Pergamon

0031-9422(95)00045-3

Phytochemistry, Vol. 39, No. 5, pp. 1099–1101, 1995
Elsevier Science Ltd
Printed in Great Britain
0031-9422/95 $9.50 + 0.00

ANGELOL-TYPE COUMARINS FROM *ANGELICA PUBESCENCE F. BISERRATA* AND THEIR INHIBITORY EFFECT ON PLATELET AGGREGATION

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(*Received* 17 *October* 1994)

Key Word Index—*Angelica pubescence f. biserrata*; Umbelliferae; roots; angelol-type coumarins; angelol K and L; anti-platelet aggregation; ^{13}C NMR.

Abstract—An ethyl acetate-soluble root extract of *Angelica pubescence f. biserrata* afforded two new angelol-type coumarins, angelols K and L, in addition to angelols B, C, D and G. Some of the compounds were tested on human platelet aggregation and showed significant activity.

INTRODUCTION

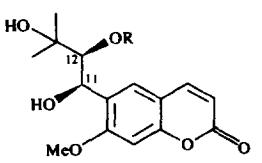
'Duhuo', the roots of *Angelica pubescence f. biserrata*, is a frequently used traditional Chinese drug for rheumatic disease and was reported to be effective on anti-platelet aggregation [1–4]. In a previous paper, we reported the isolation and identification of 11 known coumarins and four other compounds from this species [5]. Furthermore, also from the same roots, we isolated and identified five angelol-type coumarins, in addition to angelol B (**1**). Among these, angelol K (**5**) and angelol L (**6**) were new compounds and angelol C (**2**), angelol D (**3**) and angelol G (**4**) were reported for the first time from this species [6]. Tests of angelols B, D, G and K on human platelet aggregation induced by 2 μM ADP, showed significant inhibitory activity in all cases (angelol B: IC_{50} = 0.58 mM; angelol D: IC_{50} = 0.30 mM; angelol G: IC_{50} = 0.20 mM; angelol K: IC_{50} = 1.78 mM; aspirin: IC_{50} = 1.23 mM).

RESULTS AND DISCUSSION

The ethyl acetate-soluble fraction of an 80% ethanol extract of *A. pubescence f. biserrata* roots was separated by silica gel, Lobar column and HPLC to afford angelols B, C, D and G [6], and two new coumarins, **5** and **6**. All of them showed blue-violet fluorescence under 365 nm ultraviolet light.

Compound **4** was obtained as a viscid oil. Its structure was deduced from its ion peak at m/z 379 $[M + 3H]^+$ in the FAB-mass spectrum, ^{1}H NMR and ^{1}H–^{1}H COSY spectra (Table 1). The suggestion that the angeloyl group was esterified with the 11-OH was confirmed by a ^{1}H–^{1}H COSY spectrum, in which the signal at δ3.91 (*t*, J = 6.0 Hz, H-12) was not only related with that at δ6.33 (*d*, J = 6.0 Hz, H-11), but also with a hydroxyl group at δ2.56 (*d*, J = 6.0 Hz). The signals in the ^{13}C NMR spectrum were assigned according to the ^{1}H–^{13}C COSY spectrum (Table 2). Thus, some mistakes about the carbon assignments of this compound in the literature [6] were corrected. Additional wrong assignment of angelols B–D are also revised in Table 2.

Compound **5** was isolated as prisms. The EI-mass spectrum indicated a M_r of 376. The IR spectrum indicated absorption due to hydroxyl (3450 cm^{-1}) and a coumarin ring (1730, 1610 and 1560 cm^{-1}). The ^{1}H NMR spectrum showed an angelol-type coumarin pattern very similar to that of angelol G in which the



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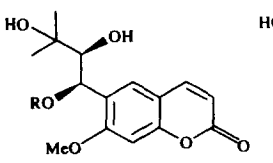
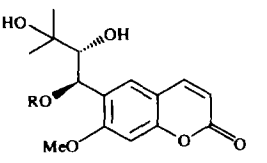
2 R=methybutyryl angelol C
7 R=isovalery angelol I



3 R=tigloyl angelol D
5 R=angeloyl angelol K
6 R=isovalery angelol L



4 R=angeloyl angelol G

Table 1. ^{1}H NMR data of angelol-type coumarins (in $CDCl_3$)

	B	C	D	K	L	G
H-3	6.21 (*d*, 9.5)	6.24 (*d*, 9.5)	6.25 (*d*, 9.5)	6.26 (*d*, 9.5)	6.21 (*d*, 9.5)	6.33 (*d*, 9.5)
H-4	7.55 (*d*, 9.5)	7.64 (*d*, 9.5)	7.64 (*d*, 9.5)	7.64 (*d*, 9.5)	7.62 (*d*, 9.5)	7.63 (*d*, 9.5)
H-5	7.55 (*s*)	7.63 (*s*)	7.38 (*s*)	7.39 (*s*)	7.43 (*s*)	7.54 (*s*)
H-8	6.72 (*s*)	6.75 (*s*)	6.82 (*s*)	6.83 (*s*)	6.80 (*s*)	6.82 (*s*)
H-11	5.62 (*br s*)	5.65 (*br s*)	6.41 (*d*, 1.2)	6.45 (*br s*)	6.36 (*br s*)	6.33 (*d*, 6.0)
H-12	5.08 (*br s*)	5.12 (*br s*)	3.62 (*d*, 1.2)	3.61 (*br s*)	3.58 (*br s*)	3.91 (*t*, 6.0)
Me	1.55 (*s*)	1.56 (*s*)	1.34 (*s*)	1.35 (*s*)	1.31 (*s*)	1.29 (*s*)
Me	1.23 (*s*)	1.21 (*s*)	1.31 (*s*)	1.31 (*s*)	1.29 (*s*)	1.27 (*s*)
OMe	3.90 (*s*)	3.92 (*s*)	3.94 (*s*)	3.94 (*s*)	3.93 (*s*)	3.97 (*s*)
Acyl	6.73 (1H, *br q*)	2.24 (1H, *m*)	7.01 (1H, *br q*)	6.23 (1H, *m*)	2.31 (2H, *d*, 6.5)	6.13 (1H, *br q*)
group	1.78 (3H, *m*)	1.30 (3H, *m*)	1.89 (3H, *s*)	2.03 (3H, *d*, 7.0)	2.04 (1H, *m*)	1.99 (3H, *d*, 7.0)
	1.69 (3H, *s*)	1.02 (3/2H, *d*, 7.0)	1.86 (3H, *d*, 7.0)	2.02 (3H, *s*)	0.95 (3H, *d*, 6.5)	1.96 (3H, *s*)
		0.77 (3/2H, *d*, 7.0)			0.93 (3H, *d*, 6.5)	
		0.72 (3/2H, *t*, 7.5)				
		0.45 (3/2H, *t*, 7.5)				

Table 2. ^{13}C NMR data for angelol-type coumarins (in $CDCl_3$)

C	B	C	D	K	L	G
2	161.4	161.7	161.1	161.0	161.5	161.0
3	112.8	113.0	113.3	112.1	113.6	113.4
4	143.7	143.6	143.6	143.6	143.7	143.5
5	126.8	126.3	126.2	125.3	126.4	128.5
6	127.9	126.4	128.3	126.3	126.3	127.1
7	159.1	159.0	158.7	158.8	158.9	160.1
8	98.4	98.5	99.0	99.0	98.4	99.2
9	111.8	111.8	112.1	113.4	111.9	112.2
10	155.1	155.3	155.4	155.4	155.0	155.5
11	67.4	67.6	68.8	68.4	68.1	69.6
12	76.2	75.5	77.2	77.7	77.5	78.7
13	74.4	74.6	73.0	72.9	72.8	72.6
Me	27.6	26.5	26.7	26.7	26.4	27.0
Me	26.7	26.5	25.6	25.5	25.1	24.6
OMe	56.1	56.1	56.2	56.2	56.1	56.3
1′	166.9	171.6	166.3	166.1	171.9	166.3
2′	126.8	41.1	125.1	125.1	42.6	124.1
3′	137.5	25.3	139.0	140.8	26.4	139.7
4′	14.2	22.1	14.6	15.9	22.2	15.8
5′	11.9	27.3	12.2	20.7	22.0	20.6

position of two methine protons (δ6.45 and 3.61) and two methyl groups (δ1.35 and 1.31) indicated that the angeloyl group was linked to C-11 [7]. The major difference in comparison with angelol G was the two broad singlets of H-11 and H-12, instead of two doublets (J = 6.0 Hz); this meant compound **5** was the stereoisomer of angelol G. According to the literature [6], the configuration of C-11 in angelol-type coumarins is determined from their ORD curve. If the configuration of C-11 was *R*, the ORD spectrum of the coumarin should have a negative plane curve. Conversely, if it was *S*, the ORD should present a positive curve. Because compound **5** showed a negative plane curve, analogous to that of angelol G, its C-11 should have the *R*-configuration. Thus, the difference of configuration between them was attributed to C-12, that is to say, the configuration of C-12 in compound **5** should be *S*, rather than *R* as in angelol G. Based on the above evidence, compound **5** was identified as 6 [(1*R*,2*S*)-1-angeloyloxy-2,3-dihydroxy-3-methylbutyl]-7-methoxycoumarin, named angelol K.

Compound **6** was obtained as a viscid oil. The EI-mass spectrum indicated a M_r of 378. The IR spectrum exhibited typical absorptions for angelol-type coumarins and the ORD spectrum showed a negative plane curve. The ^{1}H NMR spectrum displayed signals of H-11 and H-12 at δ6.36 (*br s*) and 3.58 (*br s*), and *gem*-dimethyl protons at δ1.31 and 1.29 similar to that of angelol K. The difference from angelol K was due to signals of an isovaleryl group at δ2.31 (2H, *d*, J = 6.5 Hz, H-2′), 2.04 (1H, *m*, H-3′), 0.95 and 0.93 (each 3H, *d*, J = 6.5 Hz, 2 Me), to which related signals of carbon corresponded at δ42.6, 26.4, 22.2 and 22.0 in the ^{1}H–^{13}C COSY spectrum. Therefore, compound **6** was deduced to be 6 [(1*R*,2*S*)-1-isovaleryloxy-2,3-dihydroxy-3-methylbutyl]-7-methoxycoumarin, named angelol L.

It was reported that the acyl group in angelol-type coumarins could migrate partially between 11-OH and 12-OH in the presence of acid. In the course of determination of the structure of angelol L, we found that, at room temperature, this compound was partly isomerized to angelol I (**7**). This was confirmed by comparing the ^{1}H NMR spectrum of compound **6** measured just after isolation with that measured 1 month later.

EXPERIMENTAL

General. Mps are uncorr. NMR spectra were recorded at 250 MHz for ^{1}H and 62.5 MHz for ^{13}C. Samples were measured in $CDCl_3$ with TMS as int. standard. Absorp-

tion CC was performed on silica gel (200–300 mesh). HPLC was performed on a LC-10 equipped with a shim-pack PREPSIL, solvent: $CHCl_3$–MeOH (50:1).

Plant material. Roots of *A. pubescence* Maxim *f. biserrata* Shan et Yuan were purchased from Yicahng Crude Station and identified by Professor Chun-quan Xu of our university.

Extraction and fractionation. Dried and crushed roots (35 kg) were extracted with 80% EtOH (80 l) by refluxing × 3 for 2 hr. The extract was concd *in vacuo* to 30 l and then extracted with $CHCl_3$, EtOAc and *n*-butanol, separately. The EtOAc extract (80 g) was chromatographed on silica gel column with benzene–EtOAc (3:1) as eluent to provide 8 frs. Fr. 2 (8.3 g) was rechromatographed on silica gel with the above eluent to give **1** (7.3 g) and fr. 2.2. Fr. 2.2 was subdivided by Lobar chromatography on silica gel with cyclohexane–EtOAc (1:2) to afford a mixt. A, **3** (22 mg) and **5** (16 mg). Mixt. A was further sepd by HPLC to give **2** (21 mg) and **6** (18 mg). Fr. 3 (5.7 g) was subjected to Lobar column on silica gel with cyclohexane–EtOAc (1:2) to yield **4** (26 mg).

Angelol B (**1**). Mp 152–154°. ORD, UV, IR and NMR identical with those reported previously [2].

Angelol C (**2**). Mp 112–113°. ORD (EtOH; *c* 0.5). $[\alpha]_D$ (nm): − 123.5 (589), − 224.1 (500), − 564.7 (400). IR ν_{max}^{KBr} cm^{-1}: 3415, 2970, 1730, 1619, 1562, 1460, 1381, 1222, 1206, 1184, 1128. ^1H and ^{13}C NMR: Tables 1 and 2.

Angelol D (**3**). Viscid oil. ORD (EtOH; *c* 0.5). $[\alpha]_D$ (nm): − 0.2 (589), − 0.4 (500), − 19.5 (400). IR ν_{max}^{KBr} cm^{-1}: 3600, 1725, 1622, 1570, 1500, 1460, 1380. ^1H and ^{13}C NMR: Tables 1 and 2.

Angelol G (**4**). Viscid oil. ORD (EtOH; *c* 0.5). $[\alpha]_D$ (nm): − 80.4 (589), − 142.9 (500), − 340.7 (400). IR ν_{max}^{KBr} cm^{-1}: 3600, 1726, 1621, 1564, 1500, 1460, 1380. ^1H and ^{13}C NMR: Tables 1 and 2.

Angelol K (**5**). Mp 116–118°. ORD (EtOH; *c* 0.5). $[\alpha]_D$ (nm): − 122.4 (589), − 140.1 (550), − 162.1 (500), − 256.4 (450), − 440.3 (400). IR ν_{max}^{KBr} cm^{-1}: 3450, 1730, 1610, 1560, 1495, 1455, 1380, 1020, 910. ^1H and ^{13}C NMR: Tables 1 and 2.

Angelol L (**6**). Viscid oil. ORD (EtOH; *c* 0.5). $[\alpha]_D$ (nm): − 79.0 (589), − 84.3 (550), − 101.9 (500), − 164.2 (450), − 274.6 (400). IR ν_{max}^{KBr} cm^{-1}: 3428, 1731, 1620, 1560, 1462, 1379, 1272, 1207, 914. ^1H and ^{13}C NMR: Tables 1 and 2.

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