Journal of Natural Products
Vol. 53, No. 4, pp. 909-914, Jul-Aug 1990

NEW SESQUITERPENE ALKALOIDS FROM *EUONYMUS JAPONICA*: STRUCTURES OF EUOJAPONINES D, F, J, AND K

BYUNG HOON HAN,* JAE HA RYU, YONG NAM HAN,

Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

MAN KI PARK, JEONG HILL PARK,

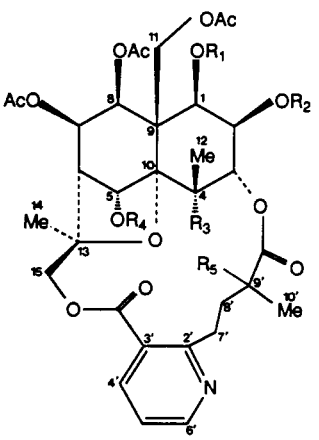
College of Pharmacy, Seoul National University, Seoul 151-742, Korea

and HIDEO NAOKI

Suntory Institute for Bioorganic Research, Mishima-Gun, Osaka 618, Japan

ABSTRACT.—Four new sesquiterpene alkaloids, euojaponines D [**1**], F [**2**], J [**3**], and K [**4**], and a known compound, euonine [**5**], were isolated from the root bark of *Euonymus japonica*. Their structures were elucidated by spectral analysis.

Euonymus japonica Thunb. (Celastraceae), a garden plant in Korea, is an evergreen tree resistant to pest attack. Recently the polyester-type sesquiterpene alkaloids of the Celastraceae (especially the genus *Tripterigium*) attracted our attention due to their potent insecticidal activity (1–4). The alkaloidal components from the root bark of *E. japonica* were studied in our laboratory, leading to the isolation of twelve alkaloids (5,6). This current paper describes the chemical structures of four new polyester-type sesquiterpene alkaloids, euojaponines D [**1**], F [**2**], J [**3**], and K [**4**], which have the commonality of wilfordic acid as their dibasic acid moiety. The structures of other alkaloids, euojaponines A, C, I, L, and M, containing evoninic acid, will be described elsewhere (7).



	R_1	R_2	R_3	R_4	R_5
1	COPh	Ac	OH	H	H[a]
2	COPh	Ac	OH	Ac	H[a]
3	COPh	H	H	Ac	H[a]
4	COPh	H	OH	Ac	H[a]
5	Ac	Ac	OH	Ac	H[a]
6	Ac	COPh	OH	Ac	OH[b]

[a]Dibasic acid moiety is wilfordic acid.
[b]Dibasic acid moiety is hydroxywilfordic acid.

RESULTS AND DISCUSSION

The MeOH extract of the root bark of *E. japonica* was partitioned between Et_2O and H_2O. The Et_2O fraction was dissolved in MeOH and treated with Pb(OH)Ac aqueous solution to remove phenolic compounds as a precipitate. Chromatography of the supernatant, as described in the Experimental section, afforded the four new alkaloids and a known alkaloid, euonine (8).

Euojaponine F [**2**] showed mass fragment ions in the secondary ion mass spectrum (sims) at *m/z* 868 $[M+H]^+$ and 105 $[C_6H_5CO]^+$, corresponding to the molecular formula of $C_{43}H_{49}NO_{18}$ and to a benzoyl group in the molecule. The ^{1}H-nmr spectrum of **2** is very similar to the reported data of wilfordine [**6**] as shown in Table 1 (9). The mol wt of **2** is 16 mass units lower than that of **6**, suggesting one fewer hydroxyl group than **6**. The ^{1}H nmr of **2** showed a doublet methyl peak for Me-10′ (δ 1.20, J = 6.8 Hz), and this peak gave ^{13}C-^{1}H long range correlation with C-9′ (δ 38.36) (Figure 1). From the above results, wilfordic acid is suggested as the dibasic acid moiety of **2** as shown in the structure. Many acetyl proton peaks overlapped with the proton peaks of the CH_2-8′ and H-9′. However, the proton couplings among CH_2-7′, CH_2-8′, H-9′, and Me-10′, including vicinal and geminal ones, could be clearly assigned by the analysis of the ^{1}H-^{1}H COSY 45 spectra of **2**.

The very weak coupling of Me-14 (δ 1.70, s) with H_B-15 (δ 5.75) in the ^{1}H-^{1}H COSY may result from W-type long range coupling. These assignments were further confirmed by ^{13}C-^{1}H long range COSY spectra that showed correlations of C-13 (δ 84.60) with Me-14, H_B-15 (δ 5.75), and H-5 (δ 6.96) peaks (Figure 1). The Me-12 (δ 1.58) was also assigned from the coupling with the 4-OH (δ 4.99) in the ^{1}H-nmr spectra. As described above, all methyl groups were assigned to their respective position in the structure, and Me singlet signals at δ 1.42, 2.13, 2.14, 2.18, and 2.32 could be assigned as acetyl groups. The C-8 acetyl group was assigned by its upfield shift (δ 1.42) due to the anisotropic effect of the C-1 benzoyl group (6,7).

The position of the benzoyl group was assigned to be at C-1 based on the ^{13}C-^{1}H long range COSY spectra of **2**. A carbonyl carbon at δ 165.00 correlated doubly with the H-1 (δ 5.95) and with the ortho proton (δ 7.83) of the benozyl group. This fact suggested that the carbonyl carbon at δ 165.00 may be assigned as the benzoyl carbonyl carbon which is linked to the C-1 position (Figure 2).

All protonated carbons were assigned from DEPT and ^{13}C-^{1}H COSY spectra by referring to ^{1}H-nmr peaks already assigned, and non-protonated carbons were assigned with the aid of ^{13}C-^{1}H long-range COSY spectra. The carbon signal due to C-9 (δ 52.46) was assigned by correlation spots with H_A-11 (δ 4.65), H-2 (δ 5.25) and H-7 (δ 5.53) (Figure 1). Similarly C-4 (δ 69.88) was assigned by correlation spots with Me-12 and H-2 (δ 5.25), and C-10 (δ 93.85) with Me-12, H-6 (δ 2.36), and H_A-11 (δ 4.65). The assignment of C-13 (δ 84.60) was described above.

Euojaponine D [**1**] was confirmed as the free C-5 hydroxy derivative of compound of **2** by the fact that the acetate of **1** was superimposed on the tlc spot of **2**. The C-5 free-hydroxyl group of **1** (δ 6.00, d, J = 3.0 Hz) was confirmed by its coupling with H-5 (δ 5.39, d, J = 3.0 Hz) and by deuteration with D_2O. Euojaponine K [**4**] was also shown to be the C-2 hydroxy compound of **2** by the same logic. The structure of euojaponine J [**3**] was established as having 4-deoxyeuonyminol as the sesquiterpene moiety, because it showed an additional secondary methyl group at Me-12 (δ 1.33, d, J = 8.0 Hz) and a coupling pattern of H-4 (δ 2.75, dq, J = 8.0, 1.0 Hz). The appearance of a highly shielded acetyl group indicated that the location of the benzoyl group is at C-1 and the acetyl group is at C-8 as described previously (6,7).

TABLE 1. ^{1}H-nmr Spectra[a] of Euojaponines D, F, J, and K, Euonine, and Wilfordine.

Proton	Multiplicity	Compound					
		1	2	3	4	5	6[b]
H-1	d	5.97 (3.5)	5.95 (3.8)	6.02 (3.5)	5.82 (3.7)	4.93 (2.6)	5.77 (3.0)
H-2	dd/ddd	5.25 (3.5, 3.0)	5.25 (3.8, 2.7)	4.12 (3.5, 2.0)	4.03 (3.7, 2.7, 4.2)[c]	5.15 (3.6, 2.6)	
H-3	d	5.05 (3.0)	5.00 (2.7)	5.08 (2.5, 1.0)	5.07 (2.7)	5.61 (3.6)	5.08 (2.8)
H-4	dq	OH[d]	OH[d]	2.75 (8.0, 1.0)	OH[d]	OH[d]	OH
H-5	d/brs	5.39 (3.0)	6.96 (brs)	6.58 (1.0)	6.97 (brs)	6.92 (brs)	6.85 (1.0)
H-6	d/dd	2.46 (4.5)	2.36 (4.0)	2.45 (4.0, 1.0)	2.33 (4.0)	2.33 (3.9)	2.40 (4.5, 1.0)
H-7	dd	5.51 (5.0, 4.5)	5.53 (5.7, 4.0)	5.58 (5.5, 4.0)	5.53 (6.0, 4.0)	5.52 (6.0, 3.9)	
H-8	d	5.40 (5.0)	5.42 (5.7)	5.42 (5.5)	5.51 (6.0)	5.36 (6.0)	
H-11	ABq	4.70, 5.35 (13.5)	4.65, 5.41 (13.4)	4.75, 5.50 (13.2)	4.79, 5.54 (13.5)	4.45, 5.23 (13.5)	4.21, 5.50 (13.0)
H-12	d	1.90 (1.0)	1.58 (1.0)	1.33 (8.0)	1.34 (1.2)	1.54 (1.1)	
H-14	s	1.70	1.70	1.65	1.67	1.65	
H-15[e]	ABq	3.77, 5.92 (13.0)	3.78, 5.75 (12.0)	3.63, 5.52 (11.5)	3.79, 5.70 (11.9)	3.77, 5.57 (11.9)	3.77, 5.82 (13.0)
H-4′[e,f]	dd	8.53	8.33	8.23	8.29	8.32	
H-5′[e,f]	dd	7.48	7.28	7.24	7.26	7.27	
H-6′[e,f]	dd	8.82	8.76	8.72	8.72	8.74	
H-7′[e]	ddd/m	3.13 (m)	2.95 (11.6, 6.8, 5.6)	3.06 (13.5, 6.5, 5.5)	3.79 (13.7, 6.3, 5.5)	2.93 (15.5, 5.5, 5.4)	
H-7′[e]	ddd/m	4.14 (m)	3.95 (11.6, 9.9, 5.9)	3.73 (13.5, 10.0, 6.5)	3.86 (13.7, 6.3, 5.5)	3.94 (15.5, 10.3, 5.9)	
H-8′[e]	m	1.93, 2.45	1.98, 2.38	2.02, 2.29	1.97, 2.18	1.98, 2.27	
H-9′[e]	m	2.35	2.41	2.29	2.36	2.34	
H-10′[e]	d	1.22 (7.0)	1.20 (6.8)	1.13 (6.5)	1.12 (7.0)	1.17 (6.6)	
(1-Bz)[g]							
ortho	dd	7.82	7.83	8.02	7.95		
meta	dd	7.38	7.40	7.45	7.40		
para	dd	7.54	7.53	7.58	7.54		

[a]Recorded at 300 MHz in $CDCl_3$, chemical shifts (in δ values) relative to internal $CHCl_3$, and coupling constants (in Hz) are given in parentheses. Signals due to acetyl groups appeared in the region of δ 1.41–2.32.

[b]These data were taken from K. Yamada *et al.* (9).

[c]This peak looks like dd (J = 3.7, 2.7).

[d]Chemical shifts of -OH protons are: **1** δ 6.3 (d, J = 1.0, 4-OH), δ 6.0 (d, J = 3.0, 5-OH); **2** δ 4.99 (d, J = 1.0, 4-OH); **3** δ 2.55 (brs, 2-OH); **4** δ 3.2 (d, J = 4.2, 2-OH), δ 4.8 (d, J = 1.2, 4-OH); **5** δ 4.96 (d, J = 1.1, 4-OH).

[e]Chemical shifts of these protons are variable due to conformational flexibility of the molecule; max ±0.05 ppm for H-15, H-8′, H-9′, and H-10′, ±0.2 ppm for H-4′, H-5′, H-6′, and H-7′.

[f]J values of pyridinic protons of wilfordic acid (in Hz); H-4′ (dd, 7.8–8.1, 1.8–2.0), H-5′ (dd, 7.8–8.1, 4.8–4.9), H-6′ (dd, 4.8–4.9, 1.8–2.0).

[g]J values of protons of benzoyl groups (in Hz); ortho (dd, 8.5, 1.4–1.5), meta (dd, 8.5, 7.5–8.0), para (dd, 7.5–8.0, 1.4–1.5).

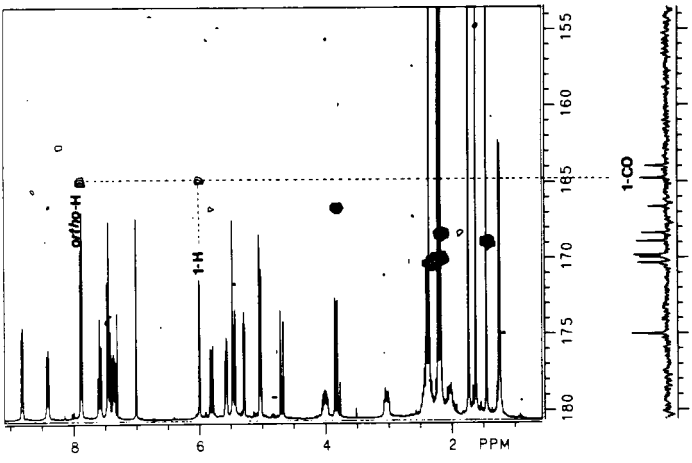


FIGURE 2. ^{13}C-^{1}H long-range COSY spectra of euojaponine F [2].

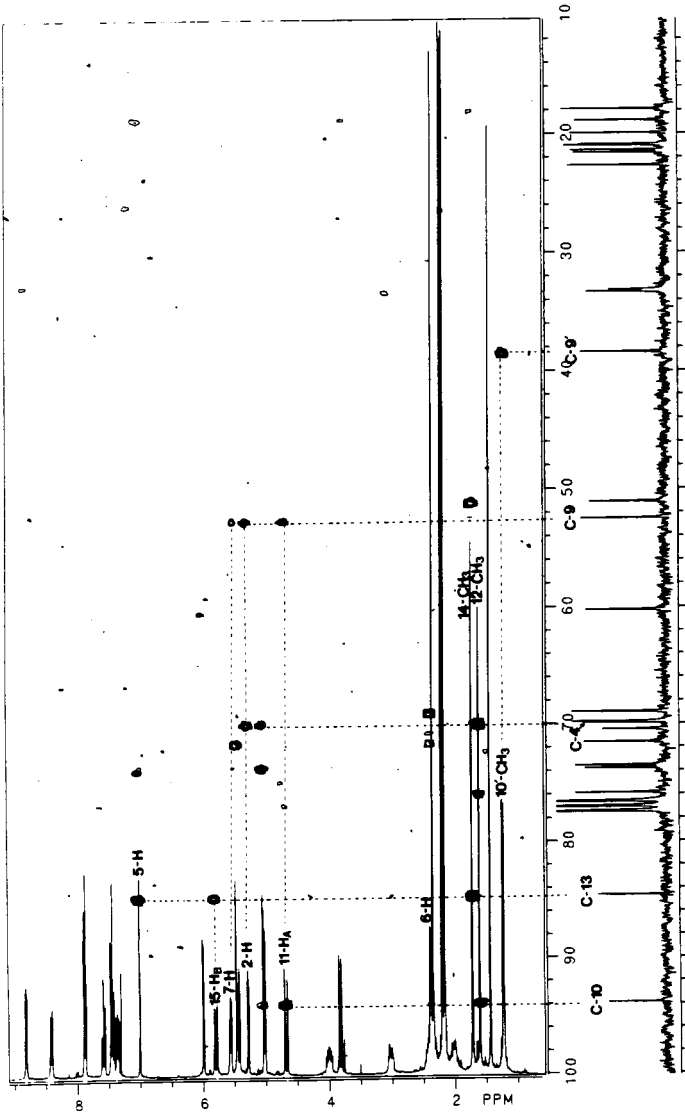


FIGURE 1. ^{13}C-^{1}H long-range COSY spectra of euojaponine F [2].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined using a Mitamura Riken heat block-MRK and are uncorrected. Uv spectra were recorded on a Gilford system 2600 uv-vis spectrophotometer. Specific rotations were determined on a Jasco DIPO-140 digital polarimeter. ^1H and ^{13}C nmr were taken with a GN-300 model at 300 MHz and 75.5 MHz, respectively, in $CDCl_3$. Ms were taken on a Hitachi M80-B double focusing mass spectrometer.

PLANT MATERIAL.—The plant material was collected in December 1987, at Mt. Naejang, Korea, and identified by Prof. S.J. Yoo, College of Pharmacy, Seoung-Kyun-Kwan University. A voucher specimen has been deposited in the Herbarium of the Natural Products Research Institute, Seoul National University.

ISOLATION PROCEDURES.—The root bark (8 kg) of *E. japonica* was powdered and extracted with MeOH (45 liters) and concentrated to give a residue (900 g). The residue was partitioned between Et_2O and H_2O, and the Et_2O layer was concentrated (80 g) and dissolved in MeOH (2 liters) and then treated with Pb(OH)Ac aqueous solution. The precipitate was filtered off and washed with MeOH, and the filtrate was concentrated to give a residue of alkaloidal mixture (11 g).

The alkaloidal mixture was chromatographed on Si gel (420 g) developed with *n*-hexane–EtOAc–iPrOH (100:100:2 and 100:300:3, successively). Fractions 1–5 (1.5 g), fraction 6 (400 mg), fractions 7–9

TABLE 2. ^{13}C-nmr Spectra[a] of Euojaponines D, F, J, and K and Euonine.

Carbon	Compound				
	1	2	3	4	5
C-1	73.47	73.53	76.20	75.46	73.50
C-2	69.90	69.78	71.28	70.64	69.31
C-3	75.03	75.86	76.01	78.16	75.96
C-4	71.89	69.88	37.08	69.81	69.85
C-5	74.12	73.76	74.42	73.95	73.70
C-6	52.38	51.03	50.36	50.98	51.13
C-7	69.13	68.95	69.04	68.97	69.02
C-8	71.76	71.54	72.05	71.86	70.91
C-9	51.19	52.46	51.21	52.80	52.13.
C-10	92.82	93.85	91.25	94.14	93.83
C-11	60.99	60.21	60.84	60.55	60.12
C-12	23.40	22.65	15.18	22.67	22.69
C-13	85.22	84.60	82.68	84.24	84.39
C-14	18.03	17.87	18.08	17.87	17.95
C-15	71.24	70.44	69.57	70.33	70.51
C-2′	165.03	163.97	162.69	163.72	163.72
C-3′	123.86	124.51	125.87	124.58	124.58
C-4′	138.72	139.09	138.48	138.71	139.56
C-5′	121.24	121.30	120.95	121.09	121.50
C-6′	153.46	152.76	152.39	152.96	152.31
C-7′	32.95	33.12	32.38	33.22	32.79
C-8′	33.43	33.28	33.26	33.40	33.28
C-9′	38.10	38.36	37.70	38.54	33.36
C-10′	19.08	18.83	18.89	18.57	18.75
(1-Bz)					
ipso	130.00	129.21	129.36	129.31	
ortho	129.45	129.46	129.69	129.64	
meta	128.36	128.46	128.55	128.50	
para	133.34	133.43	133.59	133.53	

[a]Recorded at 75.5 MHz in $CDCl_3$, chemical shifts (in δ values) relative to internal $CHCl_3$ (77.00 ppm). Assigned by evidence obtained from DEPT and ^{13}C-^1H COSY and ^{13}C-^1H long range COSY spectra. Signals of acetyl methyl carbons and carbonyl carbons appeared in the region of δ 19.90–22.57 and δ 165.00–176.23, respectively.

(1.0 g), fractions 10–11 (1.1 g), fraction 12 (1.9 g), fraction 13 (1.1 g), fraction 14 (780 mg), and fractions 15–17 (1.4 g) were collected. Fraction 12 was chromatographed on Si gel with $CHCl_3$-MeOH (30:1) to give fractions 12-1 and 12-2. Fraction 12-1 was chromatographed on Si gel with C_6H_6-EtOAc (2:1) to yield euojaponines D and F, and euonine-containing fractions (fraction D, fraction F, and fraction H). Fractions D, F, and H were subjected to semi-preparative hplc (RP-18 column, MeOH/H_2O gradient) to give euojaponine D [**1**] (18 mg), euojaponine F [**2**] (164 mg), and euonine [**5**] (12 mg). Fraction 12-2 was chromatographed on Si gel with $CHCl_3$-MeOH (50:1, 10:1) to give fractions J and K. Fractions J and K were further purified by semi-preparative hplc [RP-18 column, MeOH-H_2O (63:35)] to yield euojaponine J [**3**] (12 mg) and euojaponine K [**4**] (160 mg).

Euojaponine D [**1**].—Colorless needles (EtOH), mp 253°; $[\alpha]^{25}D$ +28.6 (c = 0.287, $CHCl_3$); uv λ max (MeCN) nm (log ε) 229 (4.27), 268 (3.59); sims *m/z* $[M+H]^+$ 826, $[M+Na]^+$ 848; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Euojaponine F [**2**].—White powder, mp 142°; $[\alpha]^{25}D$ +9.0 (c = 0.300, $CHCl_3$); uv λ max (EtOH) nm (log ε) 231 (4.28), 272 (3.58); sims *m/z* $[M+H]^+$ 868; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Euojaponine J [**3**].—Colorless needles (EtOH): mp 243°; $[\alpha]^{25}D$ +26.2 (c = 0.042, $CHCl_3$); uv λ max (EtOH) nm (log ε) 229 (4.19), 270 (3.53); sims *m/z* $[M+H]^+$ 810; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Euojaponine K [**4**].—White powder: mp 188°; $[\alpha]^{25}D$ +26.2 (c = 0.367, $CHCl_3$); uv λ max (MeCN) nm (log ε) 229 (4.30), 268 (3.60); sims *m/z* $[M+H]^+$ 826, $[M+Na]^+$ 848; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Euonine [**5**].—White powder: mp 150°; $[\alpha]^{25}D$ −2.5 (c = 6.4, $CHCl_3$); uv λ max (EtOH) nm (log ε) 230 (3.88), 270 (3.52); sims *m/z* $[M+H]^+$ 806, $[M+Na]^+$ 828; ^1H nmr see Table 1; ^{13}C nmr see Table 2.

ACKNOWLEDGMENTS

We thank Dr. P. Kenny in the Suntory Institute for Bioorganic Research, Japan, for providing the mass spectra. We also thank Prof. Yoo for identification of the plant material. This work was supported by a grant from the Korea Science and Engineering Foundation (KOSEF 86-0405-08).

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Received 27 December 1989