Lanostane Triterpenes from *Ceriporia lacerate* HS-ZJUT-C13A, a Fungal Endophyte of *Huperzia serrata*

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Two new lanostane triterpenes, 3β-acetoxy-15α-hydroxylanosta-8,24-dien-21-oic acid (**1**) and 3β-acetoxylanosta-7,9(11),24-trien-21-oic acid (**2**), along with the four known analogs, **3**–**6**, were isolated from the culture broth of *Ceriporia lacerate* HS-ZJUT-C13A, a fungal endophyte residing in the stems of the traditional Chinese herb *Huperzia serrata*. The structures of the new compounds **1** and **2** were established by spectroscopic methods, including UV, IR, HR-ESI-MS, and extensive 1D- and 2D-NMR techniques. To the best of our knowledge, this is the first report on triterpene metabolites from the genus *Ceriporia*.

Introduction. – Endophytes refer to microorganisms that reside in the internal tissues of living plants without causing any immediate overt negative effects [1]. It is believed that each plant on earth was host to one or more endophytes [2]. During the long periods of co-evolution, endophytes kept a state of balanced antagonism with the host plant [3], which regulated the metabolism of the endophytes and endowed them with limitless potential for the production of novel bioactive metabolites. The past three decades have witnessed the discovery of many natural products with unique structures and diverse bioactivities, from the endophytes [4]. As part of our ongoing program of exploring fungal endophytes for new chemical entities, the fungus *Ceriporia lacerate* HS-ZJUT-C13A was isolated from the traditional Chinese herb *Huperzia serrata*. Previous chemical investigations indicated that the fungus could produce two new tremulane sesquiterpenes when cultivated on rice medium [5]. In the present work, we describe the isolation and identification of six triterpenes, **1**–**6**, from the culture broth of the fungus fermented in liquid potato-dextrose medium, including two new lanostane triterpenes, **1** and **2**, and four known analogs, **3**–**6**. The structures of the isolates were established by spectroscopic methods, including UV, IR, HR-ESI-MS, and NMR techniques.

Results and Discussion. – An AcOEt extract of the cultures of *C. lacerate* HS-ZJUT-C13A was subjected to extensive column chromatography to afford two new triterpenes, **1** and **2**, as well as the four known ones, **3**–**6** (*Fig. 1*).

Compound **1** was obtained as a white amorphous powder with a molecular formula of $C_{32}H_{50}O_5$ on the basis of the $[M-H]^-$ peak at m/z 513.3564 in the HR-ESI-MS. The IR spectrum displayed absorption bands for OH (3336 cm^{-1}), and two CO groups of

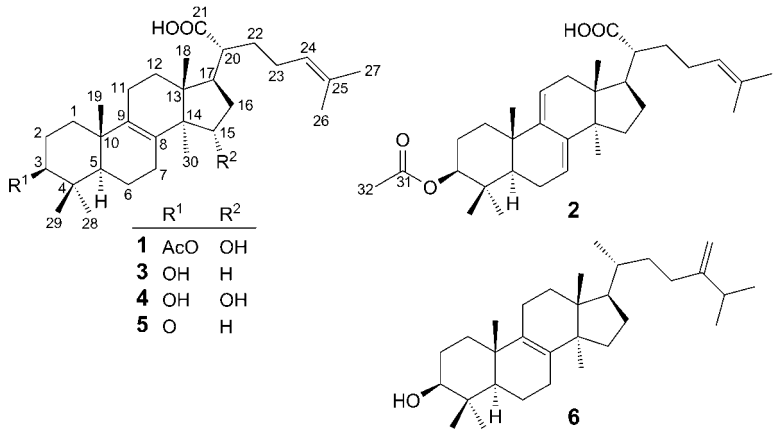

Fig. 1. *The structures of compounds* **1**–**6**

ester (1729 cm^{-1}) and COOH (1679 cm^{-1}). The ^{13}C-NMR and DEPT spectra (*Table*) indicated that compound **1** possesses a triterpene structure containing a total of 32 C-atoms. The signals at δ(C) 178.9 (C(21)) and 170.7 (C(31)) corresponded to the acid and ester COO groups, respectively, while the olefinic signals at δ(C) 135.2 (C(8)), 134.7 (C(9)), 131.8 (C(25)), and 125.0 (C(24)) were ascribed to two C=C bonds. The ^{1}H-NMR spectrum exhibited signals for eight Me groups at δ(H) 0.98 (*s*, Me(28)), 1.01 (*s*, Me(29)), 1.06 (*s*, Me(19)), 1.24 (*s*, Me(18)), 1.43 (*s*, Me(30)), 1.68 (*s*, Me(27)), 1.73 (*s*, Me(26)), and 2.12 (*s*, Me(32)). The resonance of an olefinic H-atom appeared at δ(H) 5.37 (*tt*, $J = 7.0$, 1.0, H–C(24)), and of two O-bearing CH groups at δ(H) 4.75 (*dd*, $J =$ 12.0, 4.0, H–C(3)) and 4.68 (*dd*, $J = 9.0$, 5.5, H–C(15)), corresponding to the O-bearing C-atom signals at δ(C) 80.8 (C(3)) and 72.5 (C(15)), respectively. The ^{1}H- and ^{13}C-NMR data of compound **1** provide evidences of a lanostane-type triterpene skeleton. Hence, detailed comparison of the NMR data of compound **1** and 5α-hydroxytrametenolic acid (**4**) was carried out, and revealed that **1** should be the acetate of **4**. The position of the AcO group was assigned on the basis of the downfield shift of H–C(3) from δ(H) 3.42 to 4.75 by acylation shifts as compared with **4**, and confirmed by resorting to the HMBC cross-peaks of Me(32) and H–C(3) to the ester C-atom C(31). In the ^{1}H-NMR spectrum, the signal assignable to H–C(3) appeared as a *dd* at δ(H) 4.75 with coupling constants of 12.0 and 4.0 Hz, suggesting an α-orientation of this H-atom, ensured by the NOE correlation H–C(3)/H–C(5). On the contrary, the additional NOE correlation H–C(15)/Me(18) established the β orientation of H–C(15). Thus, the structure of compound **1** was determined as 3β-acetoxy-15α-hydroxylanosta-8,24-dien-21-oic acid and confirmed by 2D-NMR analyses (*Fig. 2*).

Compound **2** was obtained as a white amorphous powder and assigned a molecular formula of $C_{32}H_{48}O_4$ on the basis of the $[M - H]^-$ peak at *m*/*z* 495.3482 in the HR-ESI-MS, indicating nine degrees of unsaturation. The IR spectrum indicated the presence of two CO groups of ester (1714 cm^{-1}) and carboxylic acid (1654 cm^{-1}). The ^{13}C-NMR and DEPT spectra (*Table*) exhibited 32 C-atom signals. Similar to compound **1**, the signals at δ(C) 182.7 (C(21)) and 170.9 (C(31)) were assigned to the carboxylic acid and ester

Table 1. *NMR Data of Compounds* **1** *and* **2**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.17–1.24 (*m*), 1.59–1.68 (*m*)	35.6	1.45 (*dt*, *J* = 12.5, 3.0), 1.92–1.98 (*m*)	35.6
2	1.63–1.72 (*m*), 1.71–1.83 (*m*)	24.6	1.66–1.74 (*m*)	24.3
3	4.75 (*dd*, *J* = 12.0, 4.0)	80.8	4.51 (*dd*, *J* = 10.5, 5.5)	80.6
4	–	38.1	–	37.7
5	1.27 (*dd*, *J* = 12.5, 1.5)	50.8	1.18 (*dd*, *J* = 10.5, 5.0)	49.3
6	1.58–1.65 (*m*), 1.74–1.81 (*m*)	18.7	2.02–2.08 (*m*), 2.09–2.14 (*m*)	22.8
7	2.56–2.64 (*m*), 2.66–2.75 (*m*)	27.6	5.48 (br. *s*)	120.4
8	–	135.2	–	142.4
9	–	134.7	–	145.8
10	–	37.4	–	37.3
11	1.98–2.03 (*m*)	21.2	5.28 (br. *d*, *J* = 6.0)	116.3
12	1.97–2.02 (*m*), 2.20–2.29 (*m*)	30.2	2.20 (*d*, *J* = 17.5), 1.83 (*dd*, *J* = 17.5, 6.5)	35.4
13	–	45.5	–	43.6
14	–	52.2	–	50.1
15	4.68 (*dd*, *J* = 9.0, 5.5)	72.5	1.40–1.45 (*m*), 1.60–1.68 (*m*)	31.0
16	2.25–2.39 (*m*)	39.4	1.30–1.38 (*m*), 2.00–2.06 (*m*)	26.9
17	2.69–2.83 (*m*)	46.8	2.11–2.19 (*m*)	47.6
18	1.24 (*s*)	17.0	0.64 (*s*)	15.8
19	1.06 (*s*)	19.4	0.99 (*s*)	22.7
20	2.67–2.78 (*m*)	49.1	2.27 (*td*, *J* = 10.5, 3.0)	47.6
21	–	178.9	–	182.7
22	1.79–1.90 (*m*), 1.94–2.09 (*m*)	33.4	1.50–1.59 (*m*), 1.58–1.66 (*m*)	32.4
23	2.30–2.38 (*m*), 2.39–2.49 (*m*)	26.8	1.91–2.05 (*m*)	26.0
24	5.37 (*tt*, *J* = 7.0, 1.0)	125.0	5.10 (br. *t*, *J* = 7.5)	123.5
25	–	131.8	–	132.3
26	1.73 (*s*)	25.9	1.70 (*s*)	25.7
27	1.68 (*s*)	17.8	1.59 (*s*)	17.7
28	0.98 (*s*)	28.1	0.88 (*s*)	28.1
29	1.01 (*s*)	16.9	0.95 (*s*)	16.9
30	1.43 (*s*)	18.3	0.89 (*s*)	25.6
31	–	170.7	–	170.9
32	2.12 (*s*)	21.3	2.05 (*s*)	21.3

[a]) Recorded in (D_5)pyridine; [b]) Recorded in $CDCl_3$.

COO groups, respectively. Three olefinic quaternary C-atom signals at δ(C) 145.8 (C(9)), 142.4 (C(8)), and 132.3 (C(25)), together with three olefinic CH C-atom signals at δ(C) 123.5 (C(24)), 120.4 (C(7)), and 116.3 (C(11)), indicated the presences of three trisubstituted C=C bonds in compound **2**. The ^{1}H-NMR spectrum showed signals for eight Me groups at δ(H) 0.64 (*s*, Me(18)), 0.88 (*s*, Me(28)), 0.89 (*s*, Me(30)), 0.95 (*s*, Me(29)), 0.99 (*s*, Me(19)), 1.59 (*s*, Me(27)), 1.70 (*s*, Me(26)), and 2.05 (*s*, Me(32)). The resonances of three olefinic H-atoms appeared at δ(H) 5.48 (br. *s*, H–C(7)), 5.28 (br. *d*, *J* = 6.0, H–C(11)), and 5.10 (br. *t*, *J* = 7.5, H–C(24)), confirming the presence of three trisubstituted C=C bonds. An O-bearing CH group signal at δ(H) 4.51 (*dd*, *J* = 10.5, 5.5, H–C(3)) corresponded to the O-bearing C-atom signal at δ(C) 80.6 (C(3)). The comparison of ^{1}H- and ^{13}C-NMR data of compound **2** with those in the related

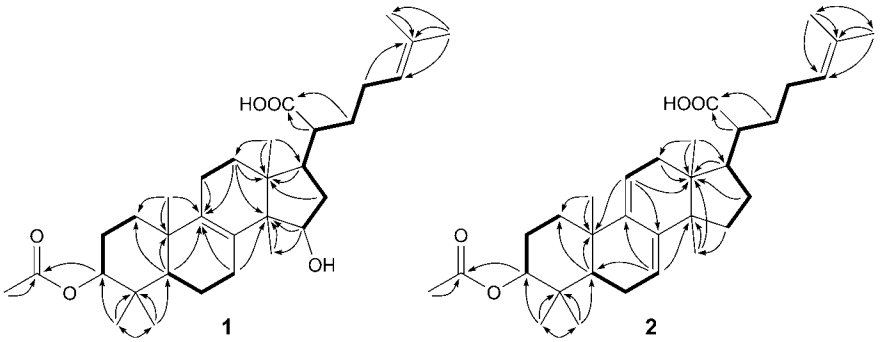

Fig. 2. *Selected* $^1H,^1H$*-COSY* (—) *and HMBC* (H → C) *features of* **1** *and* **2**

literature established a lanostane-type triterpene skeleton [6]. A further in-depth comparison of the NMR data of compounds **1** and **2**, especially regarding the olefinic signals, suggested a $\Delta^{7,9(11)}$ conjugated diene partial structure in compound **1**, which was in accordance with the maximum UV absorption at 243 nm (log ε 4.01) [6] and finally confirmed by the $^1H,^1H$-COSY plots (*Fig. 2*) of H–C(11)/CH_2(12) and H–C(5)/CH_2(6)/H–C(7), as well as HMBCs (*Fig. 2*) from H–C(7) to C(9) and C(14), and from H–C(11) to C(8), C(10) and C(13). The chemical shift of H–C(3) (δ(H) 4.51; δ(C) 80.6), along with the HMBCs from Me(32) and H–C(3) to the ester carbonyl C(3), located the AcO group at C(3) of compound **2**. The relative configuration at C(3) of **2** was established as the same as that of **1** by analyzing the coupling constants of H–C(3) and the related NOE correlation. Thus, compound **2** was determined to have the structure of 3β-acetoxylanosta-7,9(11),24-trien-21-oic acid.

The known compounds were identified as trametenolic adid (**3**) [7], 15α-hydroxytrametenolic acid (**4**) [8], 3-oxotrametenolic acid (**5**) [9], and eburicol (**6**) [10] by comparison of their spectroscopic data with those in the literature.

Ceriporia belongs to the family Polyporaceae and was always regarded to consist of white rutting fungi [11]. In the recent years, fungi of this genus have exhibited prosperous potential in the field of environmental bioremediation [12]. *Ceriporia lacerate* was first reported by *Cui et al.* as new to Chinese fungal flora in 2006 [11]. This fungus has been studied for its potential of decomposing lignocelluloses [13], removing crystal violet from aqueous solution [14], and decolorizing Alizarin Red and Methyl Orange [15]. However, no chemical investigation of this fungus or even the genus *Ceriporia* has been reported until our previous isolation and identification of two tremulane sesquiterpenes [5]. The isolation and identification of six lanostane triterpenes in the present work represents the first report of triterpenes from the genus *Ceriporia*. Furthermore, in view of the diverse biological activities [16] of lanostane triterpenes and their wide distribution in many precious medicinal fungi, *e.g.*, *Ganoderma lucidum* [17], *Poria cocos* [18], and *Inonotus obliquus* [19], it should be of great significance to launch further in-depth bioactivity studies of the fungus *Ceriporia lacerate*.

Experimental Part

General. All solvents used were of anal. grade and obtained from commercially available sources. Column chromatography (CC): silica gel (SiO_2, 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, China), *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries Ltd. Japan*), and *ODS C-18* gel (50 μm; *YMC Co. Ltd.*, Kyoto, Japan), *Toyopearl HW-40C* gel (50–100 μm; *Tosoh Corporation*, Japan). TLC: Precoated silica gel GF_{254} plates (*Qingdao Marine Chemical Inc.*, Qingdao, China); visualization with UV light and 10% H_2SO_4/EtOH. Optical rotations: *Rudolph Research Autopol III* automatic polarimeter. UV Spectra: *Shimadzu-UV-2450* spectrometer. IR Spectra: *Thermo-Nicolet-6700 FT-IR* microscope instrument (FT-IR microscope transmission). NMR Spectra: *Bruker-AM-500* apparatus; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI- and HR-ESI-MS: *Agilent- 6210-LC/TOF* mass spectrometer; in *m/z*.

Fungus and Culture Conditions. The fungus was isolated from the stems of a traditional Chinese medicinal plant *Huperzia serrata* collected in Pan-An County, Zhejiang Province, P. R. China, in July 2010. It was identified as *C. lacerate* based on the DNA sequence analysis conducted by *Sangon Biotech Co. Ltd.* (Shanghai). The original culture (voucher No. HS-ZJUT-C13A) was deposited with the China Center for Type Culture Collection (deposit No. CCTCC M 2012433). The cultivation was carried out on shakers at 28° and 185 rpm for 6 d in liquid PD (potato extracts, 200 g; glucose, 20 g; dist. H_2O, 1 l) medium, followed by static cultivation for 24 d.

Extraction and Isolation. The cultures (100 l) were filtered through cheesecloth to separate broth and mycelium. The broth was condensed under reduced pressure at 40° to *ca.* 3 l, which was partitioned with AcOEt (5×3 l). The AcOEt extract was evaporated under reduced pressure to yield 58 g of residue, which was subjected to CC (*MCI CHP20P* gel; MeOH/H_2O 10:100–100:0 (*v/v*)) give six frations, *Frs. A–F. Fr. F* was subjected to CC (SiO_2; $CDCl_3$/MeOH 25:1–5:1 (*v/v*)), followed by CC (*HW-40C*; MeOH) to give **1** (15.4 mg), **2** (35.1 mg), **3** (17.3 mg), **4** (55.7 mg), **5** (9.3 mg), and **6** (7.4 mg).

(*3β,15α)-3-(Acetyloxy)-15-hydroxylanosta-8,24-dien-21-oic Acid* (**1**). White amorphous powder. $[\alpha]_D^{20} = +27.8$ ($c = 0.07$, $CHCl_3$). IR: 3336, 2939, 1729, 1679, 1439, 1375, 1265, 1050, 905, 804. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (neg.): 513.3564 ($[M-H]^-$, $C_{32}H_{49}O_5^-$; calc. 513.3585).

(*3β)-3-(Acetyloxy)lanosta-7,9(11),24-trien-21-oic Acid* (**2**). White amorphous powder. $[\alpha]_D^{20} = +59.0$ ($c = 0.20$, $CHCl_3$). IR: 2924, 1714, 1654, 1448, 1374, 1251, 1034, 980, 882, 813. UV ($CHCl_3$): 243 (4.01). ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (neg.): 495.3482 ($[M-H]^-$, calc. for $C_{32}H_{47}O_4^-$, 495.3480).

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