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THREE TRITERPENES FROM *ASTRAEUS HYGROMETRICUS*

YOSHIHISA TAKAISHI, YOSHITO MURAKAMI, TAKASHI OHASHI*, KIMIKO NAKANO, KŌTAROU MURAKAMI and TOSHIAKI TOMIMATSU

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770, Japan; *Tokushima Prefectural Kainan Senior High School, Kaifu-gun, Tokushuma, 775-01, Japan

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Key Word Index—*Astraeus hygrometricus*; *Basidiomyces*; higher fungi; triterpenoids; Astrahygrol; 3-epi-astrahygrol; astrahygrone.

Abstract—Three new triterpenes, as well as two known steroids, were isolated from *Astraeus hygrometricus*. The structures of the new triterpenes were established by chemical and spectroscopic means.

INTRODUCTION

Astraeus hygrometricus (Pers.) Morgan (*Gasteromycetes*) is used in Chinese folk medicine and has been used as a haemastatic. The chemical constituents of the fruiting body of *A. hygrometricus* have not been studied. In this paper we wish to report the structural elucidation of three new triterpenes (**1**, **2** and **3**) and identification of two known steroids (**4** and **5**).

RESULTS AND DISCUSSION

Compound **1** was assigned the molecular formula $C_{30}H_{46}O_3$ (HRMS). Its UV spectrum showed no absorption above 210 nm. Its IR spectrum showed absorptions at 3480 (OH) and 1720 (δ-lactone) cm^{-1}. Its ^1H NMR spectrum (Table 1) showed the presence of two secondary and five tertiary methyl groups, thus suggesting a lanostane skeleton. In the mass spectrum of **1**, a fragment ion peak at *m/z* 314 [M − side chain − H]$^+$, suggested **1** had a δ-lactone moiety in the side chain. In the ^{13}C NMR spectrum (Table 2), the olefinic carbon signals at δ134.2 and 134.7 were assigned to C-8 and C-9, the signal at δ78.9 was identical to the C-3 signal of lanosterol [1]. The proton signal at δ3.24 (*dd*, *J* = 10.9 and 4.8 Hz) was assigned as H_{ax}-3 from the coupling constant.

The side chain ($C_8H_{13}O_2$) of **1** was shown by the spectroscopic evidence to be a δ-lactone [IR; 1720 cm^{-1}, ^1H NMR; δ4.39 (1H, *dd*, *J* = 11.6, 3.0 Hz, H-22), 2.61 (1H, *dq*, *J* = 10.0, 7.0 Hz, H-25), 0.99 (1H, *d*, *J* = 6.3 Hz, H-21). ^{13}C NMR: δ80.1 (*d*, C-22), 176.8 (*s*, C-26)]. All these data indicated that **1**, which we have named astrahygrol, was a 3-hydroxy-lanostane derivative with a δ-lactone in the side chain as shown.

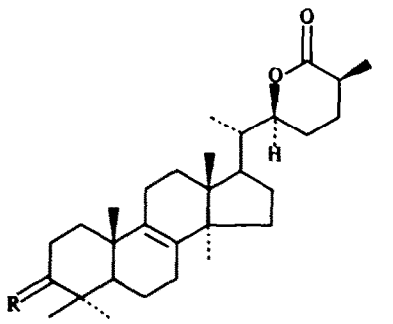
Lanostane derivatives with a lactone structure in the side chain are unknown, but derivatives with a hemiacetal structure in the side chain have been isolated from *Perenniporia ochroleuca* (Basidiomyces) [2]. The ^{13}C NMR data of compound **1** and 15-acetoxy-7,11-dihydroperennipol [3] isolated from *P. ochroleua* were very similar except for C-22 to C-28. This fact supported the structure of the tetracyclic moiety of **1**.

Compound **2**, $C_{30}H_{46}O_3$. Its IR spectrum showed the presence of hydroxy and δ-lactone groups. Its ^1H NMR spectrum was very similar to that of **1**, except for a different signal for H-3 (**1**; δ3.24 *dd*, **2**; δ3.43 *t*). In addition the ^{13}C NMR spectra of **1** and **2** were very similar except that the chemical shifts of C-1 to C-5 and C-30 for **2** were more upfield than those of **1**. These facts showed that **2** had a 3-α-hydroxy group instead of the 3-β-hydroxy group found in **1**, **2** was 3-epi-astrahygrol.

Compound **3**, $C_{30}H_{44}O_3$, showed absorptions at 1725 (δ-lactone) and 1690 (ketone) cm^{-1}. Its ^1H NMR and ^{13}C NMR spectral patterns were very similar to those of **1** and **2** except for the ketonic region. In the mass spectrum of **3**, a fragment ion peak at *m/z* 312 [M − side chain − H]$^+$, suggested it had a δ-lactone moiety in the side chain as found in **1** and **2**, and that the ketone function was present in the tetracyclic moiety. In the ^{13}C NMR spectra, the chemical shifts of C-2 and C-4 for **2** were downfield from those of **1**. This indicated that C-3 of **3** was a ketone.

The δ-lactone structure in the side chain was confirmed by hydrolysis of **3** with sodium methoxide in methanol to give the δ-hydroxy methyl ester **3a**, $C_{31}H_{50}O_4$, which showed a signal at δ3.70 (m) in its ^1H NMR spectrum which was assigned to H-22. From these spectral and chemical investigations, the structure of compound **3** was established.

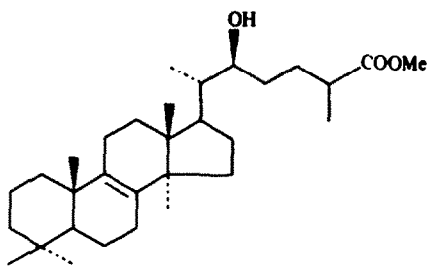
The correlation of **1**, **2**, and **3** was confirmed by chemical transformation. Compounds **1** and **2** were oxidized by Jones reagent to give compound **3** (TLC, ^1H NMR and $[\alpha]_D$). The absolute configuration of these molecules was based on the ORD curve of **3**, which had a similar shape to that of the dispersion curves of lanosta $^8\Delta$ene-3-one [4]. The absolute configuration of the side chain of these compounds was determined by comparison of the ^1H NMR data with the literature data (Table 3), for four possible model steroidal compounds (**6**) having a 26,22-lactone moiety with known C-22 and C-25 stereochemistry which were used to determine the absolute configuration of tetrahydroxyisosterocholamic acid lactone isolated from *Amyda japonica* [5]. The C-22 and C-25 stereochemistry of astrahygrol (**1**) was established to be 22*S*, 25*S* on the basis of: (i) the coupling pattern (*dd*) for the H-22 resonance of **1** indicated 22*S* configuration; (ii) the chemical shifts (δ2.61) and coupling pattern (*tq*) of the H-25 resonance of **1** were similar to those of the



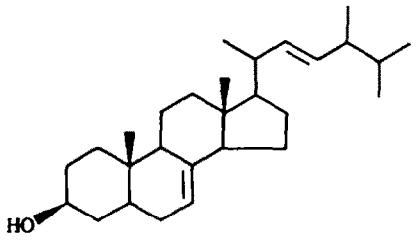
1 R = OH, H

2 R = H, OH

3 R = O



3a



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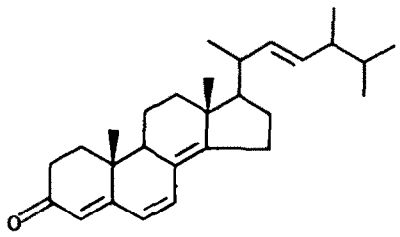

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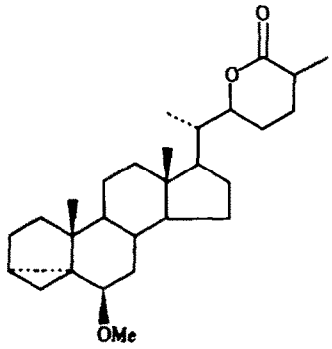

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(22*R*, 25*R*) and (22*S*, 25*S*) isomers; (iii) the chemical shift (δ1.22) of the C-27 methyl signal of **1** was agreement with those of the (22*S*, 25*S*) and (22*R*, 25*R*) isomers. Compounds **2** and **3** have the same stereochemistry as **1** as shown by spectral data and the chemical reaction.

The ^{1}H NMR spectrum of compound **4**, $C_{28}H_{46}O$, showed the presence of two tertiary methyl and four secondary methyl groups, one proton (δ3.60, *m*) attached to an oxygen bearing carbon and three protons (δ5.16, 3H, *m*) attached to a double bond, thus suggesting **4** was a ergosta-7,22-diene-3-ol. Acetylation of **4** gave the monoacetate **4a**, which was identified by spectral comparison with ergosta-7,22-diene-3-ol acetate [6].

Compound **5**, $C_{28}H_{40}O$, contained a triene-ketone [UV; 238, 282 and 345 nm, IR: 1721 cm^{-1}, ^{1}H NMR: δ5.74 (1H, *s*), 6.04, 6.60 (1H each, *d*, J = 9.4 Hz), ^{13}C NMR: δ123.0 (CH), 124.5 (C), 124.5 (CH), 135.0 (CH), 156.0 (C), 164.3 (C), 199.4 (C=O)] and a disubstituted double bond [^{1}H NMR; δ5.24 (2H, *m*), ^{13}C NMR: δ132.6 (CH), 135.0 (CH)]. Its mass spectrum contained a fragment ion (*m*/*z* 268) formed by fission of the C-17–C-20 bond and loss of a proton. These data indicated that compound **5** was ergosta-4,6,8-(14),22-tetraene-3-one. This was confirmed by spectral comparison with literature data [7].

Lanostane derivatives are widely distributed in *Basidiomyces* [8], recently, perenniporiols which have a hemiacetal structure in the side chain were isolated from culture mycelia of *Perenniporia orchroleuca*, but lanostane derivatives with a δ-lactone structure in the side chain like the withanolides [9] are unknown. The occurrence in the Basidomycetes of lanostane derivatives such as **1**, **2**, and **3** with a δ-lactone ring system has been demonstrated for the first time.

Table 1. ^{1}H NMR spectral data of compounds 1–3 (200 MHz, $CDCl_3$)

H	1	2	3
18	0.70 *s*	0.70 *s*	0.73 *s*
19	0.98 *s*	0.97 *s*	1.07 *s**
21	0.99 *d*	0.99 *d*	0.99 *d*
	(6.3)	(6.6)	(6.6)
27	1.22 *d*	1.22 *d*	1.22 *d*
	(6.6)	(6.6)	(6.6)
28	0.90 *s*	0.90 *s*	0.91 *s*
29	1.00 *s*	0.99 *s*	1.10 *s**
30	0.81 *s*	0.87 *s*	1.11 *s**
3	3.24 *dd*	3.43 *t*	
	(10.9, 4.8)	(2.7)	
22	4.39 *dd*	4.39 *dd*	4.40 *dd*
	(11.6, 3.0)	(10.7, 2.4)	(11.4, 3.4)
25	2.61 *tq*	2.61 *tq*†	2.61 *m*
	(8.0, 6.6)	(8.2, 6.6)	

Figures in parentheses are coupling constants in Hz.

*Assignment may be reversed.

†Sextet like.

Table 2. ^{13}C NMR spectral data of compounds 1–3 (50.1 MHz, $CDCl_3$)

C	1	2	3
1	35.6 *t*	30.1 *t*	36.0 *t*
2	27.7 *t*	25.8 *t*	34.5 *t*
3	78.9 *d*	76.0 *d*	217.4 *s*
4	38.9 *s*	37.6 *s*	47.3 *s*
5	50.6 *d*	44.3 *d*	51.2 *d*
6	18.3 *t*	18.2 *t*	19.5 *t*
7	27.9 *t*	27.7 *t*	27.6 *t*
8	134.2 *s*	134.0 *s*	135.3* *s*
9	134.7 *s*	134.9 *s*	135.2* *s*
10	37.1 *s*	37.1 *s*	36.9 *s*
11	21.0 *t*	21.0 *t*	21.1 *t*
12	26.5 *t*	26.1 *t*	26.3 *t*
13	44.5 *s*	44.6 *s*	44.5 *s*
14	49.9 *s*	50.0 *s*	50.0 *s*
15	31.0* *t*	31.0* *t*	30.9 *t*
16	30.8* *t*	30.8* *t*	30.9 *t*
17	46.3 *d*	46.3 *d*	46.3 *d*
18	15.7 *q*	15.7 *q*	15.8 *q*
19	19.2 *q*	19.0 *q*	18.7 *q*
20	40.6 *d*	40.6 *d*	40.6 *d*
21	12.6 *q*	12.6 *q*	12.6 *q*
22	80.1 *d*	80.1 *d*	80.0 *d*
23	24.4 *t*	24.4 *t*	24.4 *t*
24	26.0 *t*	26.0 *t*	25.9 *t*
25	33.0 *d*	33.0 *d*	33.0 *d*
26	176.8 *s*	176.7 *s*	176.7 *s*
27	16.4 *q*	16.4 *q*	16.4 *q*
28	24.4 *q*	24.4 *q*	24.4 *q*
29	28.0 *q*	28.1 *q*	26.3 *q*
30	15.4 *q*	22.4 *q*	21.3 *q*

*Assignments in the same vertical column may be interchanged.

Table 3. Selected ^{1}H NMR data for compounds 1, 2 and model compounds*

Compounds	H-22	H-25	H-27	H-21
1	4.39 *dd*	2.61 *tq*	1.22 *d*	0.99 *d*
	(11.6, 3.0)	(8.0, 6.6)	(6.6)	(6.3)
2	4.39 *dd*	2.61 *td*	1.22 *d*	0.99 *d*
	(10.7, 2.4)	(8.2, 6.6)	(6.6)	(6.6)
[22*S*, 25*R*]-6	4.39 *dd*	2.39 *ddq*	1.30 *d*	0.98 *d*
	(11.7, 3.2)	(11.2, 6.1, 6.8)	(6.8)	(6.5)
[22*S*, 25*S*]-6	4.38 *dd*	2.60 *tq*	1.22 *d*	1.01 *d*
	(11.7, 2.8)	(6.6, 6.7)	(6.7)	(6.5)
[22*R*, 25*S*]-6	4.37 *dt*	2.39 *ddq*	1.30 *d*	0.94 *d*
	(10.9, 3.6)	(11.4, 6.5, 6.8)	(6.8)	(6.5)
[22*R*, 25*R*]-6	4.34 *dt*	2.61 *tq*	1.22 *d*	0.97 *d*
	(8.9, 4.5)	(7.2, 6.8)	(6.8)	(6.5)

Figures in parentheses are coupling constants in Hz.

*The data for the model compounds was taken from ref. [5].

EXPERIMENTAL

Mps were uncorr.; ^{1}H NMR: 200 M Hz, $CDCl_3$, TMS as int. standard; ^{13}C NMR: $CDCl_3$, TMS as int. standard; MS: direct inlet, 70 eV; IR: KBr.

Material. The peridium of *A. hygrometricus* were collected from the Wajiki, Tokushima, Japan in October 1984.

Isolation of triterpenes (1, 2, 3) *and steroids* (4, 5). Fresh peridium (113 g) were cut and extracted with hot MeOH until the MeOH extract was colourless. The MeOH soln was evaporated to dryness (8.40 g), dissolved in H_2O and extracted with EtOAc. The EtOAc extract was evaporated under red. pres. to give a residue (3.78 g), which was partioned with EtOAc and 5% Na_2CO_3–H_2O. The EtOAc layer was washed with sat. NaCl, dried over $MgSO_4$ and evaporated to give a neutral and a basic fraction (2.51 g).

This fraction was chromatographed on a silica gel column (150 g) and eluted succesively with hexane–EtOAc and EtOAc–MeOH to afford 10 fractions. Fr. 9 (138 mg), 8 (288 mg) and 6 (118 mg) were crystallized from MeOH to give compounds 1 (56.0 mg), 2 (117 mg) and 3 (104 mg), respectively. Fr. 4 (199 mg) was chromatographed on a silica gel column (50 g) and eluted with hexane–EtOAc (4:1) to afford compound 4 (24.0 mg). Fr. 3 (210 mg) was chromatographed on a silica gel column (50 g) and eluted with hexane–EtOAc (9:1) to afford compound 5 (20.0 mg).

Astrahygrol (1). Colourless needles, mp 186–187°, $[\alpha]_D^{20}$ + 18.0° ($CHCl_3$, *c* 0.5). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1720 (δ-lactone); EIMS *m/z* (rel. int.): 456 $[M]^+$ (29), 441 $[M - Me]^+$, (66), 423 $[M - Me - H_2O]^+$ (100), 314 [M – side chain – H]$^+$ (12), 299 [M – side chain – H – Me]$^+$ (27), 281 $[C_{21}H_{29}]^+$ (30). HRMS *m/z*: 456.3593 for $C_{30}H_{48}O$, required 456.3604; ^{1}H NMR: Table 1. ^{13}C NMR: Table 2.

3-epi-Astrahygrol (2). Colourless needles, mp 193–194°, $[\alpha]_D^{20}$ + 101.0° (*c*, 0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1725 (δ-lactone); EIMS *m/z* (rel. int.): 456 $[M]^+$ (6), 441 $[M - Me]^+$ (9), 438 $[M - H_2O]^+$ (18), 423 $[M - Me - H_2O]^+$ (100), 281 $[C_{21}H_{29}]^+$ (43); HRMS *m/z*: 456.3577 for $C_{30}H_{48}O$, required 456.3604; ^{1}H NMR ($CDCl_3$): Table 1; ^{13}C NMR: Table 2.

Astrahygrone (3). Colourless needles, mp 168–169°, $[\alpha]_D^{20}$ + 58.0° ($CHCl_3$; c 0.5), IR ν_{max} cm^{-1}: 1720 (δ-lactone), 1700 (C=O); EIMS *m/z* (rel. int.): 454 $[M]^+$ (31), 439 $[M - Me]^+$ (100), 312 [M – side chain – H]$^+$ (27), 297 [M – side chain – H

$-Me]^+$ (51); HRMS *m/z*: 454.3444 for $C_{30}H_{46}O_6$, required 454.3447; ^1H NMR: Table 1; ^{13}C NMR: Table 2; ORD (MeOH; *c* 0.1): $[\alpha]_{340}$ + 100°, $[\alpha]_{300}$ + 650°, $[\alpha]_{278}$ + 601°, $[\alpha]_{270}$ + 710°. [8]Δ-lanostene-3-one: $[\alpha]_{589}$ + 74°, $[\alpha]_{300}$ + 610°, $[\alpha]_{277.5}$ + 498°, $[\alpha]_{270}$ + 542° [4].

Hydrolysis of compound **3**. A soln of 10 mM NaOMe–MeOH 1 ml was added to compound **3** (66.0 mg) and stirred at room temp. for 10 hr. The reaction mixture was neutralized with acid and concentrated to give a residue, which was chromatographed on a silica gel column and eluted with hexane–EtOAc to give a starting material 35.5 mg and **3a**, 29.5 mg. **3a**, colourless needles (from MeOH), $[\alpha]_D^{20}$ + 41.0° ($CHCl_3$; *c* 0.37), IR ν_{max}^{KBr} cm^{-1}: 1720 (ester); EIMS *m/z* (rel. int.): 486 $[M]^+$ (6), 468 $[M - H_2O]^+$ (14), 453 $[M - H_2O - Me]^+$ (56), 439 $[M - MeOH - H_2O]^+$ (87), 297 $[C_{21}H_{29}O]^+$ (65); ^1H NMR ($CDCl_3$): δ0.72, 0.92, 1.07, 1.10, 1.12 (3H each, *s*, 5 × Me), 0.87 (3H, *d*, *J* = 6.8 Hz, H-21), 1.14 (3H, *d*, *J* = 7.1 Hz, H-27), 2.60 (1H, *m*, H-25), 3.69 (3H, *s*, $-COOCH_3$), 3.71 (1H, *m*, 22-H).

Oxidation of compound **1**. 10.0 mg with Jones reagent followed by the usual work up gave a residue, which was separated by silica gel CC (hexane–EtOAc, 3:1) to give **1a** (4.5 mg) and starting material (1.1 mg). **1a**, Colourless needles (from MeOH), mp 168–169°, $[\alpha]_D^{20}$ + 54.0° (*c*, 0.26, $CHCl_3$), HRMS *m/z*: 454.3453 for $C_{30}H_{46}O_3$, required 454.3447. Compound **1a** was identical with **3** by mmp, TLC, IR and ^1H NMR comparison.

Oxidation of compound **2**. 25.0 mg with Jones reagent as just described gave **2a** (20.2 mg). **2a**, Colourless needles (from MeOH), mp 167–168°, $[\alpha]_D^{20}$ + 56.0° ($CHCl_3$; *c* 0.5), Compound **2a** was identical with **3** by mmp, TLC, IR, and ^1H NMR comparison.

Compound **4**. Colourless needles (from MeOH), mp 161–163°, $[\alpha]_D$ − 24.0° (*c*, 0.1, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), 1380, 1365, 968. EIMS *m/z* (rel. int.): 398 $[M]^+$ (12), 380 $[M - H_2O]^+$ (67), 365 $[M - H_2O - Me]^+$ (27), 271 $[C_{19}H_{29}O]^+$ (22); HRMS *m/z*: 398.3529 for $C_{28}H_{46}O$, required 398.3548; ^1H NMR ($CDCl_3$): δ0.55, 0.80 (3H, *s*, 2 × Me), 0.84 (3H, *d*, *J* = 6.9 Hz), 0.91 (3H × 2, *d*, *J* = 6.8 Hz, 2 × Me), 1.02 (3H, *d*, *J* = 8.1 Hz), 3.60 (1H, *m*, H-3), 5.16 (3H, *m*, H-7, H-22, H-23).

Acetylation of compound **4**. Compound **4** (10.0 mg) was acetylated with Ac_2O–C_5H_5N to give acetate **4a** (10.0 mg) which was identical with 3-acetoxy-7,22-diene ergosterol (^1H NMR and ^{13}C NMR).

Compound **5**. Colourless needles (from MeOH), mp 80–81°, $[\alpha]_D^{20}$ + 580° ($CHCl_3$; *c* 0.1), UV λ_{max} nm (ε): 238 (6600), 282 (9000), 348 (31100); IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O); EIMS (rel. int.) *m/z*: 392 $[M]^+$ (20), 268 $[M$ − side chain + $H]^+$ (100), 253 $[M$ − side chain + H − $Me]^+$ (22); HRMS *m/z*: 394.3254 for $C_{28}H_{42}O$, required 394.3236; ^1H NMR ($CDCl_3$): δ0.83 (3H, *d*, *J* = 6.6 Hz), 0.8, (3H, *d*, *J* = 6.8 Hz), 0.93 (3H, *d*, *J* = 6.8 Hz), 0.96, 1.00 (3H, each, *s*, 2 × Me), 1.06 (3H, *d*, *J* = 6.6 Hz), 5.23 (2H, *m*, 22-H, 23-H), 5.74 (1H, *s*, H-4), 6.03, 6.60 (1H each, *d*, *J* = 9.4 Hz, H-6, H-7).

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