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TWO BIFLAVONOIDS FROM *DAPHNE ACUTILOBA**

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Key Word Index—*Daphne acutiloba*; Thymelaeaceae; biflavonoids; daphnodorins M and N.

Abstract—Two spirobiflavonoids, daphnodorins M and N, were isolated from the roots and the bark of *Daphne acutiloba* and their structures established by spectral and chemical means. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Daphne acutiloba REHD. is a deciduous shrub, which grows in southern China and Formosa. The roots and bark are used in Chinese herbal medicine under the name "jin yao dai" for the treatment of adenochirapsology and bruises. In the course of our studies on the constituents of thymelaeaceous plants, we have investigated this plant and isolated two spirobiflavonoids, daphnodorin M (**1**) and daphnodorin N (**2**) as their tetramethylethers (**3**) and (**4**), together with fifteen known compounds; daphnetin, daphnin, daphnoretin, umbelliferone, daphneticin, daphnetin 8-*O*-β-D-glucopyranoside, wikstromol, pinoresinol, pinoresinol β-D-glucopyranoside and daphnodorins A, D-1, D-2, E, F and J. This paper is concerned with the structural elucidation of **1** and **2**.

RESULTS AND DISCUSSION

The methanolic root/bark extract of *D. acutiloba* was subjected to a combination of silica gel and Sephadex LH-20 chromatography in various solvent systems to afford two new spirobiflavonoids, daphnodorin M (**1**) and N (**2**) after methylation to the corresponding tetramethyl ethers (**3**) and (**4**).

Compound **3** was assigned the molecular formula $C_{34}H_{30}O_{10}$ by HR–SI mass spectrometry (*m/z* 598.1329 $[M]^+$). The UV spectrum showed absorption maxima at 319.0 sh, 293.0 sh, 281.6, 255.2 and 226.6 nm. The IR spectrum showed absorption bands at 3443 br, 3004, 2938, 2840, 1689, 1634, 1610 and 1572 cm^{-1}, suggesting the presence of hydroxyl and carbonyl groups and an aromatic ring. The ^1H NMR spectrum (Table 1) showed signals assignable to two pairs of 4-oxyphenyl groups, a 2,4,6-trioxyphenyl group, a 2,5,6-trisubstituted dihydropyran, four methoxy groups and an alcoholic hydroxyl group. Further, two singlet signals were observed at δ 5.83 (1H, *s*) and δ 5.90 (1H, *s*). In the ^{13}C NMR spectrum of **3** (Table 2) two carbonyl signals (δ 187.3 and 184.7) and two quaternary carbon signals attached to an oxygen atom (δ 84.9 and 79.9) were observed in addition to the signals described above. These signals were closely related to those of genkwanol B hexamethylether (**5**), except for the presence of signals due to a 2,5,6-trisubstituted dihydropyran instead of the signals due to a 3-hydroxy-2,5,6-trisubstituted dihydropyran. The ^{13}C NMR spectrum of **3** was also related to that of **5** except for the presence of a methylene carbon signal at δ 28.9 and the lack of a methine carbon signal at δ 75.8. Thus, **1** was assumed to be either 3-deoxygenkwanol B or its enantiomer.

Compound **4** was assigned the molecular formula $C_{34}H_{30}O_{10}$, the same as **3**, by HR–SI mass spectrometry (*m/z* 598.1839 $[M]^+$). The UV and IR spectra of **4** closely resembles those of **3**. The ^1H NMR spectrum of **4** (Table 1) showed signals due to two pairs of 4-oxyphenyl groups, a 2,4,6-trioxyphenyl group, a 2,5,6-trisubstituted dihydropyran, four methoxy groups and an alcoholic hydroxyl group. Further, one singlet signal was observed at δ 5.83 (2H, *s*). The ^{13}C NMR spectrum of **4** (Table 2) showed two carbonyl signals (δ 187.4 and 184.6) and two quaternary carbon signals linked to an oxygen atom (δ 84.9, 79.9). These signals were closely related to those of genkwanol C

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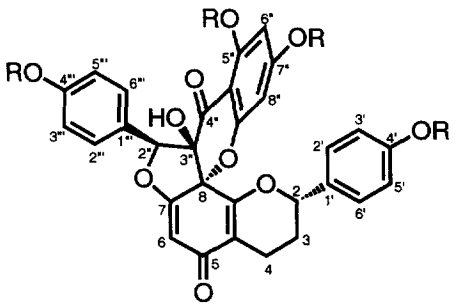
Table 1. 1H NMR spectral data for compounds **3–6** in $CDCl_3$.

H	**3**	**4**	**5**	**6**
2	4.95 *dd* (9.5, 2.8)	4.95 *dd* (9.1, 2.2)	4.93 *d* (5.1)	4.59 *d* (7.6)
3	2.10 *m*	2.11 *m*	3.51 *dt* (5.1, 4.5)	3.56 *ddd* (7.6, 7.6, 5.3)
	1.71 *m*	1.87 *m*		
4	2.55 *m*	2.56 *m*	2.52 *d* (4.5)	2.81 *dd* (17.4, 5.3)
	2.30 *m*	2.38 *m*		2.46 *dd* (17.4, 7.6)
6	5.83 *s*	5.83 *s*	5.85 *s*	5.85 *s*
2′,6′	6.71 *d* (8.9)	6.96 *d* (8.8)	6.88 *d* (8.8)	6.97 *d* (8.8)
3′,5′	6.65 *d* (8.9)	6.77 *d* (8.8)	6.72 *d* (8.8)	6.77 *d* (8.8)
2″	5.90 *s*	5.83 *s*	5.91 *s*	5.89 *s*
6″	6.20 *d* (2.2)	6.13 *d* (2.2)	6.13 *d* (2.2)	6.05 *d* (2.3)
8″	6.25 *d* (2.2)	6.17 *d* (2.2)	6.17 *d* (2.2)	6.15 *d* (2.3)
2‴,6‴	7.18 *d* (8.8)	7.17 *d* (8.8)	7.19 *d* (8.8)	7.21 *d* (8.8)
3‴,5‴	6.88 *d* (8.8)	6.88 *d* (8.8)	6.86 *d* (8.8)	6.87 *d* (8.8)
−OH	3.94 *s*	4.04 *s*		
−OMe	3.90 *s*	3.86 *s*	3.85 *s*	3.84 *s*
	3.85 *s*	3.85 *s*	3.82 *s*	3.80 *s*
	3.80 *s*	3.79 *s*	3.79 *s*	3.77 *s*
	3.75 *s*	3.74 *s*	3.77 *s*	3.76 *s*
			3.43 *s*	3.34 *s*
			3.20 *s*	3.23 *s*

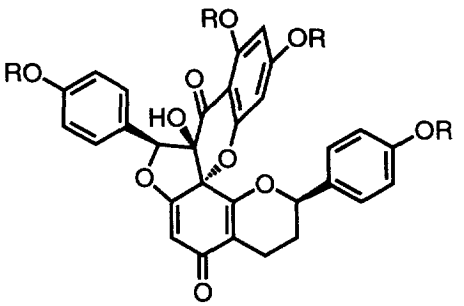
Table 2. ^{13}C NMR spectral data for compounds **3–6** in $CDCl_3$.

C	**3**	**4**	**5**	**6**
2	78.4	78.3	79.8	80.9
3	28.9	27.9	75.8	75.1
4	17.5	17.2	21.2	24.2
4a	112.2	112.8	110.3	110.9
5	187.3	187.4	187.5	187.0
6	101.9	102.0	102.4	102.0
7	167.9*	168.1*	169.3	168.8
8	84.9	84.9	84.9	84.6
8a	167.5*	167.4*	157.9	157.8
1′	132.2	131.7	130.0	128.7
2′,6′	126.2	126.3	127.5	127.9
3′,5′	113.5	113.7	114.0	113.6
4′	163.3*	163.2*	159.9	159.5
2″	90.7	91.0	91.1	90.2
3″	79.9	79.9	86.2	85.3
4″	184.7	184.6	183.3	182.9
4″a	102.2	102.1	105.4	105.2
5″	162.6*	163.2*	163.3	162.2
6″	94.3	94.0	94.4	94.0*
7″	160.3*	162.3*	167.1	166.5
8″	94.0	93.9	94.5	93.9*
8″a	158.9*	159.0*	162.8	162.4
1‴	123.6	123.7	124.5	124.0
2‴,6‴	129.1	128.9	130.0	129.3
3‴,5‴	113.7	113.7	113.9	113.5
4‴	158.1*	157.9*	160.7	160.1
−OMe	56.4	56.4	56.9	56.8
	56.0	55.9	56.6	56.3
	55.3 × 2	55.5	56.1	56.2
		55.3	55.7	55.8
			55.5	55.2 × 2
			55.4	

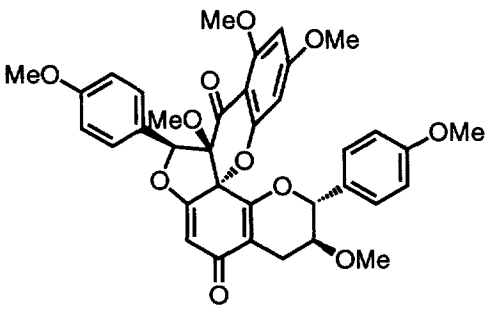
* Assignments in each column may be interchanged.



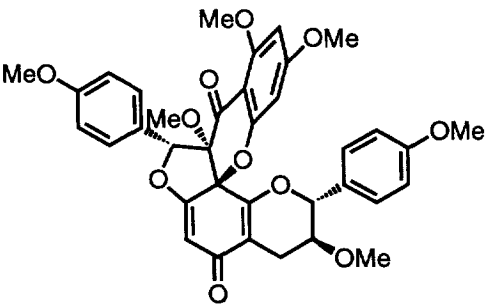
daphnodorin M(1): R = H; 3: R = Me



daphnodorin N(2): R = H; 4: R = Me



5



6

hexamethyl ether (**6**), i.e. a stereoisomer of **5**, except for the presence of signals due to a 2,5,6-trisubstituted dihydropyran instead of the signals due to a 3-hydroxy-2,5,6-trisubstituted dihydropyran. Thus, **2** was deduced to be either 3-deoxygenkwanol C or its enantiomer.

The absolute configuration at C-8, C-2″ and C-3″ positions in **1** and **2** were confirmed by comparing the CD spectra of **3** and **4** with those of **5** and **6**. The CD spectra of **5** and **6** whose C-8, C-2″ and C-3″ positions have opposite configurations showed good symmetrical curves (Fig. 1) [2]. The CD spectra of **3** and **4** both showed a negative cotton effect at 290 nm similar to that of **5**. Hence, the absolute configuration at C-8, C-2″ and C-3″ positions in **3** and **4** must be the same, that is, *S*, *S* and *R*.

Since the NMR profile of **3** and **4** were related to those of **5** and **6**, respectively, from the results of CD spectra, the absolute configuration of C-2 in **3** and **4** was concluded to be *R* and *S*, respectively. Further, it can be presumed that the transformation of **1** to **2** or **2** to **1** was a result of the enantiomer transformation shown in Fig. 2.

EXPERIMENTAL

General.

EIMS: 70 eV. SIMS: glycerol matrix. ^{1}H and ^{13}C NMR: 300 and 75.4 MHz with TMS as int. standard. CC: Merck silica gel 60 (70–230 mesh), Merck silica gel 60H and Sephadex LH-20. TLC and prep. TLC: Merck silica gel 60 F_{254} plate (0.25 mm) and Whatman silica gel 150A PLK5F (1 mm). Spots and bands were detected by UV irradiation (254 and 365 nm).

Plant material.

Roots and bark of *Daphne acutiloba* REHD. were collected on June, 1994 at Hubei province Hefeng country, China, and identified by Dr Jia-Xiang Hong, Institute for Pharmaceutical Inspection in Hefeng country, China. A voucher specimen has been deposited in the Institute of Botany Jiangsu Province and Academia Sinica.

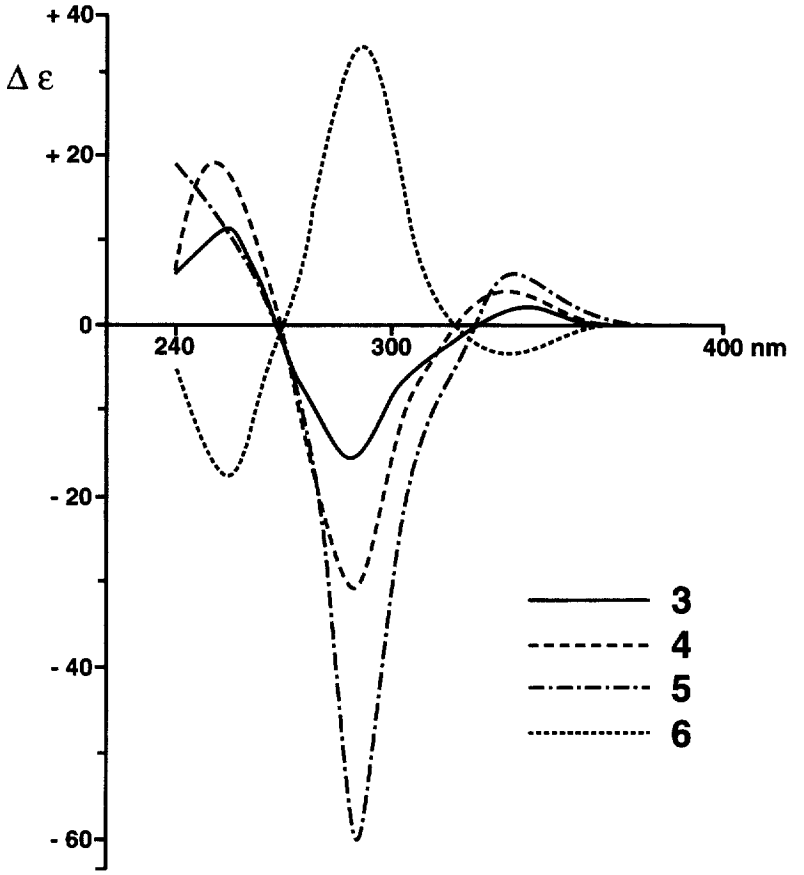


Fig. 1. The CD spectra of **3**–**6** in dioxane.

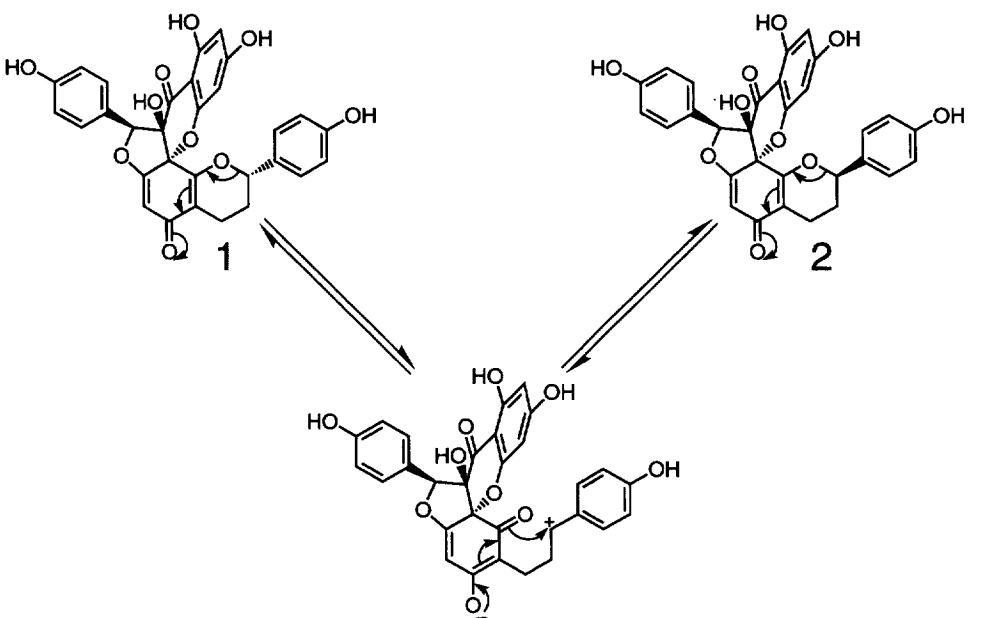


Fig. 2.

Extraction and isolation.

Air dried roots and bark (2.6 Kg) were chopped into small pieces and extracted with MeOH (10 l × 5) under reflux. The combined MeOH extracts were concd. to 2 l *in vacuo*. After removal of the ppt. by filtration, the filtrate was concd *in vacuo*. The residue was treated with *n*-hexane and the insoluble part concd *in vacuo* to give a residue (448 g), which was subjected to CC on silica gel eluted successively with $CHCl_3$-MeOH mixtures of increasing polarity. The 2% MeOH eluates were rechromatographed on silica gel with $CHCl_3$-MeOH to give daphnoretin (631 mg), daphneticin (316 mg), umbelliferone (239 mg), wikstromol (42 mg), pinoresinol (60 mg) and daphnetin (1.8 g). The 4% MeOH eluates were rechromatographed on silica gel with $CHCl_3$-MeOH followed by Sephadex LH-20 with Me_2CO to give daphnoretin 8-*O*-*β*-D-glucopyranoside (46 mg), syringin (367 mg), daphnodorin E (16 mg), daphnodorin F (10 mg), daphnodorin K (20 mg), pinoresinol *O*-*β*-D-glucopyranoside (240 mg) and a mixture (2.5 g) of daphnodorin M (**1**) and daphnodorin N (**2**). The 10% MeOH eluates were rechromatographed on Sephdex LH-20 with MeOH followed by silica gel with $CHCl_3$-MeOH to give daphnodorin A (20 mg), daphnodorin J (10 mg), daphnodorin D-1 (30 mg), daphnodorin D-2 (20 mg) and daphnin (7.7 g).

Methylation of **1** and **2**.

A solution of **1** and **2** (103 mg) in MeOH (2 ml) was methylated with CH_2N_2-Et_2O in the usual way. The product was purified by prep. TLC with $CHCl_3$-Me_2CO (30:1) to afford the tetramethyl ethers of **1** (**3**) (32.4 mg) and **2** (**4**) (23.5 mg), respectively.

Compound **3**.

Pale yellow viscous oil. HR–MS *m/z* 598.1829 $[M]^+$ (calcd. for $C_{34}H_{30}O_{10}$, 598.1839). UV $\lambda_{max}^{dioxane}$ nm (log ϵ): 319.0 sh (3.84), 293.0 sh (4.19), 281.6 (4.25), 255.2 (4.37), 226.6 (4.60). IR ν_{max}^{KBr} cm^{-1}: 3443 br, 3004, 2938, 2840, 1689, 1634, 1610, 1572. CD (dioxane; *c* 4.15×10^{-5}) $\Delta\epsilon^{20}$ (nm): 0 (390), +3.7 (335), 0 (320), −31.1 (290), 0 (269.5), +18.6 (250), +6.2 (240). ^1H and ^{13}C NMR: see Tables 1 and 2.

Compound **4**.

Pale yellow viscous oil. HR–MS *m/z* 598.1839 $[M]^+$ (calcd. for $C_{34}H_{30}O_{10}$, 598.1839). UV $\lambda_{max}^{dioxane}$ (log ϵ): 330.0 sh (3.85), 293.0 sh (4.36), 282.0 (4.42), 255.0 (4.54), 226.0 (4.78). IR ν_{max}^{KBr} cm^{-1}: 3437 br, 3006, 2940, 2839, 2361, 1668, 1610, 1570. CD (dioxane; *c* 2.68×10^{-5}) $\Delta\epsilon^{20}$(nm): 0 (380), +1.6 (340), 0 (325), −15.0 (290), 0 (268), +11.3 (255), +5.9 (240). ^1H and ^{13}C NMR: see Tables 1 and 2.

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