NPC Natural Product Communications

2010
Vol. 5
No. 10
1575 - 1578

Asporyergosterol, A New Steroid from an Algicolous Isolate of *Aspergillus oryzae*

Ming-Feng Qiao[a,b], Nai-Yun Ji[a,*], Xiang-Hong Liu[a], Fang Li[c] and Qin-Zhao Xue[a]

[a]*Yantai Institute of Coastal Zone Research*, *Chinese Academy of Sciences*, *Yantai 264003*, *China*

[b]*Graduate School of Chinese Academy of Sciences*, *Beijing 100049*, *China*

[c]*Qingdao University of Science & Technology*, *Qingdao 266042*, *China*

nyji@yic.ac.cn

Received: May 12th, 2010; Accepted: August 26th, 2010

Asporyergosterol (**1**), a new steroid with an *E* double bond between C-17 and C-20, was identified from the culture extracts of *Aspergillus oryzae*, an endophytic fungus isolated from the marine red alga *Heterosiphonia japonica*. Moreover, four known steroids including (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**2**), (22*E*,24*R*)-3β-hydroxyergosta-5,8,22-trien-7-one (**3**), (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (**4**), and (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**5**) were isolated. Structures of these compounds were unambiguously established by spectroscopic techniques and by comparison with literature values. All the isolates exhibited low activity to modulate acetylcholinesterase (AChE).

Keywords: Asporyergosterol, red alga, *Heterosiphonia japonica*, endophytic fungus, *Aspergillus oryzae*.

Marine-derived fungi have become an important source of pharmacologically active metabolites. Among all metabolites reported from marine-derived fungi, those from algicolous fungi account for 24%, but represent a slightly higher percentage (27%) of new compounds. These metabolites include anthraquinones, terpenoids, polyketones, macrolides, and alkaloids, and exhibit cytotoxic, antimicrobial, and antioxidative activities [1-3]. However, new steroids are rarely reported from algicolous fungi [4]. In our chemical investigation of an endophytic fungus *Aspergillus oryzae* that was isolated from the inner tissue of the marine red alga *Heterosiphonia japonica*, asporyergosterol (**1**), a new steroid with an *E* double bond between C-17 and C-20, was characterized. In addition, (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**2**) [5], (22*E*,24*R*)-3β-hydroxyergosta-5,8,22-trien-7-one (**3**) [6], (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (**4**) [7], and (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**5**) [8] were isolated and identified (Figure 1). The isolation, structural determination, and bioactivity of these steroids are presented here.

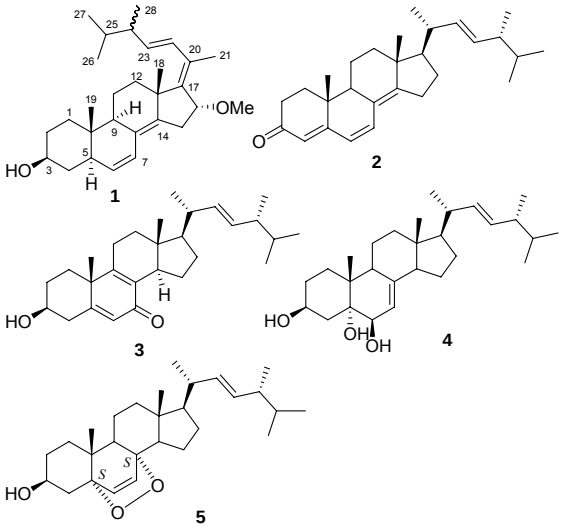


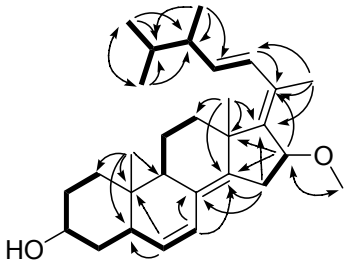
Figure 1: Chemical structures of **1**-**5**.

Compound **1** was obtained as a colorless oil. The broad IR absorption at ν_{max} 3352 cm^{-1} indicated the presence of a hydroxy group in the molecule. The molecular formula was determined to be $C_{29}H_{44}O_2$ on the basis of the HR-EI-MS (*m/z* 424.3333 $[M]^+$, calcd for $C_{29}H_{44}O_2$, 424.3341), indicates eight degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) displayed two methyl singlets at δ_H 0.75 (H-19) and 1.26 (H-18), one vinyl methyl singlet at δ_H 1.88 (H-21), one oxygenated methyl singlet at δ_H 3.24 (MeO), three methyl doublets at δ_H 0.86 (d, 7.0 Hz, H-26), 0.87 (d, 7.0 Hz, H-27), and 1.00 (d, 6.8 Hz, H-28), one multiplet at δ_H 3.60 (H-3) and one doublet at δ_H 4.33 (d, 5.4 Hz, H-16)

Table 1: ^{13}C and ^{1}H NMR data of **1** ($CDCl_3$, δ in ppm).

Position	δ_C (DEPT)	δ_H (*J* in Hz)
1a	33.7 (CH_2)	1.18 (m)
1b		1.84 (m)
2a	31.0 (CH_2)	1.30 (m)
2b		1.74 (m)
3	71.2 (CH)	3.60 (m)
4a	39.3 (CH_2)	1.30 (m)
4b		1.93 (m)
5	44.4 (CH)	1.84 (m)
6	129.5 (CH)	5.55 (dd, 9.9, 5.4)
7	124.6 (CH)	6.22 (d, 9.9)
8	126.2 (C)	
9	34.6 (CH)	2.55 (ddd, 10.6, 6.2, 3.4)
10	34.1 (C)	
11a	19.4 (CH_2)	1.56 (m)
11b		1.63 (m)
12a	35.0 (CH_2)	1.69 (m)
12b		2.39 (m)
13	43.8 (C)	
14	144.2 (C)	
15a	31.5 (CH_2)	2.27 (ddd, 15.4, 5.4, 3.4)
15b		2.84 (d, 15.4)
16	81.3 (CH)	4.33 (d, 5.4)
17	145.8 (C)	
18	26.6 (CH_3)	1.26 (s)
19	22.6 (CH_3)	0.75 (s)
20	131.7 (C)	
21	16.5 (CH_3)	1.88 (s)
22	128.5 (CH)	6.58 (d, 15.6)
23	134.9 (CH)	5.63 (dd, 15.6, 8.1)
24	43.8 (CH)	2.05 (m)
25	33.3 (CH)	1.57 (m)
26	19.8 (CH_3)	0.86 (d, 7.0)
27	20.0 (CH_3)	0.87 (d, 7.0)
28	17.5 (CH_3)	1.00 (d, 6.8)
MeO	55.3 (CH_3)	3.24 (s)

characteristic of two oxygenated methines, two double doublets at δ_H 5.55 (dd, 9.9, 5.4 Hz, H-6) and 5.63 (dd, 15.6, 8.1 Hz, H-23) and two doublets at δ_H 6.22 (d, 9.9 Hz, H-7) and 6.58 (d, 15.6 Hz, H-22) attributed to four olefinic protons. The ^{13}C and DEPT NMR spectra (Table 1) along with the HSQC experiment revealed the presence of seven methyls including one oxygenated methyl (MeO), six methylenes, ten methines containing two oxygenated methines (C-3 and C-16), and six quaternary carbon atoms. Detailed inspection of the ^{1}H and ^{13}C NMR data revealed that **1** possessed an ergosta-22-en-3β-ol skeleton, the same as found in **3**–**5**. The double bond between C-17 and C-20 was confirmed by the HMBC correlations from H-18 and H-21 to C-17 and from H-21 and H-22 to C-20. The methoxy group was assigned on C-16 by HMBC correlations from H-29 to C-16 and from H-16 to C-13, C-14, and C-20. The ^{1}H-^{1}H COSY correlation between H-15a and H-16 and HMBC correlations from H-15b to C-13 and C-17 verified the position of the methoxy group. The double bond between C-8 and C-14 was indicated by the HMBC correlations from H-18 to C-14 and from H-15b to C-8 and C-14. The last double bond was located between C-6 and C-7 based on the HMBC correlations from H-6 to C-5, C-10 and from H-7 to C-8, C-9, and C-14. The gross structure of **1** was further corroborated by analysis of the other ^{1}H-^{1}H COSY and HMBC correlations as shown in Figure 2.



1

Figure 2: Key correlations in ^{1}H-^{1}H COSY (solid lines) and HMBC (arrows) spectra of **1**.

The double bond between C-17 and C-20 was assigned the *E*-configuration by the NOESY correlations between H-16 and H-21 and between H-18 and H-22. H-16 and H-18 were located on the same face of the molecule, according to the observed NOESY correlations between H-15a and H-16, H-18. The coupling constant 15.6 Hz between H-22 and H-23 indicated the double bond at C-22 to be *trans*. The relative configurations at C-3, C-5, and C-10 were assigned to be the same as those of 5α-androst-6-ene-3β,17β-diol by detailed NMR data comparison [9]. H-9 was determined to be axial by its large coupling constant (10.6 Hz), and α-oriented based on the weak NOESY correlation between H-9 and H-5. The only configuration that could not be independently assigned was that of C-24, due to a lack of relevant correlations. Based on the above spectral evidence, compound **1** is (17(20)*E*,22*E*)-16-methoxyergosta-6,8(14),17(20),22-tetraen-3β-ol, which was named asporyergosterol. This steroid possesses an ergostane skeleton with an unusual *E* double bond between C-17 and C-20.

The four known steroids including (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**2**) [5], (22*E*,24*R*)-3β-hydroxyergosta-5,8,22-trien-7-one (**3**) [6], (22*E*,24*R*)-ergosta-7,22-dien-3β,5α,6β-triol (**4**) [7], and (22*E*,24*R*)-5α,8α-epidioxyergosta-6,22-dien-3β-ol (**5**) [8] were elucidated on the basis of detailed ^{1}H and ^{13}C NMR data comparison with literature values.

Compounds **1**–**5** were evaluated for acetylcholinesterase (AChE) inhibitory activity [10] which was low (Table 2).

Table 2: Inhibitory rates against acetylcholinesterase at 100 μg/mL of **1**–**5**.

	1	**2**	**3**	**4**	**5**	Huperzine A[a]
Inhibitory rates (%)	14.0	19.8	7.2	0.4	8.1	98.0

[a] Positive control at 100 μg/mL.

Experimental

General experimental procedures: NMR spectra were recorded in $CDCl_3$ at 500 and 125 MHz for ^{1}H and ^{13}C, respectively, on a Bruker Avance 500 MHz NMR spectrometer using TMS as internal standard. The low

resolution mass spectrum was determined on a Thermo Finnigan Trace DSQ mass spectrometer, while the high resolution mass spectrum was determined on an Autospec Premier P776 mass spectrometer. The IR spectrum was obtained on a JASCO FT/IR-4100 Fourier Transform InfraRed spectrometer. The optical rotation was determined on a JASCO P-1020 polarimeter. Column chromatography was performed with silica gel (200-300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), RP-18 reversed-phase silica gel (YMC), and Sephadex LH-20 (Pharmacia). TLC was carried out with precoated silica gel plates (GF-254, Qingdao Haiyang Chemical Co., Qingdao, China). All solvents were of analytical grade.

Microorganism and fermentation: The endophytic fungal strain *A. oryzae* cf-2 was isolated from the fresh tissue of surface-sterilized marine red alga *H. japonica* that was collected off the coast of Yantai, China. The fungus was identified based on morphological and molecular taxonomic methods by one of the authors (M.-F.Q.) and was preserved at the China Center for Type Culture Collection with access number CCTCC M 2010045.

The initial cultures were maintained on the potato dextrose agar (PDA) plate. Pieces of mycelia were cut into small segments and aseptically inoculated into 1000 mL Erlenmeyer flasks containing 300 mL potato dextrose broth (PDB) culture media. Static fermentation was carried out for 23 days at room temperature (*ca*. 25°C).

Extraction and isolation: The culture broth (9 L) was extracted with EtOAc, obtaining 2.7 g gum after removal of the solvent by evaporation (40°C) at reduced pressure. The dried and powdered mycelia (64.7 g) were extracted with a mixture of $CHCl_3$ and MeOH (1:1, v/v), concentrated and partitioned between H_2O and EtOAc, giving 5.4 g gum. Since the TLC profiles of the two extracts were nearly identical, they were combined before further separation. The total EtOAc-soluble fraction (8.1 g) was subjected to silica gel column chromatography (CC, petroleum ether (PE)/EtOAc gradient) to give 22 fractions, monitored by TLC. Fraction 13 eluted with PE/EtOAc (20:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (PE/EtOAc, 15:1) and preparative TLC (PE/EtOAc, 5:1) to give **2** (19.9 mg). Fraction 17 eluted with PE/EtOAc (5:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (PE/EtOAc, 5:1) and preparative TLC ($CHCl_3$/EtOAc, 2:1) to afford **5** (8.0 mg). Fraction 19 eluted with PE/EtOAc (1:1) and was further purified by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1), silica gel (PE/EtOAc, 2:1), and RP-18 (MeOH/H_2O, 17:3) and preparative TLC (PE/EtOAc, 1:1) to yield **1** (3.6 mg) and **3** (2.3 mg). **4** (3.8 mg) was obtained from fraction 21 (eluted with EtOAc) by CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and RP-18 (MeOH/H_2O, 17:3).

Asporyergosterol (**1**)

Colorless oil.
$[\alpha]^{17}_D$: +19.5 (*c* 0.21, $CHCl_3$).
IR (KBr): 3352, 3282, 2935, 2870, 1712, 1601, 1512, 1458, 1373, 1254, 1068, 756 cm^{-1}.
^1H and ^{13}C NMR: Table 1.
EI-MS: *m/z* (%) 424 (52), 409 (61), 392 (49), 377 (100), 359 (17), 321 (20), 285 (25), 267 (67), 249 (28), 181 (18).
HR-EI-MS: *m/z* 424.3333 $[M]^+$ (calcd for $C_{29}H_{44}O_2$, 424.3341).

Acknowledgments - This work was financially supported by the Chinese Academy of Sciences for Key Topics in Innovation Engineering (KZCX2-YW-QN209, KSCX2-YW-G-073, KZCX2-YW-225), Shandong Province Natural Science Foundation (ZR2009BQ021), Yantai Municipal Sci-Tech Development Program (2009164), and Foundation of the Chinese Academy of Sciences for President's Scholarship (awarded to N.-Y. Ji).

References

[1] Bugni TS, Ireland CM. (**2004**) Marine-derived fungi: a chemically and biologically diverse group of microorganisms. *Natural Product Reports*, ***21***, 143-163.

[2] König GM, Kehraus S, Seibert SF, Abdel-Lateff A, Müller D. (**2006**) Natural products from marine organisms and their associated microbes. *ChemBioChem*, ***7***, 229-238.

[3] Jia TZ, Liu HB, Fang YC, Zhu TJ, Gu QQ, Zhu WM. (**2006**) Advances in secondary metabolites of fungi isolated from the marine alga. *Chinese Journal of Antibiotics*, ***31***, 328-334.

[4] Zhang Y, Li XM, Proksch P, Wang BG. (**2007**) Ergosterimide, a new natural Diels-Alder adduct of a steroid and maleimide in the fungus *Aspergillus niger*. *Steroids*, ***72***, 723-727.

[5] Schulte KE, Rücker G, Fachmann H. (**1968**) Ergosta-4,6,8(14),22-tetraenon-(3) als inhaltsstoff des lärchenschwammes. *Tetrahedron Letters*, ***9***, 4763-4764.

[6] Ishizuka T, Yaoita Y, Kikuchi M. (**1997**) Sterol constituents from the fruit bodies of *Grifola frondosa* ($F_{R.}$) S.F. G_{RAY}. *Chemical & Pharmaceutical Bulletin*, ***45***, 1756-1760.

[7] Piccialli V, Sica D. (**1987**) Four new trihydroxylated sterols from the sponge *Spongionella gracilis*. *Journal of Natural Products*, ***50***, 915-920.

[8] Yue JM, Chen SN, Lin ZW, Sun HD. (**2001**) Sterols from the fungus *Lactarium volemus*. *Phytochemistry*, ***56***, 801-806.

[9] Holland HL, Chenchaiah PC, Thomas EM, Mader B, Dennis MJ. (**1984**) Microbial hydroxylation of steroids. 9. epoxidation of Δ^6-3-ketosteroids by *Rhizopus arrhizus* ATCC 11145, and the mechanism of the 6β hydroxylase enzyme. Canadian *Journal of Chemistry*, ***62***, 2740-2747.

[10] López S, Bastida J, Viladomat F, Codina C. (**2002**) Acetylcholinesterase inhibitory activity of some amaryllidaceae alkaloids and *Narcissus* extracts. *Life Sciences*, ***71***, 2521-2529.