Three New *Daphne* Diterpenes from the Buds of *Daphne genkwa* SIEB. *et* ZUCC. Processed by Rice Vinegar

by **Yi-Mei Zeng**[a]), **Wen-Jian Zuo**[a]), **Min-Ge Sun**[c]), **He Meng**[a]), **Qing-Hui Wang**[a]), **Jin-Hui Wang***[a])[b]), and **Xian Li**[a])

[a]) School of Traditional Chinese Materia Medica 49#, Shenyang Pharmaceutical University, Wenhua Road 103, Shenyang 110016, P. R. China (phone: +86-24-23986478; fax: +86-24-23986479; e-mail: Wjh.1972@yahoo.com.cn)

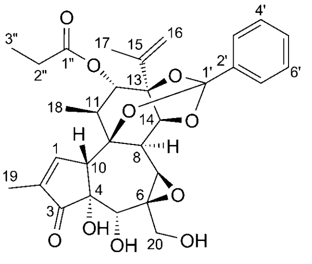
[b]) School of Pharmacy, Shihezi University, Shihezi 832002, P. R. China

[c]) Shenyang One Up-lead Pharmaceutical Technology Co., Ltd., Shenyang 110179, P. R. China

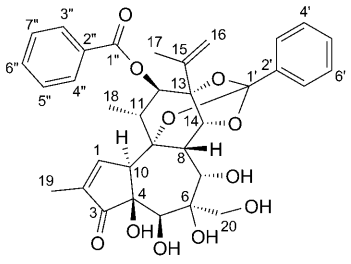
Three new *Daphne* diterpenes, yuanhuaoate A (**1**), yuanhuaoate B (**2**), and yuanhuaoate C (**3**), were isolated from the buds of *Daphne genkwa* SIEB. *et* ZUCC. processed by rice vinegar. Their structures and relative configurations were established by analysis of spectroscopic data. Their absolute configurations were assigned by the CD technique, applied for the first time to *Daphne* diterpenes.

Introduction. – *Daphne genkwa* SIEB. *et* ZUCC., belonging to the *Daphne* genus, is a famous traditional medicinal plant in China. The plant has been reported to exhibit a variety of biological effects, such as diuretic activity, induced abortive, analgesic, and antineoplastic activities [1]. In China, it has been used for induced abortion and the treatment of ascites of liver cirrhosis and tracheitis [2]. However, its efficacy is limited due to the toxicity of the plant. The toxicity can be reduced by roasting it with rice vinegar, at the same time the diuretic activity is enhanced [3]. Herein, we describe the isolation and structural elucidation of three new *Daphne* diterpenes – yuanhuaoate A (**1**), yuanhuaoate B (**2**), and yuanhuaoate C (**3**) from the buds of *Daphne genkwa* SIEB. *et* ZUCC. processed by rice vinegar.

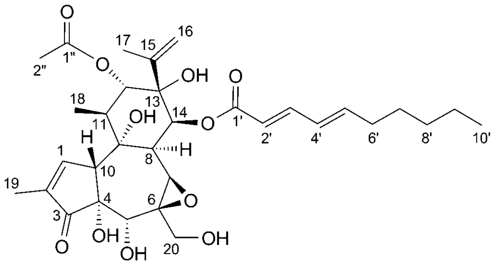
Result and Discussion. – Compound **1** was obtained as a white amorphous solid, and its molecular formula, $C_{30}H_{34}O_{10}$, was deduced from its HR-ESI-MS ($[M+Na]^+$ at *m/z* 577.2050, indicating the presence of 14 degrees of unsaturation. The IR spectrum (KBr) showed absorptions at 3440 cm^{-1} (OH) and 1707 cm^{-1} (C=O). Thirty C-atom signals comprising four Me, three CH_2, and fourteen CH groups, and nine quaternary C-atoms were evident from its ^{13}C-NMR and HSQC spectra. At the same time, one oxygenated CH_2 (δ 65.1 (C(20))) and four oxygenated CH groups (δ 72.0 (C(5)), 64.3 (C(7)), 78.1 (C(12)), and 80.7 (C(14))), four oxygenated quaternary C-atoms (δ 72.2 (C(4)), 60.4 (C(6)), 78.5 (C(9)), and 84.1 (C(13))), two carbonyl C-atoms (δ 209.4 (C(3)) and 173.1 (C(1''))), one terminal C=C bond (δ 143.0 (C(15)) and 113.5 (C(16))), and one Ph group were distinguished by analysis of the chemical shifts (*Table 1*). The resonances of C(7) (δ 64.3), C(6) (δ 60.4), and H−C(7) (δ 3.61) of **1** were evidently shifted upfield compared with genkwanine A [4], suggesting the presence of a 6,7-epoxy group. In addition, the ^{13}C-NMR and HSQC spectra revealed a



1[1])



2[1])



3[1])

quaternary C-atom signal at δ 117.9, which is the most notable spectral feature for an orthoester group in *Daphne* diterpene esters [5]. The ^{1}H-NMR and HSQC spectra of **1** indicated the presence of an oxygenated CH_2 group, showing up as an *AB* system (δ 3.82 (*d*, $J = 12.0$, $H_a-C(20)$), 3.96 (*d*, $J = 12.0$, $H_b-C(20)$)) which was attached to a quaternary C-atom. The presence of a terminal C=C bond was evident for 2 signals at δ 5.00 (*s*, 1 H−C(16)) and 5.03 (*s*, 1 H−C(16)).

Analysis of the ^{1}H- and ^{13}C-NMR, HSQC, and HMBC spectra of **1** enabled us to allot the H-atoms to their bonded C-atoms and further demonstrated that **1** was a *Daphne* diterpene ester. In the HMBC spectrum, δ 117.9 (C(1')) correlated with δ 4.90 (H−C(14)) and 7.70–7.71 (H−C(3') and H−C(7')), confirming the presence of an orthoester group in **1**; the ^{1}H,^{13}C long-range correlations between δ 173.1 (C(1'')) and δ 2.28 ($CH_2(2'')$), 1.11 (Me(3'')), and 5.06 (H−C(12)) verified that a propanoyloxy residue was connected to C(12). The linkage and oxygenation patterns of the other C-atoms were also achieved by analysis of the HMBCs (*Fig. 1*): δ 1.87 (Me(17))/δ 113.5 (C(16)), 143.0 (C(15)), and 84.1 (C(13)); δ 1.37 (Me(18))/δ 78.1 (C(12)), 44.1 (C(11)), and 78.5 (C(9)); δ 1.79 (Me(19))/δ 160.4 (C(1)), 136.9 (C(2)), and 209.4 (C(3)); δ 3.61 (H−C(7))/δ 35.5 (C(8)), 80.7 (C(14)), 78.5 (C(9)), 60.4 (C(6)), and 65.1 (C(20)). So the constitutional formula of **1** was established.

The relative configuration of **1** was determined by examination of coupling constants in the ^{1}H-NMR and the correlations in the NOESY plot (*Fig. 2*). The correlation between δ 5.06 (H−C(12)) and 1.37 (Me(18)) indicated that H−C(12) and H−C(11) are *trans*-positioned; H−C(12) (δ 5.06) appearing as a *s* in the ^{1}H-NMR

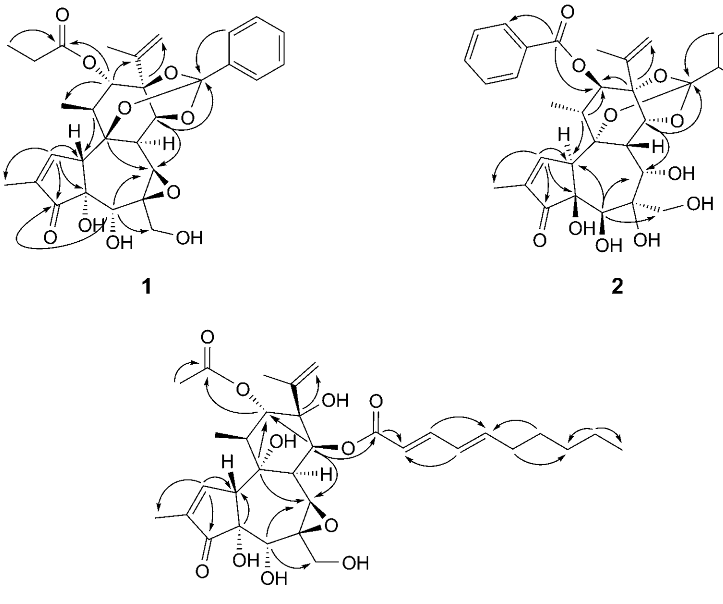
---

[1] Trivial atom numbering; for systematic names, see *Exper. Part.*

Table 1. *^{1}H- (600 MHz, CDCl$_3$) and ^{13}C-NMR Data (150 MHz, in CDCl$_3$) of* **1** *and* **2**[1]). δ in ppm, *J* in Hz.

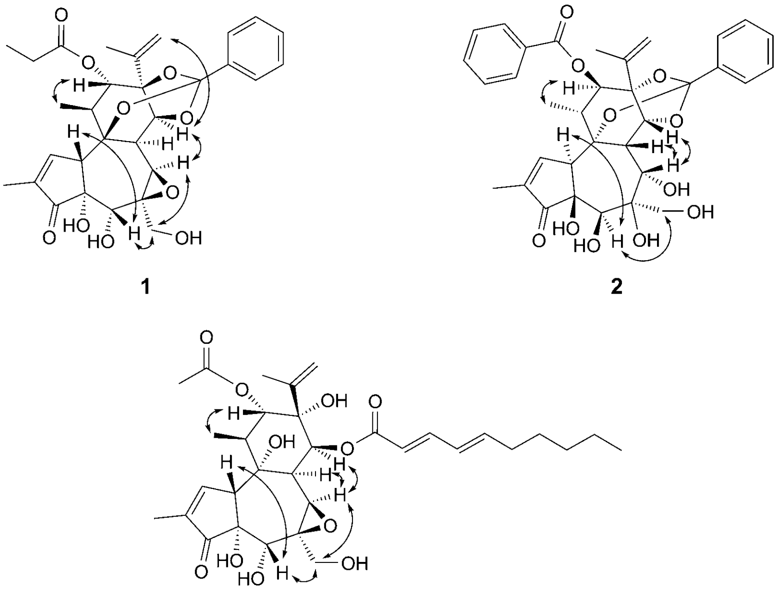
	1		**2**	
	δ(C)	δ(H)	δ(C)	δ(H)
H–C(1)	160.4	7.59 (br. *s*)	159.6	7.65 (br. *s*)
C(2)	136.9		137.2	
C(3)	209.4		208.9	
C(4)	72.2		75.2	
H–C(5)	72.0	4.27 (*s*)	73.7	4.15 (*s*)
C(6)	60.4		76.4	
H–C(7)	64.3	3.61 (*s*)	80.2	4.56 (*s*)
H–C(8)	35.5	3.59 (*d*, *J* = 2.4)	35.9	3.52 (*d*, *J* = 2.4)
C(9)	78.5		79.1	
H–C(10)	47.5	3.92–3.93 (*m*)	50.5	4.00–4.01 (*m*)
H–C(11)	44.1	2.43 (*q*, *J* = 7.8)	43.9	2.98 (*q*, *J* = 7.8)
H–C(12)	78.1	5.06 (*s*)	78.1	5.35 (*s*)
C(13)	84.1		85.0	
H–C(14)	80.7	4.90 (*d*, *J* = 2.4)	83.1	5.10 (*d*, *J* = 2.4)
C(15)	143.0		142.5	
CH$_2$(16)	113.5	5.00, 5.03 (2*s*)	114.0	5.01, 5.06 (2*s*)
Me(17)	18.8	1.87 (*s*)	18.8	1.90 (*s*)
Me(18)	18.2	1.37 (*d*, *J* = 7.2)	18.0	1.44 (*d*, *J* = 7.2)
Me(19)	9.8	1.79–1.80 (*m*)	9.9	1.80–1.81 (*m*)
CH$_2$(20)	65.1	3.82 (*d*, *J* = 12.0, H$_a$), 3.96 (*d*, *J* = 12.0, H$_b$)	70.0	3.73 (*d*, *J* = 11.4, H$_a$), 4.10 (*d*, *J* = 11.4, H$_b$)
C(1′)	117.9		117.9	
C(2′)	135.2		134.8	
H–C(3′,7′)	126.0	7.70–7.71 (*m*, 2 H)	125.8	7.67–7.69 (*m*, 2 H)
H–C(4′,6′)	128.0	7.37–7.38 (*m*, 2 H)	130.0	7.43–7.45 (*m*, 2 H)
H–C(5′)	129.6	7.39–7.40 (*m*)	133.3	7.40–7.41 (*m*)
C(1″)	173.1		165.0	
CH$_2$(2″) or C(2″)	27.7	2.28 (*qd*, *J* = 7.8, 3.0)	128.5	
Me(3″) or H–C(3″)	9.0	1.11 (*t*, *J* = 7.8)	129.6	7.94–7.96 (*m*)
H–C(4″)			128.2	7.42–7.43 (*m*)
H–C(5″)			133.3	7.55–7.56 (*m*)
H–C(6″)			128.2	7.42–7.43 (*m*)
H–C(7″)			129.6	7.94–7.96 (*m*)

further conformed this configuration [6]. δ 2.43 (H–C(11)) was correlated with δ 3.59 (H–C(8)), suggesting that they were on the same side of the ring system, and the 9,13,14-orthoester function was on the opposite side. The correlations between δ 3.92–0.93 (H–C(10)) and 4.27 (H–C(5)) showed that H–C(10) and H–C(5) were in β-configuration. The *s* of H–C(7) at δ 3.61 in the ^{1}H-NMR indicated that the angle between H–C(7) and H–C(8) was 90°, accordingly the CH$_2$(20) and H–C(7) were in α-configuration. Furthermore, δ 3.61 (H–C(7)) showed correlation with δ 4.90 (H–C(14)), confirming that H–C(7), H–C(8), and H–C(14) are in α-configuration. The CD spectrum (*Fig. 3*) showed a negative *Cotton* effect at 249 nm, which is consistent with the axiom of an α,β-unsaturated cycloketone [7] (*Fig. 4*). Therefore, the structure and absolute configuration of **1** were elucidated, and **1** was named yuanhuaoate A.



1 **2** **3**

Fig. 1. *Key HMBC of compounds* **1**–**3**



1 **2** **3**

Fig. 2. *Key NOESY of compounds* **1**–**3**

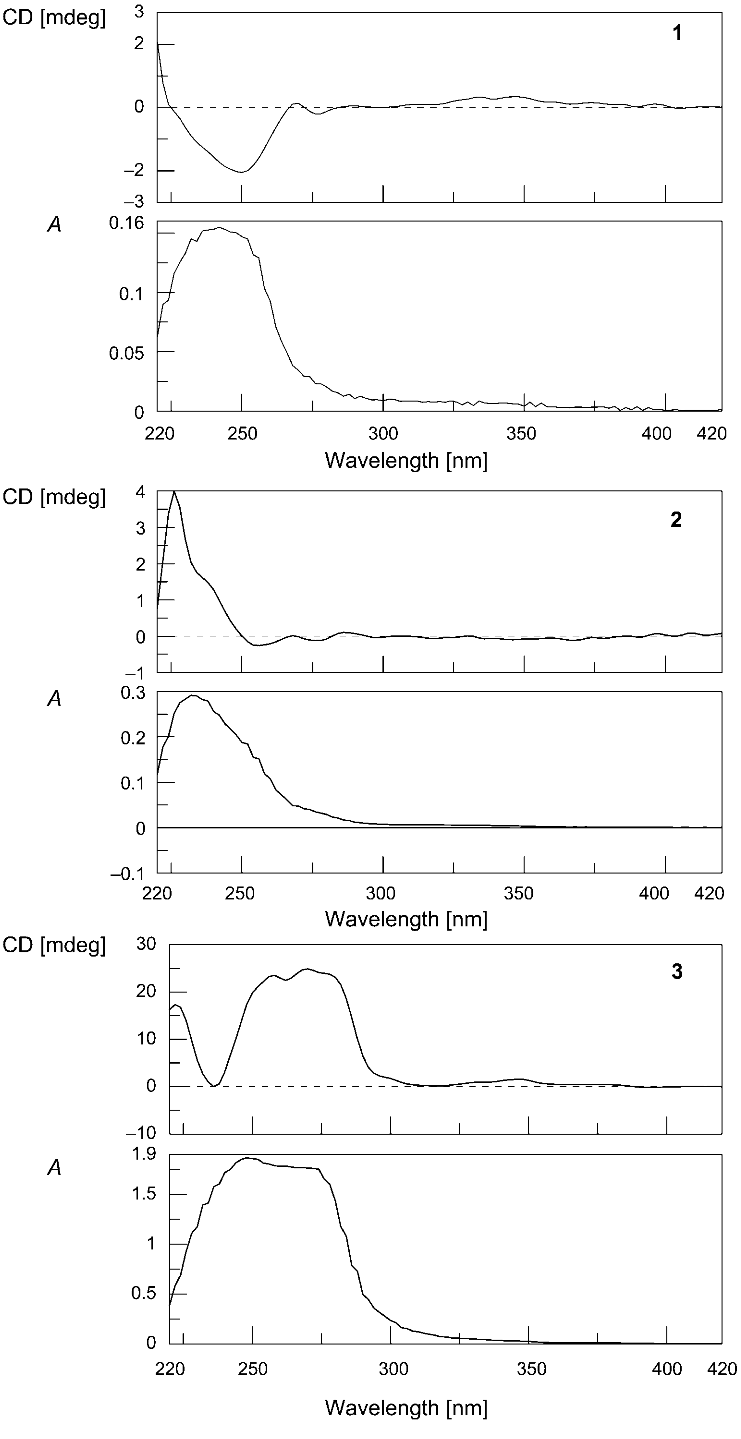


Fig. 3. *CD and UV Curves of* **1**–**3**

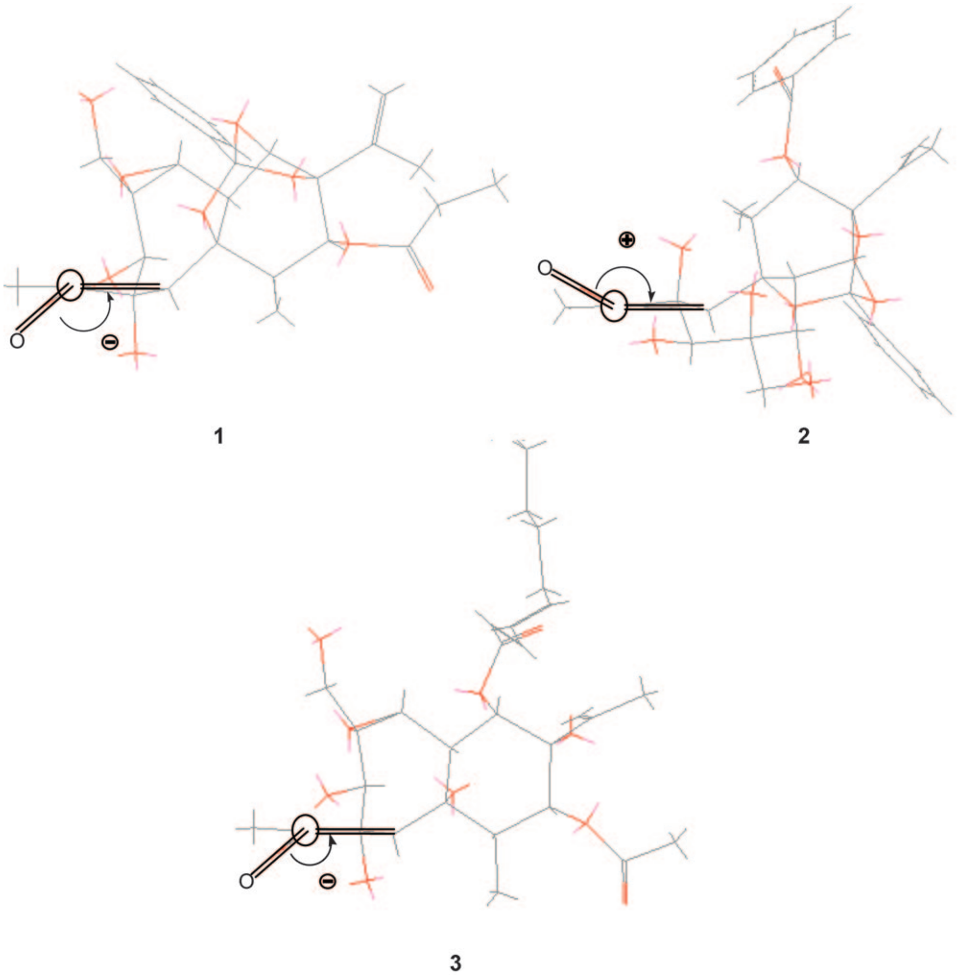


Fig. 4. *The* Cotton *effect of the α,β-unsaturated cycloketone moiety of* **1**–**3**

Compound **2**, a white amorphous powder, showed a peak at *m*/*z* 643.2151 ($[M + Na]^+$) in the HR-ESI-MS, suggesting the molecular formula $C_{34}H_{36}O_{11}$ and indicating the presence of 17 degrees of unsaturation. The IR spectrum (KBr) showed peaks at 3437 cm^{-1} (OH) and 1722 cm^{-1} (C=O). Analysis of the ^1H- and ^{13}C-NMR spectra established that **2** was a *Daphne* diterpene ester too. Compared with compound **1**, one more Ph group was found in compound **2**; the signals at δ(H) 4.56 (H−C(7)) and at δ(C) 76.4 (C(6)), 80.2 (C(7)), and 70.0 (C(20)) were all downfield shifted compared to those of **1**, suggesting the presence of a 6,7-diol in **2** instead of a 6,7-expoxy ring. In the HMBC spectrum, δ 165.0 (C(1″)) correlated with δ 7.68 (H−C(3″,7″)) and 5.35 (H−C(12)), pointing to the presence of a PhCOO group at C(12). The linkage and oxygenation patterns of the C-atoms were determined by analysis of the ^1H- and ^{13}C-NMR spectra and HMBCs (*Fig. 1*).

The relative configuration of **2** was confirmed by the NOESY data (*Fig. 2*). The significant NOEs H−C(12)/Me(18) and H−C(11)/H−C(8) permitted to assign the β-orientation for H−C(11) and H−C(8) and the α-orientation for H−C(12). The H−C(7) signal (δ 4.56) was a *s* in the ^{1}H-NMR spectrum indicating that OH−C(7) was α-oriented, since if OH−C(7) was β-oriented, the coupling constant would be 10 Hz [8]. In the NOESY plot, the significant correlations H−C(5)/H−C(10), H−C(10)/H$_a$−C(20), and H−C(7)/H−C(14) confirmed that H−C(5), H−C(10), and CH$_2$(20) were α-oriented, while H−C(14) and H−C(7) were β-oriented. In the CD spectrum of **2**, a strong positive *Cotton* effect was present at 226 nm (*Fig. 3*), which was consistent with the axiom of an α,β-unsaturated cycloketone (*Fig. 4*). The absolute configuration of **2** was established as shown in *Fig. 2*, and the compound was named yuanhuaoate B.

Compound **3**, a white amorphous powder, showed a peak at *m/z* 627.2777 ([*M* + Na]$^+$) in the HR-ESI-MS, pointing to the molecular formula $C_{32}H_{44}O_{11}$ and indicating the presence of 11 degrees of unsaturation. The IR spectrum (KBr) exhibited absorptions at 3439 cm^{-1} (OH) and 1707 cm^{-1} (C=O). The ^{1}H- and ^{13}C-NMR spectra (*Table 2*) indicated that **3** had a similar oxygenation pattern as that of **1**. In the ^{13}C-NMR and HSQC plots, one oxygenated CH$_2$ (δ 65.5 (C(20))) and four oxygenated CH groups (δ 71.0 (C(5)), 62.4 (C(7)), 79.2 (C(12)), and 72.6 (C(14))), four oxygenated quaternary C-atoms (δ 72.3 (C(4)), 61.9 (C(6)), 75.0 (C(9)), and 75.5 (C(13))), and one terminal C=C bond (δ 144.6 (C(15)), and 114.8 (C(16))) were distinguishable. Compared to compound **1**, the absence of the typical quaternary C-atom for an orthoester group and the presence, instead, of an ester C=O group at δ 166.8 (C(1′)) in **3** suggested that a (2*E*,4*E*)-deca-2,4-dienoyloxy moiety was linked to C(14), and two OH groups were positioned at C(9) and C(13) with the aid of HMBC; the propanoyloxy group of **1** was replaced by an AcO group in **3**, located at C(12). The constitution of **3** was further conformed by HMBC correlations (*Fig. 1*).

The relative configuration of **3** was confirmed by the NOESY correlations (H−C(12)/Me(18), H−C(11)/H−C(8), H−C(8)/H−C(7), H−C(7)/H−C(14) and H−C(20), H−C(20)/H−C(5), and H−C(5)/H−C(10)) (*Fig. 2*). It was not difficult to see that the molecular-skeleton parent of **3** and **1** showed almost identical relative configuration, except for that at C(9) and C(13). The negative *Cotton* effect observed at 236 nm was again due to the presence of an α,β-unsaturated cycloketone (*Figs. 3* and *4*).

This work was financially supported by the *Fok Ying Tung Education Foundation* (No. 101040). We are grateful to Prof. *Qishi Sun* (Shenyang Pharmaceutical University of China) for the identification of the plant material.

Experimental Part

General. Column chromatography (CC): silica gel (SiO$_2$; 200–300 mesh; *Qingdao Marine Chemical Group, Co.*); *Sephadex LH-20* (*Pharmacia, Co.*). Prep. HPLC: *Hitachi-L-7110* pump , *Hitachi-L-7420* UV spectrophotometric detector at 210 nm, *YMC-C$_{18}$* reversed-phase column (5 μm, 10 × 250 mm; flow rate 2.5 ml/min). CD Spectrum: *Jasco CD-2095-plus*; MeOH soln.; in nm (mdeg). UV Spectra: *Shimadzu UV-2201* spectrophotometer; MeOH soln.; in λ_{max} (log ε). IR Spectra: *IFS-55* spectrophotometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker AV-600* and *ARX-300* spectrometer; δ in ppm rel. to Me$_4$Si as internal standard, *J* in Hz. HR-ESI-MS: *BIC microTOF-Q* mass spectrometer; in *m/z* (rel. %). ESI-MS: *Finnigan LCQ* mass spectrometer; in *m/z* (rel. %).

Table 2. *^1H- (600 MHz, (D_6)DMSO) and ^{13}C-NMR Data (150 MHz, in (D_6)DMSO) of* **3**[1]). δ in ppm, *J* in Hz.

	δ(C)	δ(H)
H–C(1)	160.0	7.66 (*s*)
C(2)	137.1	
C(3)	209.2	
C(4)	72.3	
H–C(5)	71.0	4.28 (*s*)
C(6)	61.9	
H–C(7)	62.4	3.25 (*s*)
H–C(8)	39.8	4.11 (*d*, *J* = 5.4)
C(9)	75.0	
H–C(10)	50.8	3.57–3.58 (*m*)
H–C(11)	42.7	2.30 (*qd*, *J* = 7.8, 2.4)
H–C(12)	79.2	4.88 (*d*, *J* = 2.4)
C(13)	75.5	
H–C(14)	72.6	5.92 (*d*, *J* = 5.4)
C(15)	144.6	
CH_2(16)	114.8	5.04, 5.24 (2*s*)
Me(17)	19.5	1.81 (*s*)
Me(18)	16.1	1.30 (*d*, *J* = 7.8)
Me(19)	9.9	1.80 (br. *s*)
CH_2(20)	65.5	3.68 (*d*, *J* = 12.6, H_a), 3.90 (*d*, *J* = 12.6, H_b)
C(1′)	166.8	
H–C(2′)	117.9	5.90 (*d*, *J* = 15.0)
H–C(3′)	147.1	7.34 (*dd*, *J* = 15.0, 9.6)
H–C(4′)	128.2	6.17–6.19 (*m*)
H–C(5′)	146.3	6.17–6.19 (*m*)
CH_2(6′)	33.0	2.15–2.19 (*m*)
CH_2(7′)	28.3	1.40–1.45 (*m*)
CH_2(8′)	31.3	1.31–1.33 (*m*)
CH_2(9′)	22.4	1.26–1.30 (*m*)
Me(10′)	13.9	0.89 (*t*, *J* = 7.8)
C(1″)	170.1	
Me(2″)	21.1	2.00 (*s*)

Plant Material. The buds of *Daphne genkwa* Sieb. *et* Zucc. were collected in the *Pharmaceutical Market* (Shenyang, P. R. of China) and was identified by Prof. *Qishi Sun*, Shenyang Pharmaceutical University. A voucher specimen (No. 20050803) was deposited with the Research Department of Natural Medicine, Shenyang Pharmaceutical University. The dried buds of *Daphne genkwa* Sieb. *et* Zucc. (5 kg) were mixed with a blend of rice vinegar (1500 ml) and H_2O (3000 ml). When the rice vinegar was completely absorbed, the mixture was roasted in a drum-type *Herbal Medicine Roaster* up to dryness.

Extraction and Isolation. The dried buds of *Daphne genkwa* Sieb. *et* Zucc. (5 kg), processed by rice vinegar, were extracted with 95% EtOH (3 × 55 l) under reflux conditions to give a crude extract, which was suspended in H_2O and successively extracted with petroleum ether, $CHCl_3$, and AcOEt to yield a petroleum ether soluble fraction (104.2 g), a $CHCl_3$-soluble fraction (139.5 g), and an AcOEt-soluble fraction (73.5 g). A part of the $CHCl_3$-soluble fraction (100.0 g) was subjected to CC (SiO_2, gradient petroleum ether/acetone) to give a mixture (7.2 g). The latter was resubjected to CC (SiO_2, gradient $CHCl_3$/acetone): *Fractions A* and *B*. *Fr. A* was separated by CC (*Sephadex LH-20*, $CHCl_3$ containing increasing amount of MeOH) to yield *Fr. A.2* which was further subjected to prep. reversed-phase HPLC

(80% MeOH): **1** (12 mg) and **2** (8 mg). *Fr. B* was subjected to CC (*Sephadex LH-20*, $CHCl_3$ containing increasing amount of MeOH) to yield *Fr. B.3* which was further separated by prep. reversed-phase HPLC (74% MeOH): **3** (11 mg).

Yuanhuaoate A (= *(3aS,3bR,3cR,4aS,5R,5aR,8aS,8bS,9S,10S,10aR)-3a,3b,3c,4a,5,5a,8a,9,10,10a-Decahydro-5,5a-dihydroxy-4a-(hydroxymethyl)-7,9-dimethyl-10a-(1-methylethenyl)-10-(1-oxopropoxy)-2-phenyl-6H-2,8b-epoxyoxireno[6,7]azuleno[5,4-e]-1,3-benzodioxol-6-one*; **1**): White amorphous solid. UV: 210 (4.00), 243 (2.89). CD (MeOH): 220 (+1.92), 249 (−2.09). IR: 3440, 2924, 1738, 1707, 1630, 1452, 1383, 1175, 1082, 1013, 935, 754, 697. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 577.2050 ([M + Na]$^+$, $C_{30}H_{34}NaO_{10}^+$; calc. 577.2044).

Yuanhuaoate B (= *(3aR,3bS,4S,5R,6R,6aS,9aR,9bR,10R,11R,11aS)-11-(Benzoyloxy)-3a,3b,4,5,6,6a,9a,10,11,11a-Decahydro-4,5,6,6a-tetrahydroxy-5-(hydroxymethyl)-8,10-dimethyl-11a-(1-methylethenyl)-2-phenyl-7H-2,9b-epoxyazuleno[5,4-e]-1,3-benzodioxol-7-one*; **2**): White amorphous solid. UV: 203 (3.17), 231 (2.98). CD: 226 (+3.92), 237 (+1.48), 254 (−0.28). IR: 3437, 2924, 1722, 1692, 1630, 1451, 1383, 1270, 1078, 1026, 928, 713. ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 643.2151 ([M + Na]$^+$, $C_{34}H_{36}NaO_{11}^+$; calc. 643.2149).

Yuanhuaoate C (= *(1S,2S,3S,4S,4aR,4bR,5aS,6R,6aR,9aR,9bR)-1,2,3,4,4a,4b,5a,6,6a,7,9a,9b-Dodecahydro-3,6,6a,9b-tetrahydroxy-5a-(hydroxymethyl)-1,8-dimethyl-3-(1-methylethenyl)-7-oxo-2-(1-oxopropoxy)benz[7,8]azuleno[5,6-b]oxiren-4-yl (2E,4E)-Deca-2,4-dienoate*; **3**): White amorphous solid. UV: 263 (2.00). CD: 222 (+16.76), 236 (−0.21), 257 (+23.38), 270 (+24.53). IR: 3439, 2926, 1707, 1638, 1383, 1236, 1137, 1029, 914, 603. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-MS: 627.2777 ([M + Na]$^+$, $C_{32}H_{44}NaO_{11}^+$; 627.2775).

REFERENCES

[1] L. Z. Li, S. J. Song, P. Y. Gao, *Shenyang Yaoke Daxue Xuebao* **2007**, *24*, 587.
[2] B. X. Zhang, S. T. Yuan, J. X. Zhang, *Zhongguo Zhongyiyao Xinxi Zazhi* **1995**, *2*, 11.
[3] Y. Zhao, S. T. Yuan, A. Y. Li, *Zhongguo Zhongyao Zazhi* **1998**, *23*, 344.
[4] Z. J. Zhan, C. Q. Fan, J. Ding, *Bioorg. Med. Chem.* **2005**, *13*, 645.
[5] W. D. He, M. Cik, A. Lesage, *J. Nat. Prod.* **2000**, *63*, 1185.
[6] J. R. Carney, J. M. Krenisky, R. T. Williamson, *J. Nat. Prod.* **1999**, *62*, 345.
[7] L. J. Wu, 'Shiyong Tianran Youji Chanwu Huaxue', People's Medical Publishing House, 2007, p. 330.
[8] W. Adolf, E. Hecker, *J. Nat. Prod.* **1984**, *47*, 482.

Received November 20, 2008