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Insecticidal sesquiterpene pyridine alkaloids from *Euonymus* species

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Abstract

Three insecticidal sesquiterpene pyridine alkaloids with a β-dihydroagarofuran sesquiterpene skeleton, euoverrine A (**1**), B (**2**), and euophelline (**3**), and a known compound, euojaponine C (**4**), were isolated from the root bark of *Euonymus verrucosides*, *E. fortunei* and *E. phellomana* by bioassay-guided fractionation. Their chemical structures were elucidated mainly by analyses NMR and MS spectral data.

Keywords: *Euonymus verrucosides*; *E. fortunei*; *E. phellomana*; Celastraceae; Insecticide; β-Dihydroagarofuran sesquiterpene; Alkaloid

1. Introduction

Plants of the Celastraceae family produce various β-dihydroagarofuran sesquiterpene polyol esters and pyridine alkaloids, some of which exhibit insect antifeedant, insecticidal, antitumor, multidrug-resistance reversing, anti-HIV, and immunosuppressive activities (Smith, 1977; Brüning and Wagner, 1978; Tu, 1991; Takaishi et al., 1993; Kim et al., 1998, 1999; Duan and Takaishi, 1999; Duan et al., 1999, 2000, 2001). Many species of the Celastraceae family, such as *Celastrus angulatus*, *C. orbiculatus*, *Tripterygium wilfordii*, *T. forrestii*, and *T. hypoglaucum*, are widely distributed and are used in traditional Chinese medicine for cancer treatment and as insecticides in China (Jacobson, 1958; Cheng and Huang, 1999). In previous studies, some antifeedant, narcotic, and insecticidal components were isolated from *C. angulatus*, *C. flagellris*, and *C. orbiculatus* (Wakabayashi et al., 1988; Wu et al., 1992; Wang and Chen, 1995, 1997; Wu et al., 2001a,b). Various β-dihydroagarofuran sesquiterpene polyol esters and pyridine alkaloids were also isolated from the plants of the genus of *Euonymus* (Smith, 1977; Brüning and Wagner, 1978; Yamada et al., 1977, 1978; Ishiwata et al., 1983; Rozsa and Pelczer, 1989; Rozsa et al., 1989; Han et al., 1990a,b; Tu, 1990; Hohmann, 1995; Wang et al., 2000). In a continuing pursuit of the active principles of the Celastraceae family plants in China, the chemical constituents from the MeOH extracts of the root barks of *Euonymus verrucosides*, *E. fortunei* and *E. phellomana* were investigated by the activity-guided fractionation. These studies have led to the isolation of three novel sesquiterpenoid pyridine alkaloids euoverrine A (**1**), B (**2**), and euophelline (**3**), and a known alkaloid compound, euojaponine C (**4**) (Han et al., 1990a,b), each having the β-dihydroagarofuran skeleton, from the root bark of these three Enonymous species. In this paper, the isolation and structure elucidation of compounds **1**–**3** and the preliminary bioassay data for **1**–**4** against *Mythimna separate* are presented.

2. Results and discussion

Silica gel column and prep. TLC or RP-HPLC isolation of the MeOH extracts of the root barks of *Euonymus verrucosides*, *E. fortunei* and *E. phellomana* yielded four sesquiterpene alkaloids, one of which was characterized as euojaponine C (**4**) on the basis of UV, IR, HR-FAB-MS, ^{1}H NMR and ^{13}C NMR spectroscopic evidence (Han et al., 1990a,b).

Euoverrine A (**1**) was analyzed for $C_{48}H_{51}NO_{18}$ by HR-FAB-MS. Its IR spectrum revealed characteristic ester absorptions at 1720 and 1745 cm^{-1}, and a free hydroxyl absorption 3420 cm^{-1}. The UV spectrum contained an aromatic moiety (230 and 265 nm). The NMR spectral data (Tables 1 and 2) suggested the presence of four acetate esters, two benzoate esters, and one evoninate ester. The ^{1}H NMR spectrum of compound **1** showed the presence of two tertiary methyl

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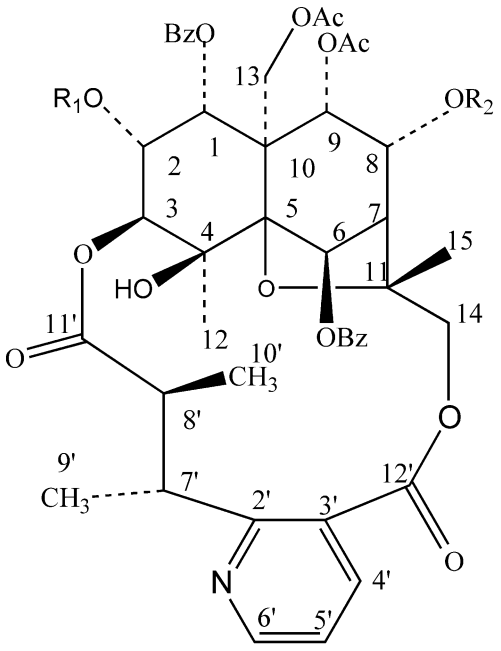

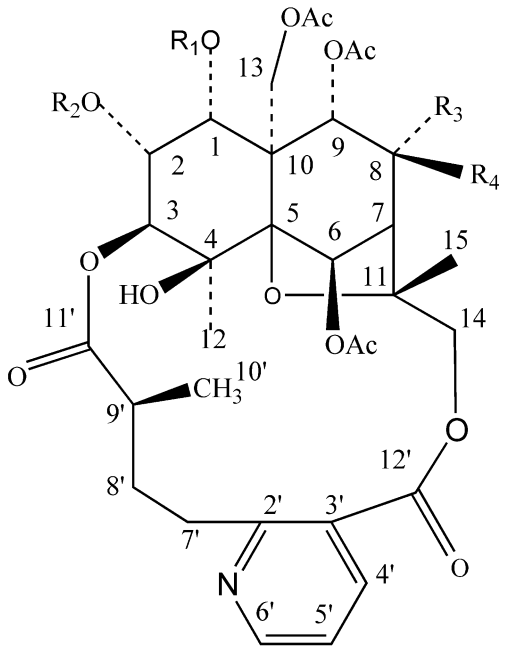

1 R_1 = Ac R_2 = Ac

2 R_1 = Bz R_2 = Ac R_3 = H R_4 = OAc

3 R_1 = H R_2 = CH_3CH_2CO

4 R_1 = H R_2 = Ac

5 R_1 = Ac R_2 = Bz R_3 = OAc R_4 = H

6 R_1 = Bz R_2 = Ac R_3 = OAc R_4 = H

Table 1
^{1}H NMR chemical shifts of compounds **1**–**4** ($CDCl_3$, 500 MHz)

No.	**1**	**2**	**3**	**4**
1	5.80 *d* (3.8)	5.95 *d* (3.4)	5.79 *d* (3.9)	5.78 *d* (3.9)
2	5.45 *dd* (3.8, 2.5)	5.29 *dd* (3.4, 2.5)	4.16 *dd* (3.9, 2.5)	4.16 *dd* (3.8, 2.5)
3	4.86 *d* (2.5)	5.04 *d* (2.5)	4.85 *d* (2.5)	4.86 *d* (2.5)
6	7.22 *s*	6.60 *s*	7.21 *s*	7.22 *s*
7	2.53 *d* (4.2)	2.48 *d* (3.3)	2.53 *d* (4.2)	2.53 *d* (4.2)
8	5.57 *dd* (4.2, 6.0)	5.65 *dd* (3.3, 9.6)	5.59 *dd* (4.2, 6.0)	5.57 *dd* (4.2, 5.9)
9	5.46 *d* (6.0)	5.80 *d* (9.6)	5.45 *d* (6.0)	5.46 *d* (5.9)
12	1.61 *s*	1.62 *s*	1.63 *s*	1.63 *s*
13	4.90 *d*, 5.53 *d* (13.8)	4.83 *d*, 5.01 *d* (12.4)	4.88 *d*, 5.54 *d* (13.7)	4.90 *d*, 5.53 *d* (13.8)
14	3.61 *d*, 6.04 *d* (11.4)	3.86 *d*, 5.67 *d* (11.5)	3.61 *d*, 6.06 *d* (11.4)	3.61 *d*, 6.05 *d* (11.4)
15	1.73 *s*	1.74 *s*	1.73 *s*	1.72 *s*
4′	8.04 *dd* (7.8, 1.8)	8.29 *dd* (7.8, 1.8)	8.04 *dd* (7.8, 1.8)	8.04 *dd* (7.8, 1.8)
5′	7.62 *m*	7.28 *m*	7.56 *m*	7.56 *m*
6′	8.70 *dd* (4.8, 1.8)	8.75 *dd* (4.8, 1.8)	8.71 *dd* (4.8, 1.8)	8.70 *dd* (4.8, 1.8)
7′	4.75 *m*	3.81 *m*, 3.01 *m*	4.74 *m*	4.75 *m*
8′	2.58 *m*	2.18 *m*, 2.26 *m*	2.58 *m*	2.58 *m*
9′	1.42 *d* (7.2)	2.56 *m*	1.42 *d* (7.2)	1.41 *d* (7.2)
10′	1.17 *d* (7.2)	1.27 *d* (6.5)	1.16 *d* (7.2)	1.17 *d* (7.2)
Ac	1.42 *s*	1.40 *s*	1.41 *s*	1.42 *s*
	2.16 *s*	1.96 *s*	2.34 *s*	2.19 *s*
	2.19 *s*	2.15 *s*		2.35 *s*
	2.35 *s*	2.20 *s*		
		2.33 *s*		
Bz	8.34 *dd*, 7.96 *dd*	8.34 *dd*, 7.96 *dd*	8.34 *dd*, 7.95 *dd*	8.34 *dd*, 7.96 *dd*
	7.57 *m*, 7.27 *m*	7.57 *m*, 7.27 *m*	7.59 *m*, 7.26 *m*	7.57 *m*, 7.26 *m*
	7.52 *dd*, 7.42 *dd*	7.52 *dd*, 7.42 *dd*	7.54 *dd*, 7.43 *dd*	7.53 *dd*, 7.42 *dd*
Pr			1.18 *t* (7.0)	
			2.45 *q* (7.0)	

groups at δ 1.61 (*s*) and 1.73 (*s*). The signals observed at δ 5.80 (*d*, $J=3.8$ Hz), 5.45 (*dd*, $J=3.8$, 2.5 Hz) and 4.86 (*d*, $J=2.5$ Hz), 2.53 (*d*, $J=4.2$ Hz), 5.57 (*dd*, $J=4.2$, 6.0 Hz), and 5.46 (*d*, $J=6.0$ Hz) were assigned to the H-1, H-2, H-3, H-7, H-8, and H-9 protons based on the COSY spectrum of **1**, and by comparison with the corresponding chemical shifts and coupling constants of euojaponine C (**4**) (Han et al., 1990a,b). The signals at δ 4.90 and 5.53 (*d*, $J=13.8$ Hz), and 3.61 and 6.04 (*d*, $J=11.4$ Hz) were assigned to the two methylene protons attached to carbon atoms bearing primary ester groups. The single signal observed at δ 7.22 (*s*) was the H-6 proton as in the spectrum of **4** (Han et al., 1990a,b). The ^{13}C NMR DEPT spectrum of the parent skeleton of **1** showed two methyls, two methylenes, seven methines, and four quaternary carbons. Their chemical shifts were very similar to those of **4** and other 1,2,3,4,6,8,9,13,14-septasubstituted β-dihydroagarofuran sesquiterpene pyridine alkaloids (Smith, 1977; Brüning and Wagner, 1978; Han et al., 1990a,b; Cheng et al., 1992; Liu et al., 1993; Kuo et al., 1995; Duan et al., 1997, 1999).

From the HMQC spectrum of compound **1** and comparison with the corresponding carbon atom chemical shifts of euojaponine C (**4**) (Han et al., 1990a,b) and other β-dihydroagarofuran sesquiterpene alkaloids (Han et al., 1990a,b; Cheng et al., 1992; Liu et al., 1993; Kuo et al., 1995; Duan et al., 1997, 1999), the ^{13}C NMR signals were assigned and are given in Table 2. Generally, H-1, H-2, and H-6 in this class of compounds have axial, equatorial, and axial stereochemistry, respectively. The small coupling constant ($J_{2,3}=2.5$ Hz) between H-2 and H-3 suggested that both H-2 and H-3 had an equatorial orientation. The coupling constants ($J_{7,8}=4.2$ Hz, $J_{8,9}=6.0$ Hz) between H-7 and H-8, and between H-8 and H-9, suggested that H-7, H-8, and H-9 had equatorial, equatorial, and axial stereochemistry, respectively, which were confirmed by the cross peaks between H-1 and H-9 in the NOESY spectrum of **1**, and the similar coupling pattern and constants of **4** in the ^{1}H NMR spectrum (Han et al., 1990a,b).

One free hydroxyl group was situated at C-4 (Han et al., 1990a,b). Careful comparison of the ^{1}H NMR chemical shift of **1** with the known compound **4** (Han et al., 1990a,b) isolated from *Euonymus japonica*, indicated that the chemical shift of the H-2 proton was shifted downfield from δ 4.16 to δ 5.45, and that compound **1** had one acetate ester more than **4**, while the other signals were very similar. This result suggested that the hydroxyl group in **4** was changed into the acetate ester group in **1** at C-2, while the other ester groups are the same as in **4**, which were confirmed by the cross peaks between H-1 and the carbonyl at δ 165.0, H-3 and the carbonyl at δ 174.4, H-6 and the carbonyl at δ 165.7, H-8 and the carbonyl at δ 170.1, H-9 and the carbonyl at δ 169.0, H-13 and the carbonyl at δ 170.0, and H-14 and the carbonyl at δ 168.5 in the HMBC spectrum of 1

Table 2
The ^{13}C NMR chemical shifts for compounds 1–4 ($CDCl_3$, 500 MHz)

No.	1	2	3	4
1	75.3	72.6	75.4	75.4
2	70.1	69.3	70.1	70.0
3	78.3	75.3	78.4	78.5
4	70.6	70.2	70.7	70.7
5	94.2	93.8	94.2	94.2
6	75.0	74.8	75.0	75.0
7	50.3	50.1	50.4	50.3
8	69.1	73.2	68.9	69.1
9	71.7	74.5	71.9	71.8
10	53.0	51.9	53.0	53.0
11	84.1	85.9	84.2	84.1
12	23.0	23.3	23.0	23.0
13	60.4	60.6	60.6	60.4
14	71.7	69.8	70.2	70.1
15	18.3	18.2	18.3	18.3
2′	164.6	163.3	164.9	164.9
3′	125.4	124.6	125.5	125.4
4′	137.5	138.9	137.6	137.5
5′	121.0	121.1	121.1	121.1
6′	151.3	152.9	151.3	151.4
7′	36.4	33.1	36.4	36.4
8′	45.0	33.7	45.1	45.1
9′	11.8	38.8	11.9	11.9
10′	9.3	18.9	9.3	9.0
11′	174.4	175.0	174.5	174.6
12′	168.5	167.1	168.5	168.6
Ac	19.1, 168.9	20.2, 168.1	19.9, 169.0	19.9, 169.1
	19.9, 169.0	20.7, 169.6	21.5, 170.2	21.0, 170.1
	20.9, 170.0	20.8, 169.7		21.5, 170.2
	21.5, 170.1	21.2, 169.8		
		21.4, 170.1		
Bz	128.5, 128.8	128.5	128.6, 128.8	128.6, 128.8
	129.2, 129.7	129.4	129.3, 129.4	129.3, 129.4
	130.3, 130.8	130.5	129.8, 130.3	129.8, 130.3
	133.4, 133.5	133.3	133.4, 133.6	133.5, 133.6
	165.0, 165.7	164.7	165.0, 165.8	165.0, 165.8
Pr			9.2	
			27.6	
			173.8	

(Wang and Chen, 1997; Duan et al., 1999; Wu et al., 2001a,b). Thus, the chemical structure of euoverrine A (**1**) was elucidated as 2-acetyleuojaponine C, which was confirmed by the same MS, ^{1}H and ^{13}C NMR spectroscopic data of the acetylation product of euojaponine C with Ac_2O/pyridine (Han et al., 1990a,b).

Euoverrine B (**2**) was determined to be $C_{43}H_{49}NO_{18}$ by HR-FAB-MS. Its IR spectrum exhibited the characteristic ester absorptions at 1720 and 1750 cm^{-1}, and a free hydroxyl absorption at 3410 cm^{-1}. The UV spectrum revealed an aromatic moiety (232 and 266 nm). Its ^{1}H NMR and ^{13}C NMR spectral data suggested the presence of five acetate esters, one benzoate ester, one wilfordate ester, and a free hydroxyl group. The ^{1}H and ^{13}C NMR spectral data of the parent skeleton of **2** were very similar to those of **1** and **4**, which suggested that compound **2** also contained the 1,2,3,4,6,8,9,13,14-septa-substituted-β-dihydroagarofuran skeleton (Smith, 1977;

Brüning and Wagner, 1978; Han et al., 1990a,b; Cheng et al., 1992; Liu et al., 1993; Kuo et al., 1995; Duan et al., 1997, 1999). Based on the COSY, HMQC spectra of **2**, and the NMR spectroscopic data of other sesquiterpene alkaloids (Ishiwata et al., 1983; Han et al., 1990a,b; Cheng et al., 1992), the ^{1}H and ^{13}C NMR signals were assigned and are presented in Tables 1 and 2. Compound **2** was found to have the same stereochemistry for H-1, H-2, H-3, H-6 and H-9 as **1** and **4** because of the similar coupling patterns, coupling constants, and the cross peaks in the NOESY spectrum. However, the coupling constant ($J_{8,9}=9.6$ Hz) is different from **1** and **4**, and is greater than wilforine (**5**) ($J_{8,9}=5.8$ Hz) and euojaponine F (**6**) ($J_{8,9}=5.7$ Hz) (Han et al., 1990a,b; Cheng et al., 1992), which indicated that both H-8 and H-9 are in an axial orientation. These results are in agreement with the cross peaks between H-1 and H-9, and H-6 and H-8 in the NOESY spectrum of **2**.

As with **1**, in euojaponine C (**4**), wilforine (**5**), and euojaponine F (**6**), the free hydroxyl groups were at C-4. The ester group distributions were determined by the HMBC spectrum, which showed cross peaks between H-1 and the carbonyl at δ 164.7, H-2 and the carbonyl at δ 168.1, H-3 and the carbonyl at δ 175.0, H-6 and the carbonyl at δ 169.6, H-8 and the carbonyl at δ 169.8, H-9 and the carbonyl at δ 170.1, H-13 and the carbonyl at δ 169.7, H-14 and the carbonyl at δ 167.1, respectively. These observations indicated that **2** has the same ester group distributions as euojaponine F (**6**), but that the stereochemistry at C-8 was different from euojaponine F (**6**). Thus, the structure of euoverrine (**2**) was elucidated as the epimer of euojaponine F (**6**) at C-8. Careful comparison with wilforine (**5**), indicated that the benzoate was at C-1 and the acetate at C-2 in euoverrine (**2**) and euojaponine F (6), but that the benzoate was at C-2 and the acetate at C-1 in wilforine (**5**).

Euophelline (**3**) was found to be $C_{47}H_{51}NO_{17}$ by HR-FAB-MS. Its IR spectrum revealed characteristic ester absorptions at 1720 and 1740 cm^{-1}, and a free hydroxyl absorption at 3440 cm^{-1}. The UV spectrum also showed an aromatic moiety (231 and 265 nm). Its ^{1}H NMR and ^{13}C NMR spectral data suggested the presence of two acetate esters, two benzoate esters, one propionate ester, and one evoninate ester. The ^{1}H and ^{13}C NMR spectroscopic data of the parent skeleton of **3** were very similar to those of **1**, **2**, **4**, **5**, and **6**, which suggested that compound **3** also contained the 1,2,3,4,6,8,9,13,14-septasubstituted-β-dihydroagarofuran skeleton (Smith, 1977; Brüning and Wagner, 1978; Han et al., 1990a,b; Cheng et al., 1992; Liu et al., 1993; Kuo et al., 1995; Duan et al., 1997, 1999). Based on the COSY spectrum and comparison with the corresponding chemical shifts and coupling constants of **1**, **2**, **4**, **5**, and **6** (Han et al., 1990a,b; Cheng et al., 1992), H-1, H-2, H-3, H-6, H-7, H-8, H-9, and the other protons were assigned and shown in Table 1. The signals at δ 4.88 and 5.54 (*d*, $J=13.7$ Hz) and 3.61 and 6.06 (*d*, $J=11.4$ Hz) were assigned to H-13 and H-14, respectively. The H-2 proton also showed one cross peak with a free hydroxyl proton at δ 2.89 (disappeared when exchanged with D_2O) in the COSY spectrum, which indicated that the substituent group at C-2 was a hydroxyl group. The ester group distributions were determined from the HMBC spectrum, which showed cross peaks between H-1 and the carbonyl carbon at δ 165.0, H-3 and the carbonyl carbon at δ 174.5, H-6 and the carbonyl carbon at δ 165.8, H-8 and the carbonyl carbon at δ 173.8, H-9 and the carbonyl carbon at δ 169.0, H-13 and the carbonyl carbon at δ 170.2, and H-14 and the carbonyl carbon at δ 168.5. These observations indicated that two acetate esters were at C-9 and C-13, two benzoate esters at C-1 and C-6, and the propionate ester at C-8. The H-1, H-2, H-3, H-6, H-7, H-8, and H-9 protons had the same orientation as **1**, euojaponine C (**4**), and wilforine (**5**) because of the very similar coupling constants (Han et al., 1990a,b; Cheng et al., 1992). Therefore, the structure of **3** was elucidated. We found that the propionate ester is at C-8 in **3**, but that the acetate ester is at C-8 in euojaponine C (**4**), the other esters are at the same positions as in **3** and **4** by careful comparison with euojaponine C (**4**). To our knowledge, this is the first time that a propionate ester is found in the β-dihydroagarofuran sesquiterpene polyol esters and alkaloids.

The preliminary bioassay results (for methodology see Wu et al., 1992) showed that the KD_{50} values (the dose required to knock down 50% of the population of *Mythimna separate*) for compounds **1**–**4** were 269.9, 21.6, 168.2, and 102.5 μg/g, respectively. It was very interesting that compound **2** exhibited much more stronger activity than **1**, **3**, **4**, celangulin II (KD_{50} 46.0 μg/g) and celangulin IV (KD_{50} 260.0 μg/g) (Wu et al., 1992). These data confirmed further that the number and orientation of the ester groups, and the existence of the pyridine alkaloids, have characteristic influence on the insecticidal activity of β-dihydroagarofuran sesquiterpene polyol esters and alkaloids (Smith, 1977; Brüning and Wagner, 1978; Wu et al., 2001a,b).

3. Experimental

3.1. General

Melting points were measured on a Yanagimoto apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. UV spectra in MeOH were obtained on a 756MC spectrophotometer. IR spectra were determined on IR-450 instrument (KBr plate). ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC, and NOESY spectra were recorded on Brüker DRX 500

and DPX 300 NMR Spectrometers with $CDCl_3$ as solvent and TMS as internal standard. HR-FAB-MS were obtained on Brüker Apex II mass spectrometer using nitrobenzyl alcohol and sodium chloride as matrix.

3.2. Plant material

The root barks of *Euonymus verrucosides* and *E. fortunei* were collected in 1999 in Songpingsi and Yuntou village, respectively, in Meixian county, Shanxi Province, China. *E. phellomana* was collected in the campus of Northwestern Agricultural University (China, 1999). The plants were authenticated by Professor Yang Jianqiang and Liu Huqi of the Department of Plant Protection, Northwestern Agricultural University. Voucher specimens (samples No. NWAU99-E01, NWAU99-E02 and NWAU99-E03) were deposited at the Department of Plant Protection, Northwestern Agricultural University.

3.3. Extraction and isolation

The dried and pulverized root bark (6.2 kg) of *E. verrucosides* was extracted with MeOH (25 l) under reflux (4 h). The extracted material was re-extracted with acetone (5 l) under reflux (2 h). The acetone extract was re-extracted with petroleum ether (1 l) under reflux (2 h), and evaporated to afford a residue (99 g). This crude extract was chromatographed on a silica gel (200–300 mesh) column using EtOAc-petroleum ether (1:1), EtOAc, and MeOH as eluents to give 3 fractions. Active fractions 1 and 2 were combined (62.5 g) and re-chromatographed on a silica gel (200–300 mesh) column using EtOAc–petroleum ether (1:9–9:1) to give 37 fractions (each 500 ml). Active fractions 25–32 (24.1 g) was re-chromatographed on a silica gel (200–300 mesh) column using MeOH–H_2O to produce an active fraction (8.6 g). The fraction was re-chromatographed on a silica gel (200–300 mesh) column using acetone-petroleum ether (1:9–9:1) as eluent to give 15 fractions. Combined fractions 5–10 were subjected to RP-HPLC (RP-18, MeOH–H_2O 6:4, 7:3 and 9:1) to give 3 fractions. Fractions 1–3 were re-subjected to HPLC (RP-18, MeOH–H_2O 65:35) to afford compounds **1** (18 mg), **2** (22 mg), and **4** (27 mg).

The dried and pulverized root bark (2.5 kg) of *E. fortunei* was extracted with benzene (10 l) under reflux (4 h). The extracted material was re-extracted with acetone under (2 l) reflux (2 h). This crude acetone extract was chromatographed on a silica gel (200–300 mesh) column using EtOAc and MeOH as eluents to give 2 fractions. Active fraction 1 (40 g) was re-chromatographed on a silica gel (200–300 mesh) column using EtOAc–petroleum ether (95:5–50:50) to give 93 fractions. Combined active fractions 71–90 (25 g) were chromatographed on a silica gel (200–300 mesh) column using EtOAc–petroleum ether (1:9–9:1) and MeOH to produce an active fraction (4.62 g). The active fraction was subjected to RP-HPLC (RP-18, MeOH–H_2O 6:4, 7:3 and 9:1) to give 5 fractions. Active fraction 3 (0.65 g) was re-subjected to HPLC (RP-18, MeOH–H_2O 50:50) to afford compound **2** (23 mg).

The dried and pulverized root bark (5.0 kg) of *E. phellomana* was extracted with MeOH (20 l) under reflux (2 h). The extracted material was re-extracted with acetone (3 l) under reflux (2 h). The extracted material was re-extracted with petroleum ether under reflux to produce an oil (40 g) and a precipitate (83 g). This precipitate was chromatographed on a silica gel (200–300 mesh) column using EtOAc–petroleum ether, EtOAc, and MeOH as eluents to give 3 fractions. Active fractions 1 and 2 were combined (54 g) and re-chromatographed on a silica gel (200–300 mesh) column using petroleum ether–EtOAc (9:1–1:9), EtOAc, and MeOH as eluents to give 6 fractions. Active fraction 4 (24.2 g) was re-chromatographed on a silica gel (200–300 mesh) column using acetone-petroleum ether (3:7–10:0) to produce 25 fractions. Combined active fractions 18–20 were subjected to prep. silica gel TLC using toluene–EtOAc-1,4-dioxolane (7:2:1) to afford compounds **3** (20 mg) and **4** (25 mg).

3.3.1. Euoverrine A (*1*)

Amorphous white powder; mp 145–146 °C. $[\alpha]_D^{24}$ +5.5°($CHCl_3$; *c* 0.55). UV λ_{max}^{MeOH} nm: 230, 265. IR υ_{max} cm^{-1}: 3420 (OH), 3060, 2910, 1745, 1720 (C=O), 1600, 1580, 1560, 1450, 1410, 1370, 1315, 1270, 1255, 1110, 1080, 890, 710. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m/z*: 952.3105 $[M+Na]^+$ (calc. for $C_{48}H_{51}NO_{18}Na$, 952.3107).

3.3.2. Euoverrine B (*2*)

Amorphous white powder; mp. 148–149 °C. $[\alpha]_D^{24}$ +10.9° ($CHCl_3$; *c* 0.55). UV λ_{max}^{MeOH} nm: 232, 266. IR υ_{max} cm^{-1}: 3410 (OH), 2950, 1750, 1720 (C=O), 1600, 1580, 1560, 1450, 1435, 1370, 1270, 1155, 1090, 1020, 715. For ^1H NMR and ^{13}C spectral data, see Tables 1 and 2. HR-FAB-MS *m/z*: 890.2820 $[M+Na]^+$ (calc. for $C_{43}H_{49}NO_{18}Na$, 890.2841).

3.3.3. Euophelline (*3*)

Amorphous white powder, mp 168–169 °C. $[\alpha]_D^{24}$ +2.2° ($CHCl_3$; *c* 0.45). UV λ_{max}^{MeOH} nm: 231, 265. IR υ_{max} cm^{-1}: 3440 (OH), 3050, 2960, 1740, 1720 (C=O), 1580, 1560, 1540, 1450, 1370, 1310, 1270, 1180, 1115, 1060, 890, 710. For ^1H and ^{13}C NMR spectral data, see Tables 1 and 2. HR-FAB-MS *m/z*: 924.3072 $[M+Na]^+$ (calc. for $C_{47}H_{51}NO_{17}Na$, 924.3043).

3.3.4. Bioassay

Leaf discs of known area were treated with known amounts of the test samples dissolved in acetone, with acetone itself being used as a negative control). The 5th instar larvae of *M. separata* were fed with the discs for 12 h (repeated 10 times for every sample). After 24 h,

the numbers of knocked-down larvae (symptoms: the larvae were narcotized and could not move; the bodies were immobilized and very soft; and the response disappeared completely) were recorded, and the toxicity was ascertained by estimating the median knock-down dose (KD_{50} value) of the test sample (Wu et al., 1992).

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