,15-dioxolanosta-8-en-26-oic acid (4)

Colorless amorphous solid; $[\alpha]_D^{26}$ = +117.5° (*c* 0.211, $CHCl_3$), UV (MeOH) λ_{max} (log ε) 252 nm (4.34); IR ν_{max} 3430 (OH), 1773 (five-membered ring C=O), 1711 (COOH), 1647 (α,β-unsaturated C=O), 1458 (CH_2), 1377 (CH_3), 1181, 1034 (OH), 926 cm^{-1}; EIMS *m/z* (rel. int., %) 516 $[M - H_2O]^+$ (2.5), 498 $[M - 2H_2O]^+$ (0.8), 470 $[M - (2H_2O + CO)]^+$ (5.8), 358 [M - side chain (SC) - H]$^+$ (1.7), 313 $[M - SC - (CO + H_2O)]^+$ (0.8), 175 $[C_8H_{15}O_4, SC]^+$ (8.3), 157 $[SC - H_2O]^+$ (8.3), 99 (45.5), 69 (100), 55 (100); (+)-FABMS *m/z* 539 $[M + Na - H_2O]^+$; (+)-HRFABMS *m/z* 539.2982 (calcd for $C_{30}H_{44}O_7Na$, 539.2985).

7β,20,23ξ-Trihydroxy-3,11,15-trioxolanosta-8-en-26-oic acid (5)

Colorless amorphous solid; $[\alpha]_D^{26}$ = +225.5° (*c* 0.216, $CHCl_3$), UV (MeOH) λ_{max} (log ε) 250 nm (4.07); IR ν_{max} 3569 and 3491 (OH), 1767 (five-membered ring C=O), 1734 (six-membered ring C=O), 1699 (COOH), 1661 (α,β-unsaturated C=O), 1458 (CH_2), 1377 (CH_3), 1171 and 1069 (OH), 924 cm^{-1}; EIMS *m/z* (rel. int., %) 514 $[M - H_2O]^+$ (43.8), 468 $[M - (2H_2O + CO)]^+$ (100), 376 (20.8), 329 $[M - (SC + CO)]^+$ (6.9), 175 $[C_8H_{15}O_4, SC]^+$ (8.5), 157 $[SC - H_2O]^+$ (8.3), 99 (13.8), 69 (13.5); (+)-FABMS *m/z* 537 $[M + Na - H_2O]^+$; (+)-HRFABMS *m/z* 537.2807 (calcd for $C_{30}H_{42}O_7Na$, 537.2828).

Cerevisterol (6)

m.p. 224-226°C; EIMS (%): *m/z* 412 $[M - H_2O]^+$ (20.5),

397 [M - H_2O - CH_3]$^+$ (6.6), 394 [M - $2H_2O$]$^+$ (10.6), 382 [M - H_2O - $2CH_3$]$^+$ (11.5), 379 [M - $2H_2O$ - CH_3]$^+$ (18.0), 376 [M - $3H_2O$]$^+$ (3.3), 369 [M - H_2O - C_3H_7]$^+$ (2.5), 287 [M - H_2O - $SC(C_9H_{17})$]$^+$ (2.5), 269 (43.4), 69 (100), 55 (94.3); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 0.65 (3H, s, 18-CH_3), 0.84 (3H, d, *J* = 6.6 Hz, 26-CH_3), 0.85 (3H, d, *J* = 6.9 Hz, 27-CH_3), 0.94 (3H, d, *J* = 6.9 Hz, 28-CH_3), 1.05 (3H, d, *J* = 6.3 Hz, 21-CH_3), 1.54 (3H, s, 19-CH_3), 4.33 (1H, br s, H-6), 4.84 (1H, m, H-3), 5.16 (1H, dd, *J* = 7.8, 15.3 Hz, H-22), 5.24 (1H, dd, *J* = 6.9, 15.3 Hz, H-23), 5.74 (1H, t, *J* = 2.4 Hz, H-7); ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 32.7 (C-1), 30.0 (C-2), 67.6 (C-3), 39.9 (C-4), 76.1 (C-5), 74.2 (C-6), 120.5 (C-7), 141.5 (C-8), 43.0 (C-9), 33.8 (C-10), 22.4 (C-11), 38.0 (C-12), 43.7 (C-13), 55.2 (C-14), 23.5 (C-15), 28.35 (C-16), 56.1 (C-17), 12.5 (C-18), 18.8 (C-19), 40.9 (C-20), 19.8 (C-21), 132.1 (C-22), 136.2 (C-23), 42.0 (C-24), 33.3 (C-25), 20.1 (C-26), 214 (C-27), 17.8 (C-28).

7β,23ξ-Dihydroxy-3,11,15-trioxolanosta-8,20*E*(22)-dien-26-oic acid (7)

Colorless amorphous solid; $[\alpha]_D^{27}$ = +95.4° (*c* 0.2, MeOH), UV (MeOH) λ_{max} (log ε) 254 nm (3.94); IR ν_{max} 3437 (OH), 1761 (five-membered ring C=O), 1719 (six-membered ring C=O), 1655 (α,β-unsaturated C=O), 1458 (CH_2), 1377 (CH_3) cm^{-1}; EIMS *m/z* (rel. int., %) 496 [M - H_2O]$^+$ (7.3), 468 [M - H_2O - CO]$^+$ (9.8), 450 [M - $2H_2O$ - CO]$^+$ (1.6), 435 [M - $2H_2O$ - CO - CH_3]$^+$ (1.6), 395 (7.3), 358 [M - SC + H]$^+$ (16.3), 273 (13.0), 175 (39.0), 157 [$C_8H_{13}O_3$, SC]$^+$ (12.2), 149 (42.3), 121 (59.3), 93 (87.8), 69 (74.8), 55 (100); (+)-FABMS *m/z* 497 [M + H - H_2O]$^+$, 479 [M + H - $2H_2O$]$^+$; (+)-HRFABMS *m/z* 497.2913 (calcd for $C_{30}H_{41}O_6$, 497.2903).

7β-Hydroxy-3,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester (8)

Colorless amorphous solid; $[\alpha]_D^{27}$ = +106.8° (*c* 0.5, MeOH), UV (MeOH) λ_{max} (log ε) 245 nm (3.74); IR ν_{max} 3429 (OH), 1736 (five-membered ring C=O), 1730 (six-membered ring C=O), 1710 ($COOCH_3$), 1657 (α,β-unsaturated C=O), 1385 (CH_3), 1170 and 833 cm^{-1}; (+)-FABMS *m/z* 527 [M + H]$^+$; (+)-HRFABMS *m/z* 527.3008 (calcd for $C_{31}H_{43}O_7$, 527.3009).

Aldose reductase activity *in vitro*

Rat lenses were removed from Sprague-Dawley rats weighing 250-280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to the procedures of Hayman and Kinoshita (1965). The partially purified enzyme was routinely used to test for enzyme inhibition. The partially purified material was separated into 1.0 mL aliquots and stored at -40°C. The rat lenses were homogenized and centrifuged at 12,000×g and the supernatant was used as an enzyme source. The aldose reductase activity was assayed spectrophotometrically by measuring the decrease in the absorption of NADPH (16 mM) at 340 nm over a 5 min period, with DL-glyceraldehyde as the substrate (Sato and Kador, 1990). The concentration of the inhibitors giving rise to a 50% inhibition of the enzyme activity (IC_{50}) was calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity.

RESULTS AND DISCUSSION

The bioassay-guided fractionation of the CH_2Cl_2 fraction from *G. applanatum* was subjected to repeated chromatography on a silica gel and led to the isolation of eight compounds. They were identified as 2-methoxyfatty acids (**1**), 5-dihydroergosterol (**2**), ergosterol peroxide (**3**) 3β,7β,20,23ξ-tetrahydroxy-11,15-dioxolanosta-8-en-26-oic acid (**4**), 7β,20,23ξ-trihydroxy-3,11,15-trioxolanosta-8-en-26-oic acid (**5**), cerevisterol (**6**), 7β,23ξ-dihydroxy-3,11,15-trioxolanosta-8,20*E*(22)-dien-26-oic acid (**7**), and 7β-hydroxy-3,11,15,23-tetraoxolanosta-8,20*E*(22)-dien-26-oic acid methyl ester (**8**) by spectral analysis and the comparison of their spectroscopic data with those reported in the literature (Domínguez *et al.*, 1972; Ishizuka *et al.*, 1998; Jinming *et al.*, 2001; Kawagishi *et al.*, 1988; Kobayashi *et al.*, 1992; Wright, 1981). Among them, compounds **1**, **4**, **5**, **7**, and **8** had already been reported (Lee *et al.*, 2005; Shim *et al.*, 2004). To the best of our knowledge, all compounds were isolated for the first time from the fruiting bodies of *G. applanatum*.

All of the compounds were tested for their effects on RLAR *in vitro* using DL-glyceraldehyde as a substrate, and the results are summarized in Table I. Among them, compound **3** was found to exhibit potent RLAR inhibition,

Table I. Inhibitory effects of compounds **1** - **8** on RLAR

Compound	Concentration (μg/mL)	Inhibition (%)	IC_{50} (μg/mL)
TMG	10	91.5	0.6
	1	62.9	
	0.1	18.4	
1	100	0.73	>100
2	100	1.0	>100
3	100	92.2	15.4
	50	57.0	
	10	45.2	
4	100	7.2	>100
5	100	2.6	>100
6	100	20.3	>100
7	100	10.0	>100
8	100	7.2	>100

Inhibition rates were calculated as percentages with respect to the control value.
TMG: Tetramethylene glutaric acid

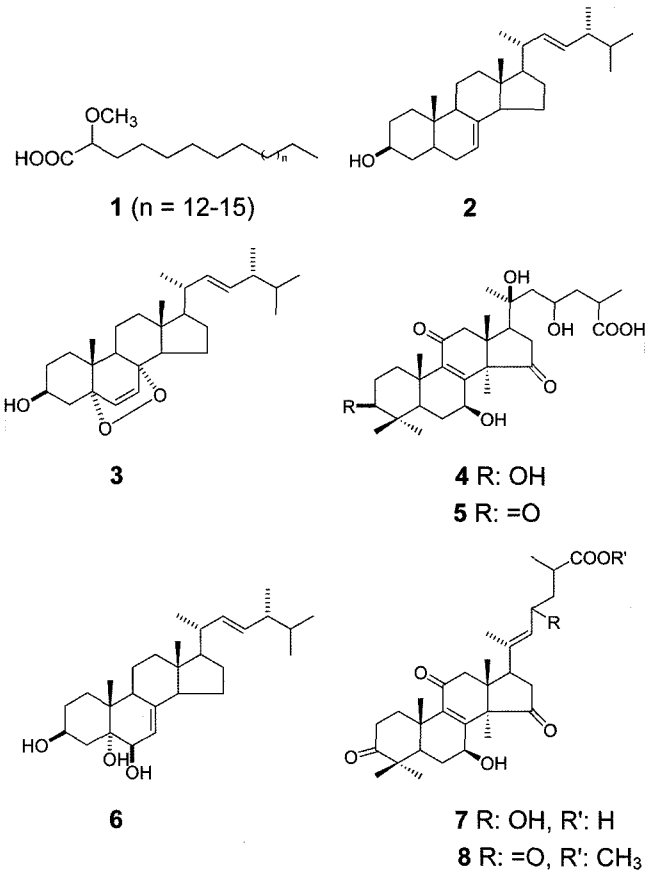

Fig. 1. Structures of compounds **1** - **8**

its IC_{50} value being 15.4 μg/mL, while that of TMG, a known AR inhibitor, was 0.6 μg/mL. It has been acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors of AR (Haraguchi *et al.*, 1996; Matsuda *et al.*, 1999; Matsuda *et al.*, 2002). So far, a number of compounds have been isolated from plants which are known to act as AR inhibitors and these have been classified as flavonoids (Aida *et al.*, 1990; Haraguchi *et al.*, 1996; Haraguchi *et al.*, 1998; Haraguchi *et al.*, 2003; Shin *et al.*, 1994; Yoshikawa *et al.*, 1998), stilbens (Matsuda *et al.*, 2002), terpenoids (Shimizu *et al.*, 1987), ellagic acid and its derivatives (Ueda *et al.*, 2004), and alkaloids (Lee, 2002).

The present study was carried out in a search for new potential AR inhibitors from the fruiting bodies of *G. applanatum*, and ergosterol peroxide (**3**) was isolated as an active principle that may be useful for the treatment of diabetic complications.

ACKNOWLEDGEMENTS

This study was supported by a grant from the St. Clair Milk and Grocery (Niagara Falls, Canada).

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