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Steroids 70 (2005) 127–130
Steroids
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Two new steryl esters from the basidiomycete *Tricholomopsis rutilans*

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Received 18 June 2004; received in revised form 12 October 2004; accepted 18 October 2004
Available online 30 November 2004

Abstract

Two new steryl esters with a polyhydroxylated ergostane-type nucleus, 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6β-yl oleate (**1**) and 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-22-en-7-one-6β-yl oleate (**2**), were isolated from the fruiting bodies of the basidiomycete *Tricholomopsis rutilans* along with three known sterols (**3**, **4**, and **5**). The structures of compounds **1** and **2** were established on the basis of spectroscopic means and chemical methods.

Keywords: *Tricholomopsis rutilans*; Steryl ester; 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6β-yl oleate; 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-22-en-7-one-6β-yl oleate

1. Introduction

Tricholomopsis rutilans (Schaeff. Fr.) Singer is a widespread basidiomycete of the northern temperate zone, which fruits solitarily or in clusters on conifer stumps and logs and is occasionally found on wood chips [1]. A series of non-protein amino acids were previously reported from this fungus [2–5]. As a part of our search for naturally occurring secondary metabolites of higher fungi in the Yunnan Province [6–9], we have carried out a chemical investigation on this fungus and isolated two new steryl esters from its ethanol extract, 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6β-yl oleate (**1**) and 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-22-en-7-one-6β-yl oleate (**2**) (Fig. 1), along with three known sterols, (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**3**), 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**4**), and (22*E*,24*R*)-5α,6α-epoxyergosta-8(14),22-dien-3β,7α-diol (**5**). Previously, a few monohydroxylated steryl esters: ergosta-7,22-dien-3β-yl palmitate, ergosta-7,22-dien-3β-yl linoleate, 5α,8α-epidioxyergosta-6,22-dien-3β-yl linoleate, ergosta-7,24(28)-dien-3β-yl linoleate, and ergosta-7-en-3β-yl linoleate, have been reported [10–12], but only one steryl ester with a polyhydroxylated ergostane-type nucleus, 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6β-yl linoleate, has been isolated from the fungus *Catathelasma imperiale* [13]. This report deals with the isolation and the structural elucidation of these two new steryl esters (**1** and **2**).

2. Experimental

2.1. General

Melting points obtained on a XRC-1 apparatus are uncorrected. Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Bio-Rad FTS-135 using KBr pellets. NMR spectra were recorded on Bruker AV-400 and Bruker DXR-500 spectrometers in $CDCl_3$ solvent with TMS as an internal standard. MS (EI, FAB) were recorded with a VG Autospec-3000 spectrometer. ESI and HR-ESI were recorded with an API QSTAR Pulsar 1 spectrometer. GC–MS was carried out on an Agilent 5973N instrument.

Silica gel (200–300 mesh) and pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Factory, PR China) were used for column chromatography and TLC, respectively.

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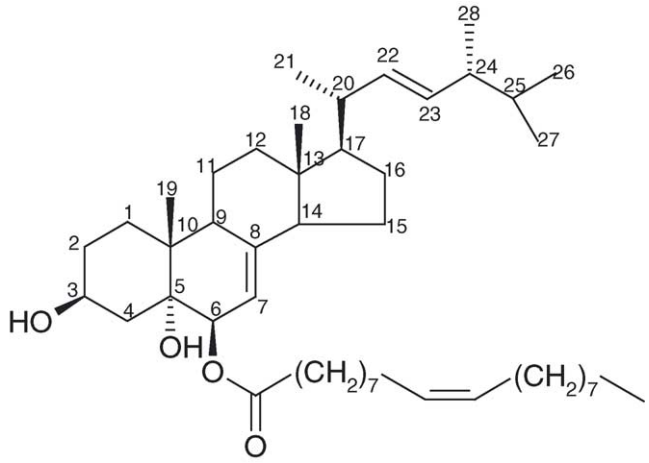
0039-128X/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2004.10.007



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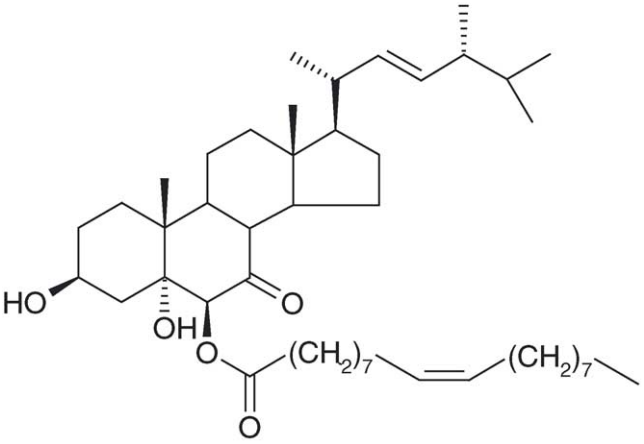

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Fig. 1. Structures of **1** and **2**.

Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

2.2. Extraction and isolation

The fungus *T. rutilans* was collected on the Ailao Mountain of Yunnan Province, PR China, in July, 2003 and identified by Prof. *Zang Mu*, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimen was deposited in the Herbarium of the Kunming Institute of Botany, the Chinese Academy of Sciences.

Air-dried and crushed fruiting bodies of *T. rutilans* (2.5 kg) were extracted successively with ethanol four times at room temperature. The combined extraction was concentrated to dryness in vacuo to afford a syrup (180 g), which was subjected to silica gel column chromatography using petroleum ether/acetone (98:2, 95:5, and 90:10, v/v) to give fractions A (98:2, 3000 ml), B (95:5, 3000 ml), C (90:10, 3000 ml), respectively. The residue (2 g) of fraction A was further purified by silica gel column chromatography using a gradient of petroleum ether/acetone (99:1, 98:2, v/v) to afford **3** (710 mg, petroleum ether/acetone 98:2, v/v). The residue (1 g) of fraction B was further purified by silica gel column chromatography using a gradient of petroleum ether/acetone (98:2, 97:3, v/v) to afford **1** (156 mg, petroleum ether/acetone 97:3, v/v). The residue (1.5 g) of fraction C was further purified by silica gel column chromatography using a gradient of petroleum ether/acetone (95:5, 94:6, 93:7, v/v) to afford **4** (148 mg, petroleum ester/acetone 95:5), **2** (21 mg, petroleum ester/acetone, 94:6), and **5** (103 mg, petroleum ester/acetone, 93:7).

2.2.1. 3β,5α-Dihydroxy-(22E,24R)-ergosta-7,22-dien-6β-yl oleate (*1*)

Colorless, oily solid, $[\alpha]_D^{24} = -53.3^\circ$ (c 1.02, $CHCl_3$). TLC: (R_F 0.80, $CHCl_3$: MeOH 20:1). IR (KBr) *ν*: 3440, 2956, 2927, 2855, 1714, 1659, 1635, 1460, 1381, 1251, 1166, 1048, 1025, 970, 942 cm^{-1}. ^1H NMR and ^{13}C NMR data are listed in Table 1. ESI–MS (pos.): 717 $[M+Na]^+$, 395 $[M+1-C_{18}H_{34}O_2-H_2O]^+$, 377 $[M+1-C_{18}H_{34}O_2-2H_2O]^+$. HR–ESI–MS (pos.) calc. for $C_{46}H_{78}O_4Na$ $[M+Na]^+$: 717.5797; found: 717.5774.

2.2.2. Methanolysis of compound *1*

A solution of **1** (25 mg) in methanol (10 ml) was treated with 0.1 M HCl (2 ml) at 75 °C for 18 h. The resultant mixture was extracted with petroleum ether to obtain the organic layer, which was dried (Na_2SO_4) and then analyzed by GC–MS.

2.2.3. 3β,5α-Dihydroxy-(22E,24R)-ergosta-22-en-7-one-6β-yl oleate (*2*)

Colorless, oily solid, $[\alpha]_D^{26} = -63.7^\circ$ (c 0.46, $CHCl_3$). TLC: (R_F 0.45, $CHCl_3$: MeOH 20:1). IR (KBr) *ν*: 3426, 2956, 2926, 2855, 1731, 1639, 1461, 1381, 1237, 1164, 1034, 971, 944 cm^{-1}. ^1H NMR and ^{13}C NMR data are listed in Table 1. FAB-MS (pos.): 711 $[M+1]^+$, 429 $[M+1-C_{18}H_{34}O_2]^+$, 411$[M+1-C_{18}H_{34}O_2-H_2O]^+$, 393 $[M+1-C_{18}H_{34}O_2-2H_2O]^+$. HR–ESI–MS (pos.) calc. for $C_{46}H_{78}O_5Na$ $[M+Na]^+$: 733.5766; found: 733.5746.

3. Results and discussion

Compound **1** was obtained as a colorless, oily solid. The molecular formula of **1** was determined to be $C_{46}H_{78}O_4$ by positive ion HR–ESI–MS (calc. for $[M+Na]^+$: 717.5797; found: 717.5774). The ^1H NMR spectrum of **1** (Table 1) displayed singlets (δ 0.54, 1.00) for two tertiary methyl groups, doublets (δ 0.78, 0.79, 0.87, 0.98) for four secondary methyl groups, and a doublet (δ 5.18, $J = 5.0$) typical for the 7-H of the trisubstituted olefinic proton of ergosta-7,22-dien-3β,5α,6β-triol derivatives. A characteristic downfield doublet (δ 4.82, $J = 5.0$) of 6α-H caused by esterification

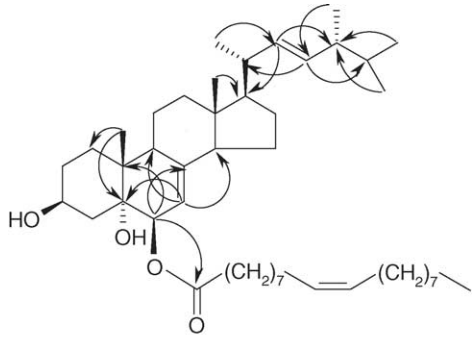
Table 1
^{1}H and ^{13}C NMR data ($CDCl_3$) for **1** and **2**

Position	**1**	**1**	**2**	**2**
1	1.22 (m)	32.33 (CH_2)	1.25 (m)	32.06 (CH_2)
2	1.77 (m)	30.40 (CH_2)	1.83 (m)	30.13 (CH_2)
3	4.03 (m)	67.09 (CH)	4.09 (m)	66.85 (CH)
4	2.00 (m), 1.81 (m)	39.17 (CH_2)	1.99 (m), 1.74 (m)	40.42 (CH_2)
5	–	75.10 (C)	–	78.94 (C)
6	4.82 (brd, 5.0)	73.34 (CH)	4.55 (s)	82.47 (CH)
7	5.18 (brd, 5.0)	114.09 (CH)	–	207.44 (C)
8	–	145.49 (C)	2.63 (t, 11.1)	46.28 (CH)
9	1.97 (m)	43.06 (CH)	1.78 (m)	47.66 (CH)
10	–	37.11 (C)	–	39.30 (C)
11	1.55 (m)	21.88 (CH_2)	1.47 (m)	21.63 (CH_2)
12	1.65 (m), 1.28 (m)	39.24 (CH_2)	1.64 (m), 1.15 (m)	38.63 (CH_2)
13	–	43.64 (C)	–	42.39 (C)
14	1.86 (m)	54.76 (CH)	1.50 (m)	48.10 (CH)
15	1.24 (m)	22.79 (CH_2)	1.24 (m)	24.62 (CH_2)
16	1.67 (m)	27.83 (CH_2)	1.64 (m)	28.46 (CH_2)
17	1.24 (m)	55.88 (CH)	1.14 (m)	54.93 (CH)
18	0.54 (s)	12.19 (CH_3)	0.65 (s)	12.31 (CH_3)
19	1.00 (s)	18.10 (CH_3)	1.30 (s)	17.36 (CH_3)
20	1.97 (m)	40.33 (CH)	1.99 (m)	39.94 (CH)
21	0.98 (d, 6.6)	21.04 (CH_3)	0.98 (d, 6.6)	21.04 (CH_3)
22	5.12 (dd, 15.2, 7.9)	135.33 (CH)	5.16 (m)	135.61 (CH)
23	5.18 (dd, 15.2, 7.3)	132.04 (CH)	5.16 (m)	131.89 (CH)
24	1.80 (m)	42.78 (CH)	1.84 (m)	42.82 (CH)
25	1.46 (m)	33.00 (CH)	1.44 (m)	33.07 (CH)
26	0.78 (d, 7.2)	19.57 (CH_3)	0.78 (d, 6.7)	19.63 (CH_3)
27	0.79 (d, 7.4)	19.88 (CH_3)	0.80 (d, 6.7)	19.95 (CH_3)
28	0.87 (d, 6.8)	17.55 (CH_3)	0.88 (d, 6.8)	17.64 (CH_3)
1′	–	173.42 (C)	–	172.20 (C)
2′	2.26 (t, 7.6)	34.62 (CH_2)	2.32 (t, 7.8)	34.23 (CH_2)
3′	1.58 (m)	24.87 (CH_2)	1.60 (m)	24.82 (CH_2)
4′–7′, 12′–15′	1.22–1.29 (m)	29.02–29.64 (CH_2)	1.22–1.30 (m)	29.04–29.75 (CH_2)
8′	1.96 (m)	27.13 (CH_2)	2.00 (m)	27.17 (CH_2)
9′	5.30 (m)	129.65 (CH)	5.32 (m)	129.66 (CH)
10′	5.30 (m)	129.88 (CH)	5.32 (m)	130.03 (CH)
11′	1.96 (m)	27.13 (CH_2)	2.00 (m)	27.17 (CH_2)
16′	1.22–1.29 (m)	31.84 (CH_2)	1.22–1.30 (m)	31.89 (CH_2)
17′	1.22–1.29 (m)	22.61 (CH_2)	1.22–1.30 (m)	22.67 (CH_2)
18′	0.84 (t, 7.0)	14.04 (CH_3)	0.85 (t, 7.0)	14.10 (CH_3)

Assignments made on the basis of ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments.

suggested that the oleate moiety was clearly located at the 6β position of the sterol nucleus [13]. These data were compatible with those reported for cerevisterol ((22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol) and its derivatives [14,15,19]. Furthermore, the ^{1}H NMR spectrum of **1** also showed the characteristic signals at δ 0.84 (t, J = 7.0) for a terminal methyl group, at δ 2.26 (t, J = 7.6) for a methylene group in α-position to an ester function, at δ 5.30 (m) for two olefinic protons, suggesting a disubstituted C=C bond, and other signals at δ 1.96, 1.58, 1.22–1.29 (overlapped) all related to a monounsaturated long-chain fatty-acid ester moiety. Following methanolysis, **1** yielded the unsaturated fatty-acid methyl ester, which was identified as methyl oleate ((9*Z*)-octadec-9-enoic acid methyl ester) by GC–MS analysis, and this was consistent with the ESI-MS of **1**, which exhibited characteristic fragment ion peaks at *m*/*z* 395 $[M+1-C_{18}H_{34}O_2-H_2O]^+$ and 377 $[M+1-C_{18}H_{34}O_2-2H_2O]^+$. Supporting evidence was also obtained from the ^{13}C NMR spectrum (Table 1), which had typical signals at δ 173.42 (C-1′), 34.62 (C-2′), 24.87 (C-3′), 27.13 (C-8′ and C-11′), 129.65 (C-9′), and 129.88 (C-10′), for the oleate moiety with (*Z*)-configuration. The characteristic upfield values for both the allylic C-atoms C-8′ and C-11′ were typical for *cis*-olefins as compared to *trans*-olefins [16]. The linked position of the oleate moiety was also reasonably confirmed by the HMBC spectrum (Fig. 2), which clearly displayed the strong correlation peak between 6α-H (δ 4.82) and C-1′ (δ 173.42). The stereochemistry of the side chain was determined by comparison of the ^{1}H and ^{13}C NMR data of **1** with those of (22*E*,24*R*)-methyl-Δ^{22}-sterol side chain [17]. From all of these data mentioned above, the structure of **1** was, therefore, determined to be 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-7,22-dien-6β-yl oleate.

Compound **2** was also obtained as a colorless, oily solid. A quasi-molecular ion peak at *m*/*z* 711 $[M+1]^+$ and a series of characteristic fragment ion peaks at *m*/*z*



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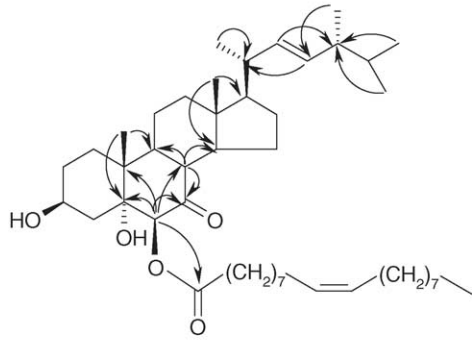

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Fig. 2. Selected HMBC correlations of **1** and **2**.

429 $[M+1-C_{18}H_{34}O_2]^+$, 411$[M+1-C_{18}H_{34}O_2-H_2O]^+$, 393 $[M+1-C_{18}H_{34}O_2-2H_2O]^+$ were given in positive ion FAB-MS. The positive ion HR–ESI–MS of **2** exhibited a molecular formula of $C_{46}H_{78}O_5$ (calc. for $[M+Na]^+$: 733.5766; found: 733.5746), which was consistent with the analysis of its ^{13}C NMR spectrum. The ^1H NMR and ^{13}C NMR (Table 1) spectra of **2** showed very similar signals compared with those of **1**, indicating **2** also possessed the same steryl ester skeleton as **1**, and the only difference between **2** and **1** was that the Δ^7 C=C group of **1** was replaced by a keto group in **2**. The presence of the 7-keto function also led to the appearance of the 8β-H signal further downfield at δ 2.63 (t, $J = 11.1$) in the ^1H NMR spectrum **2**. By comparing this data of the sterol nucleus with that in the literature [18], **2** was inferred to be a derivative of 3β,5α,6β-trihydroxy-(22*E*,24*R*)-ergosta-22-dien-7-one, and the oleate moiety was also assigned to the 6β position. From all of these data mentioned above, the structure of **2** was, therefore, determined to be 3β,5α-dihydroxy-(22*E*,24*R*)-ergosta-22-en-7-one-6β-yl oleate.

Comparison of the physicochemical properties with the reported data allowed for the identification of compounds **3**, **4**, and **5**, isolated from the same fungus, as (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**3**) [19], 3β-hydroxy-(22*E*,24*R*)-ergosta-5,8,22-trien-7-one (**4**) [18], (22*E*,24*R*)-5α,6α-epoxyergosta-8(14),22-dien-3β,7α-diol (**5**) [19], respectively.

Acknowledgement

This project was supported by the National Natural Science Foundation of China (30225048).

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