STRUCTURES OF RISHIRILIDES A AND B, α_2-MACROGLOBULIN INHIBITORS PRODUCED BY *STREPTOMYCES RISHIRIENSIS* OFR-1056

Sir:

Plasmin plays an important role in the fibrinolytic enzyme system and is known to degrade the insoluble fibrin[1]. However, the activity of plasmin is reduced by the presence of plasmin inhibitors such as α_2-macroglobulin and α_2-plasmin inhibitor, hence little effect on fibrinolytic thrombosis can be expected[2]. Considering the suppressing control in the fibrinolytic enzyme system, specific inhibitors of the plasmin inhibitors may be useful in prevention and treatment of thrombosis caused by fibrinolytic accentuation. In the course of our screening for α_2-macroglobulin inhibitors[3], rishirilides A and B were isolated from the culture broth of a new *Streptomyces* strain designated as *Streptomyces rishiriensis* OFR-1056. They have been shown to have highly-oxygenated anthracene skeleton and to inhibit plasma α_2-macroglobulin.

Rishirilides A and B were produced in a 30-liter jar-fermenter culture of *S. rishiriensis* OFR-1056 maintained at 28°C for 4 days in a medium (20 liters) composed of 3% soluble starch, 0.5% glucose, 0.05% Polypepton, 0.5% yeast extract, 0.05% $MgSO_4 \cdot 7H_2O$, 0.02% KH_2PO_4, 0.05% Na_2HPO_4, 0.0001% $FeSO_4 \cdot 7H_2O$, 0.0001% $MnCl_2 \cdot 4H_2O$ and 0.0001% $ZnSO_4 \cdot 7H_2O$ (pH 7.0). The 1-butanol extract (40 g) of the culture filtrate (18 liters) was chromatographed on silica gel. The active fractions were concentrated to yield crude rishirilide. Further purification using a combination of Sephadex LH-20 chromatography and HPLC led to the isolation of rishirilide A (110 mg) as colorless prisms and rishirilide B (200 mg) as a yellow powder. Rishirilide A (**1**): mp 122~124°C; $[\alpha]_D^{22}$ $-411°$ (*c* 1.04, EtOH); UV λ_{max}^{EtOH} 207 (ε 19,500), 223 (13,800) and 319 (16,800) nm; IR ν_{max}^{KBr} 3510 (hydroxyl), 1740 (ester), 1680 (conjugated ketone), 1610 (double bond) and 1575 cm^{-1} (aromatic). The ^1H NMR and ^{13}C NMR spectra of **1** are summarized in Tables 1 and 2. The ^1H NMR spectrum of **1** indicated the presence of a 1,2,3-substituted benzene ring [δ 7.06 (dd, J=7.9, 1.4 Hz), 7.26 (dd, J=7.9, 8.0 Hz) and 7.08 (ddd, J=8.0, 1.4, 0.4 Hz)], one proton of which was found to have a long-range coupling (0.4 Hz) with the proton at δ 7.56. Moreover, the following groups are present in **1**: An α,β-un-

Table 1. ^1H NMR spectral data (400 MHz) of rishirilides A (**1**) and B (**2**).

	1*	**2****
H-2	2.84 (q, 7.6)	3.06 (m)
H-6	7.06 (dd, 7.9, 1.4)	6.89 (d, 7.4)
H-7	7.26 (dd, 7.9, 8.0)	7.24 (dd, 7.4, 7.4)
H-8	7.08 (ddd, 8.0, 1.4, 0.4)	7.41 (d, 7.4)
H-9	7.56 (d, 0.4)	8.41 (s)
H-10	5.61 (s)	8.37 (s)
H-11	1.60~1.75 (m)	1.45~1.73 (m)
H-12	2.61 (m)	2.34 (m)
H-13	1.44 (m)	1.38 (m)
H-14	0.85 (d, 6.4)	0.70 (d, 6.6)
H-15	0.86 (d, 6.4)	0.81 (d, 6.6)
H-17	1.22 (d, 7.6)	1.31 (br d)
OH	3.07, 3.85	—

* In $(CD_3)_2CO$ with TMS as internal standard. The multiplicities and J values (in Hz) are in parentheses.

** In CD_3OD with TMS as internal standard.

Table 2. ^{13}C NMR spectral data (100.61 MHz) of rishirilides A (**1**), B (**2**) and compound **3**.

Carbon	**1**[a]	**2**[b]	**3**[c]
1	197.9 s	200.0 s	197.1 s
2	49.8 d	33.1 d	48.7 d
3	81.3 s	79.0 s	78.1 s
4	84.7 s	86.0 s	84.8 s
5	156.1 s	155.0 s	155.8 s
6	120.6 d	121.8 d	119.4 d
7	130.6 d	128.0 d	128.0 d
8	123.5 d	121.8 d	122.1 d
9	138.1 d	128.3 d	126.8 d
10	65.8 d	111.8 d	106.6 d
4a	81.0 s	140.0 s	138.9 s
10a	130.8 s	131.5 s	130.0 s
8a	130.9 s	134.7 s	133.2 s
9a	122.8 s	128.9 s	128.0 s
11	32.1 t	37.4 t	36.5 t
12	—*	30.7 t	31.5 t
13	—*	29.9 d	28.5 d
14	22.5 q	22.9 q	22.6 q
15	22.6 q	23.1 q	22.7 q
16	175.7 s	176.0 s	174.0 s
17	12.1 q	10.0 q	10.3 q
$COOCH_3$			53.0 q
OCH_3			55.8 q

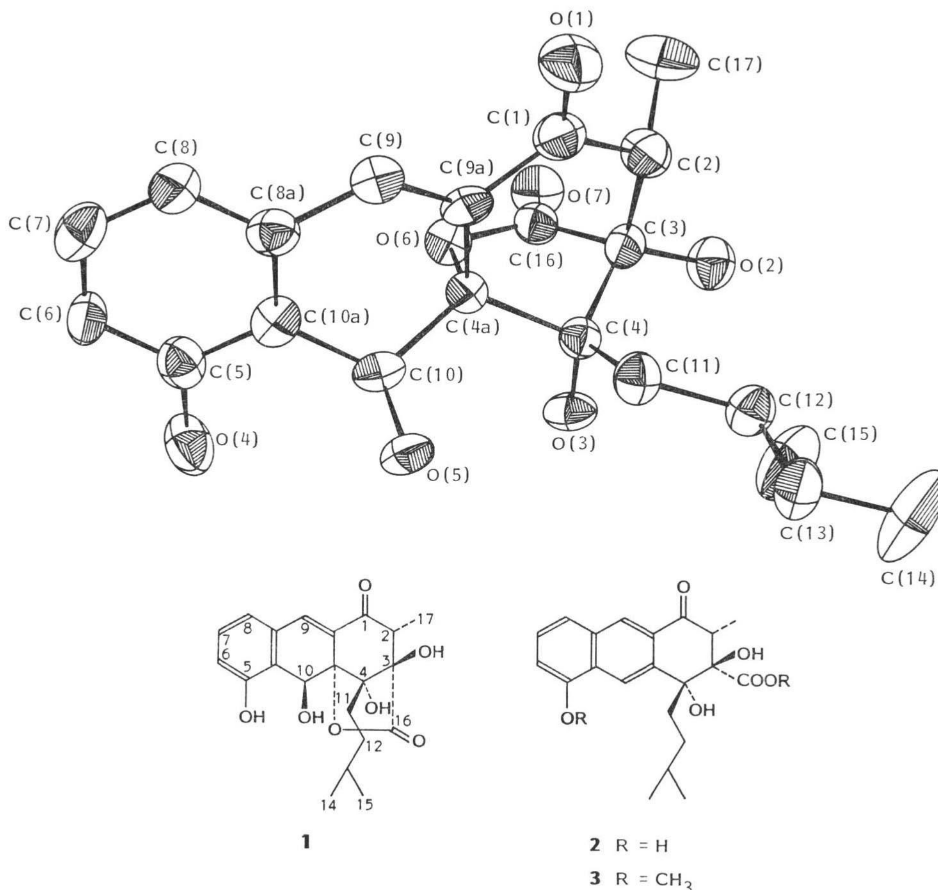
[a] In $(CD_3)_2CO$. [b] In DMSO-d_6. [c] In $CDCl_3$.

* Obscured by solvent peaks.

saturated ketone (1680 cm^{-1}, δ 197.9), an ester (1740 cm^{-1}, δ 175.7), an isopentyl [δ 0.85 (3H, d, J=6.4 Hz), 0.86 (3H, d, J=6.4 Hz), 1.44 (1H, m), 1.6~1.75 (3H, m), 2.61 (1H, m)] and a secondary methyl [δ 1.22 (3H, d, J=7.6 Hz), 2.84 (1H, q, J=7.6 Hz)] group. These spectral data revealed that **1** has a highly-oxygenated anthrancene skeleton. In order to establish the position and stereochemistry of the functional groups in **1**, an X-ray analysis was carried out. The crystal and intensity data were derived from measurements on a Syntex R3 four-circle diffractometer with graphite-monochromated MoKα radiation. Crystal data: $C_{21}H_{24}O_7 \cdot H_2O$, orthorhombic, space group $P2_12_12_1$, a=9.578(3), b=11.573(5), c=17.991(11) Å, Dx=1.35 g·cm^{-3}, Z=4, and μ(MoKα)=1.1 cm^{-1}. A total of 1,372 reflections [I>1.96σ(I)] were considered to be observed. The structure was solved by direct methods using MULTAN[4] on a Syntex XTL program. The refinement of atomic parameters was carried out by a block-diagonal least squares method. The final R-value was 0.042. The final atomic parameters have been deposited with the Crystallographic Data Center. Fig. 1 shows a stereoscopic drawing of the relative configuration of molecule **1**.

Rishirilide B (**2**): mp >280°C; $[\alpha]_D^{22}$ +12.8° (c 0.448, EtOH); UV λ_{max}^{EtOH} 218 (ε 22,300), 264 (25,300), 305 (4,400) and 370 (2,500) nm; IR ν_{max}^{KBr} 3400 (hydroxyl), 1675 (conjugated ketone), 1625 (double bond), 1600 and 1575 (aromatic) cm^{-1}. Treatment of **2** with diazomethane yielded compound **3** (M^+ m/z 400.1881 for $C_{23}H_{28}O_6$, ν_{max}^{KBr} 1725 cm^{-1}), which indicated the presence of both carboxylic acid and phenolic hydroxyl groups. The ^{1}H NMR spectrum of **2** was found to be similar to that of **1** except for the signals due to H-9 and H-10. These occurred at lower field in **2** [δ 8.37 and 8.41 (each

Fig. 1. A perspective view of rishirilide A.



s)] than in **1** [δ 5.61 (s, H-10), 7.56 (d, H-9)]. Since **2** also lacked the IR absorption (1740 cm^{-1}) due to a lactone carbonyl, this spectral evidence and chemical transformation suggested that a $\Delta^{4a,10}$ double bond had been produced by a reductive opening of the lactone group in **1**. This assumption was substantiated by the observation of olefinic carbons [δ 106.6 (d) and 138.9 (s)] which were not present in **1**. Thus, structure **2** was proposed for rishirilide B. It is noted that the absolute configurations of **1** and **2** were not determined at the present paper. The inhibitory activity of rishirilide B (**2**) on α_2-macroglobulin is IC_{50} 35 μg/ml, whereas rishirilide A (**1**) exhibits weak activity (100 μg/ml).

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