Pergamon

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BICOUMARINS FROM *PLEUROSPERMUM RIVULORUM*

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Key Word Index—*Pleurospermum rivulorum*; Umbelliferae; underground parts; bicoumarin; rivulobirins A and B.

Abstract—Two new bicoumarins, rivulobirins A and B, were isolated from the underground part of *Pleurospermum rivulorum* together with 11 known coumarins. Their structures were established by spectral evidence. © 1997 Elsevier Science Ltd. All rights reserved

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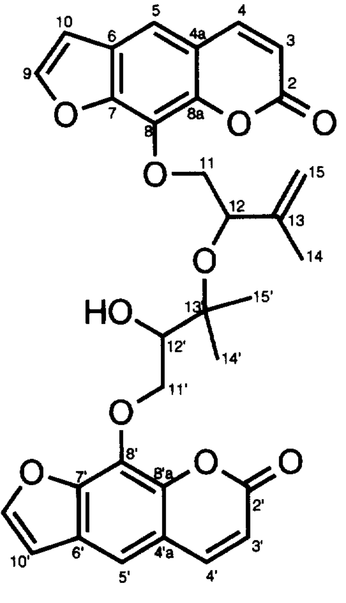
INTRODUCTION

Pleurospermum rivulorum 'Yunnan Qiang Huo' is a Chinese folk medicine used as an antipyretic, analgesic and diaphoretic agent in local areas of Yunnan province, China. It has been proved by pharmacological experiments that the water extract possesses an anti-arrhythmic effect [1]. In the course of our studies on the phenolic components of Umbelliferous plants, we investigated the constituents of the underground part of this plant and isolated two new bicoumarins, rivulobirins A and B (**1** and **2**) together with 11 known coumarins, 8-geranyloxy psoralen (**3**), imperatorin (**4**), isopimpinellin (**5**), pabularinone (**6**), bergapten (**7**), xanthotoxin (**8**), isogosferol (**9**), marmesin (**10**), heraclenol (**11**), xanthotoxol (**12**) and 8-(3-chloro-2-hydroxy-3-methylbutyloxy)-psoralen (**13**). This paper is concerned with structure elucidation of compounds **1** and **2**.

RESULTS AND DISCUSSION

The ethyl acetate extract of the underground part of this plant yielded the two new bicoumarins (**1** and **2**) after chromatographic purification.

Compounds **1** and **2** fluoresced yellowish green under UV light (265 nm) and had UV spectral characteristics of a linear type of furanocoumarin. The IR absorption of **1** and **2** indicated the presence of an aromatic ring and an α,β-unsaturated lactone. Compound **1** was isolated as a colourless amorphous powder and assigned the molecular formula $C_{32}H_{28}O_{10}$ by HR mass spectrometry (m/z 572.1668 $[M]^+$). The NMR spectra (Table 1) showed the presence of two C-8 substituted linear furanocoumarin fragments with signals at δ 7.93 (1H, *d*, $J = 9.6$ Hz), 6.27 (1H, *d*, $J = 9.6$ Hz), 7.38 (1H, *s*), 6.91 (1H, *d*, $J = 2.4$ Hz), 7.93 (1H, *d*, $J = 2.4$ Hz) and 7.94 (1H, *d*, $J = 9.6$ Hz), 6.27 (1H, *d*, $J = 9.6$ Hz), 7.43 (1H, *s*), 6.90 (1H, *d*, $J = 2.4$ Hz), 7.96 (1H, *d*, $J = 2.4$ Hz). The signals assignable to a 3-methyl-3-butenyl-1,2-dioxy group were observed at δ 4.53 (1H, *m*), 4.22 (1H, *m*), 4.18 (1H, *m*), 5.11 (1H, *s*), 4.91 (1H, *s*) and 1.75 (3H, *s*) and signals due to a 2-hydroxy-3-methylbutyl-1,3-dioxy group were seen at δ 3.78 (1H, *m*), 5.20 (1H, *d*, $J = 6.3$ Hz), 4.37 (1H, *dd*, $J = 9.9$, 8.4 Hz), 4.73



Rivulobirin A (1)

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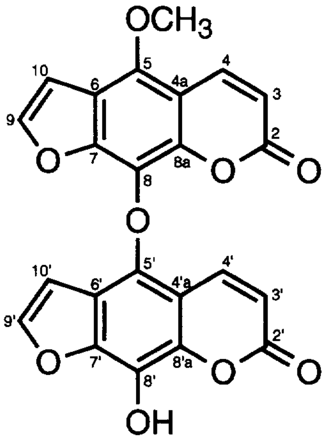

Rivulobirin B (2)

Table 1. ^1H NMR spectral data for compound **1** in DMSO-d_6

H	**1**	H	**1**
3	6.27 *d* (9.6)	3′	6.27 *d* (9.6)
4	7.93 *d* (9.6)	4′	7.94 *d* (9.6)
5	7.38 *s*	5′	7.43 *s*
9	7.93 *d* (2.4)	9′	7.96 *d* (2.4)
10	6.91 *d* (2.4)	10′	6.90 *d* (2.4)
11	4.18 *m*	11′	4.37 *dd* (9.9, 8.4)
	4.22 *m*		4.73 *dd* (9.9, 1.8)
12	4.53 *m*	12′	3.78 *ddd* (8.4, 6.3, 1.8)
14	1.75 *s*	14′	1.11 *s*
15	4.91 *s*	15′	1.22 *s*
	5.11 *s*	–OH	5.20 *d* (6.3)

Table 2. ^{13}C NMR spectral data for compounds **1**, **9** and **11** in DMSO-d_6

C	**1**		**9**	**11**
2, (2′)	159.6	159.8	159.8	159.9
3, (3′)	114.0	114.1	114.2	114.2
4, (4′)	145.1	145.1	145.1	145.3
4a, (4′a)	116.2	116.3	116.4	116.4
5, (5′)	113.2	113.8	113.9	113.6
6, (6′)	125.7	125.7	125.8	125.8
7, (7′)	146.9	146.9	147.1	147.1
8, (8′)	131.0	131.7	131.1	131.6
8a, (8′a)	142.2	142.5	142.6	142.6
9, (9′)	147.5	147.6	147.8	147.8
10, (10′)	106.9	106.9	107.1	107.0
11, (11′)	75.2	75.4	75.9	76.7
12, (12′)	74.7	75.0	73.1	75.5
13, (13′)	145.1	78.1	145.3	70.9
14, (14′)	18.7	21.5	18.5	27.3
15, (15′)	113.0	23.3	112.1	24.4

Table 3. Long range ^{13}C–^1H COSY spectral data for compound **1**

C	Correlated H	C	Correlated H
2	3	2′	3′
4	5	4′	5′
4a	3	4′a	3′
6	10	6′	10′
7	5, 10	7′	5′, 10′
8	11	8′	11′
8a	5	8′a	5′
10	9	10′	9′
12	14, 15	12′	14′, 15′
14	15	13′	14′, 15′
15	14	14′	15′
		15′	14′

Table 4. NMR spectral data for compound **2** in $CDCl_3$

	C	H		C	H
2	159.6		2′	158.7	
3	112.4	6.38 *d* (9.9)	3′	112.1	6.30 *d* (9.8)
4	138.7	8.13 *d* (9.9)	4′	138.8	8.46 *d* (9.8)
4a	106.8		4′a	106.3	
5	145.3		5′	137.5	
6	113.5		6′	114.0	
7	148.8		7′	146.1	
8	123.4		8′	126.3	
8a	142.9		8′a	138.7	
9	145.1	7.52 *d* (2.3)	9′	144.9	7.37 *d* (2.3)
10	104.7	7.07 *d* (2.3)	10′	102.5	5.78 *d* (2.3)
–OCH_3	59.8	4.28 *s*			

(1H, *dd*, $J = 9.9, 1.8$ Hz), 1.11 (3H, *s*) and 1.22 (3H, *s*). The functional groups were also identified by ^{13}C NMR spectroscopy (Table 2). From the spectral data, compound **1**, was assumed to be tert-O-isogosferyl heraclenol. This presumption was confirmed by comparison of the ^{13}C NMR spectrum of **1** with those of **9** and **11** (Table 2) and by analysis of long range ^{13}C–^1H COSY spectra (Table 3) and HR mass spectrometry of **1**.

Compound **2** was isolated as a pale yellow crystalline powder and assigned the molecular formula $C_{23}H_{12}O_9$ by HR mass spectrometry (*m*/*z* 432.0489 $[M]^+$). The ^1H NMR spectrum of **2** (Table 4) exhibited signals arising from two 5,8-dioxyfurocoumarin units at δ 8.13 (1H, *d*, $J = 9.9$ Hz), 6.38 (1H, *d*, $J = 9.9$ Hz), 7.52 (1H, *d*, $J = 2.3$ Hz), 7.07 (1H, *d*, $J = 2.3$ Hz) and 8.46 (1H, *d*, $J = 9.8$ Hz), 6.30 (1H, *d*, $J = 9.9$ Hz), 7.37 (1H, *d*, $J = 2.3$ Hz), 5.78 (1H, *d*, $J = 2.3$ Hz). A methoxy group was repeated by the signal at δ 4.28 (3H, *s*). These spectral data suggested that **2** might be a furanocoumarin dimer linked through an oxygen atom and have a methoxy group and an additional hydroxyl group. The HMBC spectrum of **2** (Table 5) showed that the methoxy signal at δ 4.28 was correlated to the signal assignable to C-5 (δ 145.3). Therefore, the methoxy group must be linked at C-5 and so the C-8 of this coumarin ring should be linked through

Table 5. HMBC data for compound **2**

C	Correlated H
2	3
4a	3
5	4, $-OCH_3$
6	9, 10
7	9, 10
8	4
8a	4
2′	3′
4′a	3′
5′	4′
6′	9′, 10′
7′	9′, 10′
8′	4′

an oxygen atom at either C-5 or C-8 of the other coumarin ring. In the NMR spectrum of **2**, the signals due to the two furanocoumarin moieties were shifted towards each other unlike those of **1**. In particular, the β-proton signal of one furan ring was shifted to a higher field (δ 5.78) by shielding of the other furanocoumarin ring. From the above spectral data, the structure of **2** was established as shown. This structure was further supported by analyses of the HMBC spectrum and the HR mass spectrometry of **2**.

EXPERIMENTAL

General. Mps: uncorr. EIMS: 70 eV. ^1H and ^{13}C NMR: 300 and 75.4 MHz with TMS as int. standard. CC: Qing Dao silica gel (100–200 mesh) and Merck RP-18. TLC: Qing Dao GF_{254} plate (0.25 mm). Spots and bands were detected by UV irradiation (253.6 and 365 nm).

Plant material. Underground parts of *Pleurospermum rivulorum* were collected in October 1993 at Ynnan Province, China and identified by Professor Hu-Si Ling (Institute of Chinese Materia Medica, Academy of Traditional Chinese Medicine, Beijing). A voucher specimen is deposited in the Herbarium of this institute.

Extraction and isolation. Air dried underground parts (10 kg) were chopped into small pieces and extracted with 95% EtOH at room temp. and concd *in vacuo*. The extract (1.2 kg) was treated with petrol and EtOAc. The EtOAc soluble part was concd *in vacuo* to give a residue (500 g), which was subjected to CC on silica gel eluted successively with petrol–EtOAc solvent mixts of increasing polarity. The frs with similar fluorescent spots on TLC were combined. Rechromatography on silica gel and ODS and purification by PTLC afforded **1** (1.2 g), **2** (10 mg), **3** (450 mg), **4** (2.4 g), **5** (560 mg), **6** (6.8 g), **7** (250 mg), **8** (12 mg), **9** (1.2 g), **10** (25 mg), **11** (1.5 mg), **12** (10 mg) and **13** (24 mg). The 11 compounds (**3**–**13**) were identified by direct comparison with authentic sample.

Rivulobirin A (**1**). Colourless amorphous powder, mp 147–149°. Optically inactive. HR-MS *m/z* 572.1668 $[M]^+$ (calcd for $C_{32}H_{28}O_{10}$, 572.5632), 371.1503 ($C_{21}H_{23}O_6$, 371.4067), 287.0915 ($C_{16}H_{15}O_5$, 287.2895), 269.0810 ($C_{16}H_{13}O_4$, 269.2747), 201.1073 ($C_{11}H_5O_4$, 201.1565). UV λ_{max}^{EtOH} nm (log ε): 300.0 (4.44), 262.0 sh (4.54), 248.0 (4.74), 244.5 (4.74). IR ν_{max}^{KBr} cm^{-1}: 3441, 1724, 1710, 1694, 1651, 1624, 1587, 1548. ^1H and ^{13}C NMR in Table 1 and 2.

Rivulobirin B (**2**). Pale yellow crystalline powder, mp 277–278°. HR-MS *m/z* 432.0489 $[M]^+$ (calcd for $C_{23}H_{12}O_9$, 432.0480), 232.0361 ($C_{12}H_8O_5$, 232.0371), 217.0134 ($C_{11}H_5O_5$, 271.0137), 189.0190 ($C_{10}H_5O_4$, 189.0187). UV λ_{max}^{EtOH} nm (log ε): 271.0 (4.22), 311.0 (4.03). IR ν_{max}^{KBr} cm^{-1}: 3451, 1738, 1725, 1597, 1474. ^1H and ^{13}C NMR in Table 3.

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