

ORIGINAL ARTICLE

Pentanol derivatives from basidiomycete *Catathelasma imperiale* and their 11 β -hydroxysteroid dehydrogenases inhibitory activity

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Five new secondary metabolites derived from pentanol, namely catathelasmols A–E (1–5), were isolated from the fruiting bodies of the basidiomycete *Catathelasma imperiale*. Their structures were elucidated on the basis of spectroscopic analysis, and the absolute configurations were determined by computational chemistry. Compounds 3, 4 and 5 showed inhibitory activities against two isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2), with IC₅₀ values of 28.7–62.3 $\mu\text{g ml}^{-1}$ (human 11 β -HSD1), 30.4–149.2 $\mu\text{g ml}^{-1}$ (mouse 11 β -HSD1), 5.1–177 $\mu\text{g ml}^{-1}$ (human 11 β -HSD2) and 32.3–129.1 $\mu\text{g ml}^{-1}$ (mouse 11 β -HSD2), which catalyze the interconversion of cortisol and cortisone.

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INTRODUCTION

Catathelasma imperiale (Fr.) Sing. (Tricholomataceae) is a conifer-loving basidiomycete defined by its large size, white spore print, sticky brownish cap, mealy odor and double ring. This mushroom is mainly distributed in the southwest of China. Only a few ergosterols have been reported from the chemical investigation of this fungus.¹ As a part of our efforts to discover the structurally diverse and biologically active secondary metabolites from higher fungi,^{2–5} the investigation of the fruiting bodies of *C. imperiale* has led to the isolation of five new compounds, catathelasmols A–E (1–5). Herein, details of the isolation and structural elucidation of 1–5 are described, including assignment of the absolute configurations by computational chemistry. Among them, compounds 3–5 showed inhibitory activities against two isozymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2), which catalyze the interconversion of cortisol and cortisone. Although these secondary metabolites are structurally simple, there are very few reports on these types of natural products.

MATERIALS AND METHODS

General

Optical rotations were obtained on a Horiba SEPA-300 polarimeter (Horiba, Tokyo, Japan). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer (Bruker GmbH, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded with a Bruker DRX-500 instrument (Bruker GmbH) in CDCl₃

($\delta_{\text{H}}=7.26$ p.p.m., $\delta_{\text{C}}=77.00$ p.p.m.) at room temperature. EI-MS, electrospray ionization mass spectrum (ESI-MS) and high resolution electrospray ionization mass spectrum (HR-ESI-MS) were measured on Finnigan-MAT 90 (Finnigan, Somerset, NJ, USA) and API QSTAR Pulsar i (MDS Sciex, Concord, ON, Canada) mass spectrometers, respectively. Silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with vanillin-H₂SO₄ in ethanol.

Fungus material

The fresh fruiting bodies of *C. imperiale* were purchased at a market in Nanhua County of Yunnan Province, China, in August 2005 and were identified by Professor Mu Zang. The voucher specimen (HFG 05112) was deposited in the herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dry fruiting bodies of *C. imperiale* (700 g) were extracted thrice with EtOAc (total 9 l) at room temperature for 3 days each time. The extract was filtered and concentrated under reduced pressure to give a residue (18.8 g), which was subjected to silica gel column chromatography eluted with CHCl₃/MeOH (from 100:0 to 0:100). The fraction (6.6 g) eluted with pure CHCl₃ was subjected to further silica gel column chromatography using a gradient of petroleum ether:acetone (150:1; 50:1; 20:1) followed by pure MeOH to give subfractions A–D. Subfraction A (300 mg) (petroleum ether:acetone 150:1, v/v) was further isolated over a silica gel column eluted with petroleum ether:

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acetone (150:1) to give a residue (160 mg) mainly containing **1** and **2**, which was repeatedly subjected to silica gel column chromatography to yield **1** (40.0 mg) and **2** (1.7 mg). Subfraction B (180 mg) (petroleum ether:acetone 50:1, v/v), containing mainly **3** and **4**, was further isolated on a silica gel column eluted with CHCl_3 to yield **3** (14.6 mg) and **4** (74.1 mg). Subfraction D (121 mg) eluted with MeOH was purified by Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}$ 1:1, v/v) and silica gel column chromatography using $\text{CHCl}_3/\text{MeOH}$ (150:1, v/v) as the eluent, to yield **5** (61.4 mg).

Computational methods

The stable geometries of **1** with low energy were investigated using HyperChem 7.0 (HyperCube, Gainesville, FL, USA). These low-energy conformations were then optimized at the B3LYP/6-31G (d) level again. The B3LYP/6-31G (d)-optimized structures were then used for optical rotation calculations at the B3LYP/6-31G (d) level. The calculated optical rotation for (*R*) configuration was $+14.5^\circ$. This value is very close to the experimental magnitude of $+10.6^\circ$. Thus, the absolute configuration of (+)-**1** was assigned as (*R*). Compound **5** has a linear structure and it is difficult to use the above method to compute its optical rotation to determine the absolute configuration. Our recent matrix method was used in the study. The value of the determinant ($\det(D)$) for (*R*)-**5** was -2.50 . According to the principle of matrix prediction, the $\det(D)$ and k_0 value were needed to use both. As the optical rotation was -10.4 (*c* 0.40, CHCl_3), the calculated k_0 value for this chiral secondary alcohol was 4.2, which is located in the window of coefficients of chiral secondary alcohols in chloroform. Thus, compound **5** was assigned (*R*) configuration.

Physicochemical properties

Catathelasmol A (**1**): amorphous powder; $[\alpha]_D^{25} +10.6$ (*c* 0.30, CHCl_3); IR (KBr): 3450, 1175, 1115, 1058, 1033 cm^{-1} ; ^1H - and ^{13}C -NMR: see Table 1; ESI-MS (pos.): 101 $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, 83 $[\text{M}-2\text{H}_2\text{O}+\text{H}]^+$; HR-ESI-MS (pos.): 101.0600 ($[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, calcd. 101.0602).

Catathelasmol B (**2**): colorless oil; $[\alpha]_D^{25} +8.9$ (*c* 0.30, CHCl_3); IR (KBr): 3441, 1191, 1167, 1104, 1062, 1034 cm^{-1} ; ^1H - and ^{13}C -NMR: see Table 1; ESI-MS (pos.): 218 $[\text{M}]^+$, 201 $[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, 183 $[\text{M}-2\text{H}_2\text{O}+\text{H}]^+$; HR-ESI-MS (pos.): 201.1125 ($[\text{M}-\text{H}_2\text{O}+\text{H}]^+$, calcd. 201.1126).

Catathelasmol C (**3**): colorless oil; IR (KBr): 1736, 1661, 1371, 1236, 1191 cm^{-1} . ^1H - and ^{13}C -NMR: see Table 2; ESI-MS (pos.): 225 $[\text{M}+\text{Na}]^+$; HR-ESI-MS (pos.): 225.0739 ($[\text{M}+\text{Na}]^+$, calcd. 225.0738).

Catathelasmol D (**4**): colorless oil; IR (KBr): 3441, 2966, 1738, 1369, 1248, 1045 cm^{-1} ; ^1H - and ^{13}C -NMR: see Table 2; EI-MS: 160 (M^+ , 2), 129 (42), 112 (17), 100 (14), 87 (100); HR-FAB-MS (pos.): 161.0838 ($[\text{M}+\text{H}]^+$, calcd. 161.0814).

Catathelasmol E (**5**): colorless oil; $[\alpha]_D^{25} -10.4$ (*c* 0.40, CHCl_3); IR (KBr): 3458, 1740, 1450, 1247 cm^{-1} . ^1H - and ^{13}C -NMR: see Table 2; ESI-MS (pos.): 227 $[\text{M}+\text{Na}]^+$; HR-ESI-MS (pos.): 227.0889 ($[\text{M}+\text{Na}]^+$, calcd. 227.0895).

Biological testing

The inhibitory activities of the compounds on human or mouse 11β -HSD1 and 11β -HSD2 enzymatic activities were determined by the scintillation proximity assay using microsomes containing 11β -HSD1 or 11β -HSD2, according to our earlier studies.⁶ Briefly, the full-length cDNAs of human or murine 11β -HSD1 and 11β -HSD2 were isolated from the cDNA libraries provided by the NIH Mammalian Gene Collection and cloned into pcDNA3

expression vector. HEK-293 cells were transfected with the pcDNA3-derived expression plasmid and selected by cultivation in the presence of $700 \mu\text{g ml}^{-1}$ of G418. The microsomal fraction overexpressing 11β -HSD1 or 11β -HSD2 was prepared from the HEK-293 cells stably transfected with either 11β -HSD1 or 11β -HSD2 and was used as the enzyme source for scintillation proximity assay. Microsomes containing human or mouse 11β -HSD1 were incubated with NADPH and $[\text{H}^3]\text{cortisone}$. Then the product, $[\text{H}^3]\text{cortisol}$, was specifically captured by a monoclonal antibody coupled to protein A-coated scintillation proximity assay beads. The 11β -HSD2 screening was performed by incubating 11β -HSD2 microsomes with $[\text{H}^3]\text{cortisol}$ and NAD⁺ and monitoring substrate disappearance. IC₅₀ values were calculated by using Prism Version 4 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Catathelasmol A (**1**), obtained as an amorphous powder, has a molecular formula of $\text{C}_5\text{H}_{10}\text{O}_3$ based on the positive-ion HR-ESI-MS, showing a quasi-molecular ion peak at *m/z* 101.0600 (calcd. for $[\text{C}_5\text{H}_{10}\text{O}_3-\text{H}_2\text{O}+\text{H}]^+$, 101.0602) and requiring only one degree of unsaturation. The IR spectrum showed the presence of one or more hydroxyl groups (3450 cm^{-1}). The ^{13}C -NMR spectrum (Table 1) exhibited five signals: one quaternary carbon bearing two oxygens at δ 102.7, two oxymethylenes at δ 68.0 (t), 65.2 (t) and two up-field methylenes at δ 33.8 (t), 23.5 (t). It was obvious that only one degree of unsaturation was attributed to a ring. The ^1H -NMR spectrum (Table 1) showed eight protons: two oxygenated methylenes at δ 3.45 (1H, d, $J=11.5 \text{ Hz}$), 4.14 (1H, d, $J=11.5 \text{ Hz}$), 3.97 (2H, t, $J=6.9 \text{ Hz}$), and up-field resonances at δ 1.61 (1H, m), 1.94 (1H, m), 1.88 (1H, m), 2.05 (1H, m). The above NMR data suggested that **1** possessed a tetrahydrofuran moiety connected with a hydroxyl and a hydroxymethyl group. The heteronuclear multi-bond correlations (HMBC) (Figure 1) from H-6 to C-2 and C-3, and from H-5 to C-2 and C-3 were observed; consequently, the hydroxyl and hydroxymethyl groups were doubtless both emplaced at C-2. The absolute configuration of (+)-**1** was assigned as (*R*) using the B3LYP/6-31G (d) methods⁷⁻⁹ based on the comparison of experimental optical rotation ($+10.6^\circ$) and calculated optical rotation ($+14.5^\circ$). Therefore, the structure of **1** was determined as (*R*)-(+)-2-(hydroxymethyl)-tetrahydrofuran-2-ol and named catathelasmol A, as shown in Figure 2.

Catathelasmol B (**2**), a colorless oil, was obtained as a minor constituent with a molecular formula of $\text{C}_{10}\text{H}_{18}\text{O}_5$, based on the positive-ion HR-ESI-MS: 201.1125 (calcd. for $[\text{C}_{10}\text{H}_{18}\text{O}_5-\text{H}_2\text{O}+\text{H}]^+$, 201.1126). In the NMR spectra (Table 1) of **2**, signals for the number of protons and carbon that were observed were only half of the number that would correspond to the molecular formula. This indicated that **2** is a symmetrical structure. The NMR data were considerably in accordance with those of **1**, but their TLC behavior was discriminable, which suggested that **2** was unambiguously a dimer of **1**, and there were just two possible condensed positions: C-2 or C-6 hydroxyl. There was no reaction and no corresponding product obtained in acetylation, which suggested that there was no free

Table 1 NMR spectral data for compounds **1** and **2** in CDCl_3

No.	1		2	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
2	102.7 (s)		103.8 (s)	
3	33.8 (t)	1.61, 1.94 (each 1H, m)	33.7 (t)	1.77, 2.11 (each 1H, m)
4	23.5 (t)	1.88, 2.05 (each 1H, m)	24.1 (t)	1.89, 2.03 (each 1H, m)
5	68.0 (t)	3.97 (2H, 6.9)	67.9 (t)	3.94, 4.00 (each 1H, m)
6	65.2 (t)	3.45 (1H, d, 11.5), 4.14 (1H, d, 11.5)	66.3 (t)	3.55 (1H, d, 11.8), 3.94 (1H, d, 11.8)

Table 2 NMR spectral data for compounds 3–5 in CDCl₃

No.	3		4		5	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	67.9 (t)	4.65 (2H, s)	63.1 (t)	4.26 (2H, s)	68.6 (t)	3.95 (1H, dd, 11.3, 7.2), 4.11 (1H, dd, 11.3, 7.2)
2	202.9 (s)		208.9 (s)		69.3 (d)	3.84 (1H, m)
3	35.1 (t)	2.50 (2H, t, 7.2)	34.4 (t)	2.52 (2H, t, 7.2)	29.6 (t)	1.71, 1.80 (each 1H, m)
4	22.2 (t)	1.95 (2H, m)	22.3 (t)	1.97 (2H, m)	24.6 (t)	1.52 (2H, m)
5	66.3 (t)	4.06 (2H, t, 6.3)	67.8 (t)	4.08 (2H, t, 6.3)	64.2 (t)	4.08 (2H, t, 6.5)
a ₁	170.2 (s)				171.3 (s)	
a ₂	20.4 (q)	2.16 (3H, s)			20.9 (q)	2.08 (3H, s)
b ₁	171.0 (s)		170.9 (s)		171.3 (s)	
b ₂	20.9 (q)	2.04 (3H, s)	20.5 (q)	2.04 (3H, s)	21.0 (q)	2.03 (3H, s)

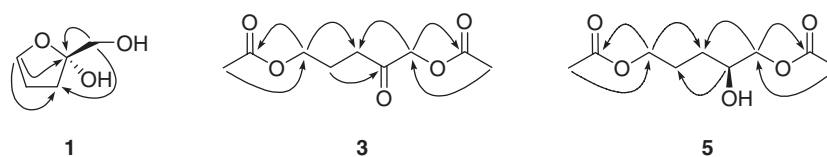


Figure 1 Key HMBC correlations of compounds 1, 3 and 5.

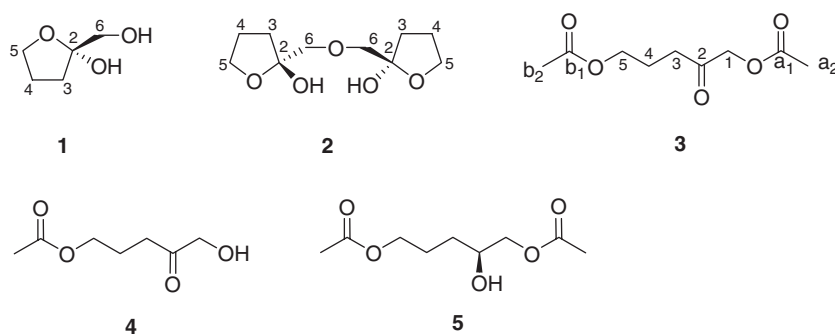


Figure 2 Structures of compounds 1–5.

primary hydroxyl group in the structure of **2**, but that **2** was formed from two molecules of **1** through the intermolecular dehydration at C-6 hydroxyl. Thus, the structure of **2** was proposed as (2*R*,2'*R*)-2,2'-oxybis(methylene)bis(tetrahydrofuran-2-ol), as shown in Figure 2.

Catathelasmol C (**3**) was isolated as a colorless oil possessing the molecular formula C₉H₁₄O₅, based on the positive-ion HR-ESI-MS: 225.0739 (calcd. for C₉H₁₄O₅Na, 225.0738). The IR spectrum showed the absorption bands of ester carbonyl (1736 cm⁻¹) and keto carbonyl (1661 cm⁻¹) groups. The ¹³C-NMR spectrum (Table 2) exhibited nine carbon resonances, including one keto carbonyl at δ 202.9 (s), two oxymethylenes at δ 67.9 (t), 66.3 (t), two up-field methylene carbons at δ 35.1 (t), 22.2 (t), as well as characteristic signals at δ 170.2 (s), 20.4 (q); 171.0 (s), 20.9 (q) contributed to two acetoxy groups. The ¹H-NMR spectrum (Table 2) of **3** showed signals for two oxygenated methylenes at δ 4.65 (2H, s), 4.06 (2H, t, *J*=6.3 Hz), two up-field methylenes at δ 2.50 (2H, t, *J*=7.2 Hz), 1.95 (2H, m) together with two acetoxy methyl singlets at δ 2.16 (3H, s), 2.04 (3H, s). The above NMR character allowed us to conclude that **3** was a diacetylated pentanediol containing a keto group. By analysis of the HMBC spectrum (Figure 1), the position of the ketone was determined at C-2, in which the correlations from H-1 to C-3 and C-a₁, from H-5 to C-3 and C-b₁ and

from H-4 to C-2 were observed. Therefore, the structure of **3** was elucidated as 2-oxopentane-1,5-diyl diacetate named catathelasmol C, which was a new natural product.¹⁰

Catathelasmol D (**4**), also obtained as a colorless oil, has a molecular formula of C₇H₁₂O₄, based on the EI-MS showing a molecular ion peak at *m/z* 160, in combination with the ¹³C-NMR (DEPT) spectrum. The NMR data (Table 2) of **4** were similar to those of **3**, but there was only one set of acetoxy signals: δ 170.9 (s), 20.5 (q). Considering that the signal at δ 4.26 (2H, s, H-1) in **4** evidently shifted up-field (δ =0.39 p.p.m.) compared with that of **3**, the acetoxy group must be connected at C-5 in **4**. Thus, the structure of **4** was determined as 5-hydroxy-4-oxopentyl acetate and named catathelasmol D, which was also a new natural product,¹¹ as shown in Figure 2.

Catathelasmol E (**5**), a colorless oil, was assigned the molecular formula C₉H₁₆O₅ by the positive-ion HR-ESI-MS: 227.0889 (calcd. for C₉H₁₆O₅Na, 227.0895). The NMR data of **5** were similar to those of **3**, and comparison of ¹³C-NMR data showed that instead of a ketone, one oxymethine carbon at δ 69.3 (d) newly appeared in **5**. The obvious differences in the ¹H-NMR spectra were as follows: (a) the oxymethylene singlet in **3** was observed separately from each other at δ 3.95 (1H, dd, *J*=11.3, 7.2 Hz) and 4.11 (1H, dd, *J*=11.3, 7.2 Hz),

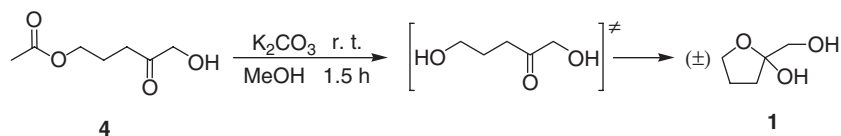


Figure 3 Chemical transformation from 4 to 1.

respectively in 5; and (b) an oxygen-bearing methine proton at δ 3.84 (1H, m) was newly detected in 5. The above NMR character indicated that the keto group in 3 was hydrogenated in 5. Initially, the modified Mosher's method^{12,13} was applied to determine the absolute configuration of the hydroxyl at C-2, but failed. Alternatively, we resorted to the computational method to establish the absolute configuration. The absolute configuration of 5 was assigned as (R) using matrix model.¹⁴ The det(D) value for this chiral alcohol was -2.50 and the calculated k_0 value was 4.2, which is located in the window of coefficients of chiral secondary alcohols in chloroform. Accordingly, the structure of 5 was established as 2-hydroxypentane-1,5-diyl diacetate, shown in Figure 2, and was named catathelasmol E.

It was not until quite recently that such simple pentanol derivatives were found, which suggested that this type of metabolites were distributed in a narrow range, so we believe that they may possess an important chemotaxonomic significance. Besides, the realization of the chemical transformation from 4 to 1 validated the structural correctness (Figure 3).

Chemical transformation from 4 to 1:¹⁵ A solution of 4 (50 mg) in MeOH (5.0 ml) was treated with K_2CO_3 (97.4 mg) at room temperature. After stirring for 1.5 h, saturated aqueous ammonium chloride was added and extracted with ethyl acetate, and the organic layer was washed with water and brine, dried over sodium sulfate, and evaporated to a residue that was purified by a silica gel column using pure $CHCl_3$ to yield 1 (12 mg). The determined optical rotation was expectedly zero. Thus, the cyclization of 4 to 1 obtained in the experiments must involve the enzyme catalysis.

Glucocorticoid hormones play important roles in many biological and physiological processes, including regulation of energy metabolism, inflammatory, immune and stress responses, and cardiovascular homeostasis. The action of glucocorticoid on target tissue is not dependent inevitably on the circulating levels, but is regulated in a tissue-specific manner by the enzymes of 11 β -hydroxysteroid dehydrogenases (11 β -HSD1 and 11 β -HSD2), which catalyze the interconversion of active 11-hydroxy-glucocorticoids (cortisol in humans and corticosterone in rodents) and their respective inert 11-keto forms (cortisone in humans and 11-dehydrocorticosterone in rodents).¹⁶ 11 β -HSD1 is highly expressed in the liver, gonad, adipose tissue and brain, in which it acts as a reductase regenerating the active glucocorticoids from its inactive forms, thus amplifying local glucocorticoid action.¹⁷ 11 β -HSD2 is predominantly expressed in aldosterone target cells, such as the kidney and colon, in which it catalyzes the inactivation of glucocorticoids, thereby preventing the excessive activation of the mineralocorticoid receptor and sequelae, including sodium retention, hypokalemia and hypertension.

We tested the inhibitory effect of the compounds on both human and mouse 11 β -HSD1 and 11 β -HSD2. Compound 3 showed inhibitory activities against 11 β -HSD1 (human IC_{50} =28.7 μ g ml⁻¹; mouse IC_{50} =30.4 μ g ml⁻¹) and 11 β -HSD2 (human IC_{50} =5.1 μ g ml⁻¹; mouse

IC_{50} =32.3 μ g ml⁻¹). Compound 4 showed inhibitory activities against 11 β -HSD1 (human IC_{50} =47.4 μ g ml⁻¹; mouse IC_{50} =149.2 μ g ml⁻¹) and 11 β -HSD2 (human IC_{50} =38.9 μ g ml⁻¹; mouse IC_{50} =129.1 μ g ml⁻¹). Compound 5 showed inhibitory activities against human 11 β -HSD1 (IC_{50} =62.3 μ g ml⁻¹) and 11 β -HSD2 (IC_{50} =177.0 μ g ml⁻¹). Therefore, compounds 3–5 showed inhibitory activities against 11 β -HSD1 and 11 β -HSD2 and provide the possibility for modulating local cortisone/cortisol availability *in vivo*.

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