

Aurovertin E, a New Polyene Pyrone from the Basidiomycete *Albatrellus confluens*

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Abstract A new polyene pyrone, aurovertin E (**2**), was isolated along with aurovertin B (**1**) from the culture mycelia of the basidiomycete *Albatrellus confluens*. Their structures were elucidated on the basis of spectroscopic studies including 2D NMR experiments. This is the first example of the occurrence of aurovertins in basidiomycetes.

Keywords aurovertin E, *Albatrellus confluens*, basidiomycete, polyene pyrone

Introduction

The aurovertins, metabolites from the fungus (anamorphic ascomycetes) *Calcarisporium arbuscula* Preuss [1–4], are a group of acute neurotoxic substances which act as potent inhibitors of ATP synthesis and ATP hydrolysis catalyzed by mitochondrial enzyme systems [5, 6]. It was also reported that aurovertin B is a bovine F_1 -ATPase inhibitor [7]. The sites at which aurovertin B binds to bovine F_1 -ATPase have been determined by X-ray analysis of crystals soaked in the inhibitor. Aurovertin B has been totally synthesized from D-glucose and its absolute configuration has been unambiguously determined [8, 9]. The biosynthetic origins of the aurovertins have also been reported [10, 11]. In a continuation of our investigation of the bioactive principles from the fungus (basidiomycetes) *Albatrellus confluens* (Alb. et Schw.: Fr.) Kolt. et Pouz. [12–15], a new polyene pyrone, aurovertin E (**2**), was isolated along with aurovertin B (**1**) from its culture

mycelia. We describe herein the isolation and structural elucidation of aurovertin E (**2**).

Experimental

General

Optical rotations were measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tensor 27 with KBr pellets. UV spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. NMR spectra were recorded on Bruker AV-400 and Bruker DRX-500 spectrometers in $CDCl_3$ solvent with TMS as an internal standard. EI-MS were recorded with a VG Autospec-3000 spectrometer. ESI-MS and HRESI-MS were recorded with an API QSTAR Pulsar 1 spectrometer.

Silica gel (200~300 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol.

Fermentation and Isolation

The mushroom *A. confluens* was collected at Ailao Mountain of Yunnan Province, 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 Kunming Institute of Botany, the Chinese Academy of Sciences.

The culture medium consisted of potato (peel off) 200 g,

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glucose 20 g, KH_2PO_4 3 g, MgSO_4 1.5 g, citric acid 0.1 g and thiamin hydrochloride 10 mg in 1 liter of deionized water. Reagent bottles were used as flask (size: 500 ml; volume of media: 300 ml). The pH was adjusted to 6.5 before autoclaving. Fermentation was carried out on a shaker at 22°C and 150 rpm for 10 days.

The 10-day old whole culture broth (12 liters) was filtered and then extracted twice with EtOAc. The organic layer was concentrated *in vacuo* to give an oily residue (1.1 g) that was applied on a silica gel column and eluted stepwise with CHCl_3 -MeOH solvent system. Fr. I (18 mg), eluted with CHCl_3 -MeOH (100:1, v/v), was further purified on a Sephadex LH-20 column, eluting with CHCl_3 -MeOH (1:1, v/v) to give **1** (2.3 mg) as a pale yellow syrup. Fr. II (24 mg) from CHCl_3 -MeOH (60:1, v/v) was repeatedly purified on a Sephadex LH-20 column, eluting with CHCl_3 -MeOH (1:1, v/v), to afford **2** (2.9 mg) as a pale yellow syrup.

Physico-chemical Properties

Aurovertin B (6-[(1*E*,3*E*,5*E*)-6-[8-acetyloxy-7-ethyl-4-hydroxy-1,5-dimethyl-2,6-dioxabicyclo[3.2.1]oct-3-yl]-1,3,5-hexatrienyl]-4-methoxy-5-methyl-2*H*-pyran-2-one, **1**). Pale yellow syrup, $[\alpha]_{\text{D}}^{16.4} -25.3$ (*c* 0.47, CHCl_3), Rf=0.76, CHCl_3 -MeOH, 12:1 (v/v). EI-MS *m/z* 460 (M, 2), 442 (M-H₂O, 5), 418 (M-CH₂CO, 2), 400 (M-H₂O-CH₂CO, 2), 341 (5), 325 (15), 302 (14), 247 (29), 219 (67), 109 (41).

Aurovertin E (6-[(1*E*,3*E*,5*E*)-6-[4,8-dihydroxy-7-ethyl-1,5-dimethyl-2,6-dioxabicyclo[3.2.1]oct-3-yl]-1,3,5-hexatrienyl]-4-methoxy-5-methyl-2*H*-pyran-2-one, **2**). Pale yellow syrup, $[\alpha]_{\text{D}}^{17.2} -14.1$ (*c* 0.59, CHCl_3), Rf=0.43, CHCl_3 -MeOH, 12:1 (v/v). UV $\lambda_{\text{max}}^{\text{CHCl}_3}$ nm (ϵ) 370 (34,400), 360 (sh), 277 (34,800), 270 (33,300). IR (KBr) 3432, 2956, 2919, 2850, 1682, 1623, 1539, 1462, 1406, 1378, 1251, 1092, 1037, 1002, 972 cm^{-1} . ESI-MS *m/z* 419 (M+1)⁺, 441 (M+Na)⁺, 859 (2M+Na)⁺. HRESI-MS *m/z* 419.2054 (M+1, Calcd. for C₂₃H₃₁O₇ 419.2069). EI-MS *m/z* 418 (M, 3), 400 (M-H₂O, 7), 341 (5), 325 (9), 302

Table 1 ¹H and ¹³C NMR data for **1** and **2** in CDCl₃.

Position	1		2	
1	1.10 (3H, t, 7.5)	11.8	1.08 (3H, t, 7.5)	11.9
2	1.70 (2H, m)	20.1	1.68 (2H, m)	20.2
3	3.93 (1H, dd, 8.8, 4.3)	85.5	3.97 (1H, dd, 8.7, 4.5)	84.8
4		82.7		83.6
5	4.81 (1H, s)	80.5	3.46 (1H, s)	80.4
6		83.4		84.1
7	3.29 (1H, d, 8.2)	76.3	3.17 (1H, d, 8.1)	76.3
8	4.13 (1H, dd, 8.2, 6.1)	78.0	4.13 (1H, dd, 8.1, 6.3)	77.9
9	5.93 (1H, dd, 14.2, 6.1)	134.0	5.91 (1H, dd, 14.2, 6.3)	134.7
10	^a	131.8	^b	131.6
11	^a	137.0	^b	137.2
12	^a	132.1	^b	131.9
13	7.19 (1H, dd, 15.0, 11.2)	135.6	7.18 (1H, dd, 15.0, 11.2)	135.7
14	6.36 (1H, d, 15.0)	119.6	6.35 (1H, d, 15.0)	119.4
15		154.2		154.3
16		108.1		108.1
17		170.6		170.6
18	5.50 (1H, s)	88.8	5.50 (1H, s)	88.8
19		163.8		163.8
20	1.19 (3H, s)	16.4	1.29 (3H, s)	16.4
21	1.27 (3H, s)	15.0	1.38 (3H, s)	14.6
22	1.97 (3H, s)	8.9	1.97 (3H, s)	8.9
23	3.83 (3H, s)	56.2	3.83 (3H, s)	56.2
COCH ₃	2.17 (3H, s)	20.8		
COCH ₃		169.9		

^{a,b} Complex signals δ 6.35~6.55.

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

(28), 259 (16), 247 (35), 219 (57), 218 (80), 139 (100).

Results and Discussion

Compound **2** was obtained as yellow syrup. The molecular formula of **2** was determined to be $C_{23}H_{30}O_7$ on the basis of HRESI-MS m/z 419.2054 ($M+1$, calcd. for $C_{23}H_{31}O_7$ 419.2069) and ^{13}C NMR spectrum (DEPT: five methyls, one methylene, eleven methines and six quaternary carbons). The IR spectra of **2** showed absorptions at 1682, 1623 and 1539 cm^{-1} consistent with the presence of a 4-oxy- α -pyrone unit and at $3432\text{ (br, OH)}\text{ cm}^{-1}$. The 1H and ^{13}C NMR spectra (Table 1) of **2** were similar to those of aurovertin B (**1**) [3, 11]. The distinct differences between compound **2** and aurovertin B (**1**) are that: (a) the acetyl signals of aurovertin B [δ_H 2.17 (3H, s), δ_C 20.8 (q, Me) and 169.9 (s, C=O)] are absent in **2**; (b) a characteristic downfield singlet [δ_H 4.81 (1H, s)] caused by esterification is markedly shifted upfield [δ_H 3.46 (1H, s)] in **2**. In the light of the evidences mentioned above and the key HMBC correlations (Fig. 2), the structure of **2** was therefore elucidated as shown in Fig. 1, named aurovertin E. Because the NMR spectral data and other physico-chemical properties of **2** and those of **1** in literature are very similar, the absolute configuration and geometrical isomerism of both compounds are suggested to be the same.

The molecule consists of a substituted pyrone ring linked

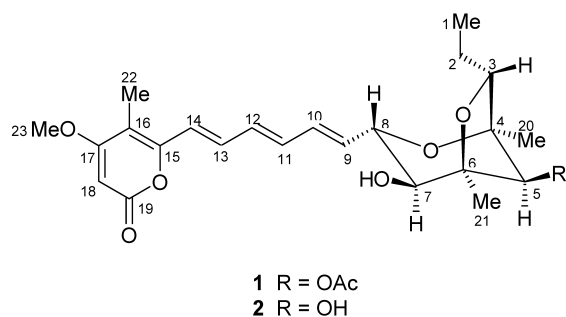


Fig. 1 Structures of **1** and **2**.

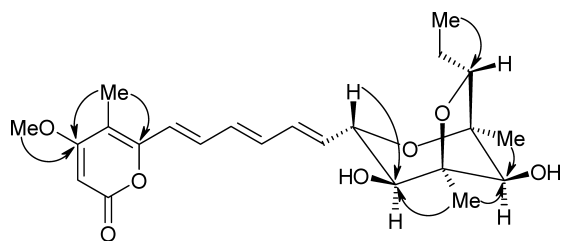


Fig. 2 Key HMBC correlations of **2**.

by a rigid spacer containing conjugated double bonds to a substituted dioxabicyclo[3.2.1]octane. It is important to note that this is the first isolation of the aurovertins from basidiomycetes.

Aurovertin E (**2**) has been mentioned as a product of the reaction from aurovertin B (**1**) by alkaline hydrolysis [3]. It is of interest if aurovertin E that is the deacetylated derivative of **1** has a F_1 -ATPase inhibiting activity as **1**.

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