

Article

## A New Insecticidal Sesquiterpene Ester from *Celastrus Angulatus*

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Received: 12 March 2009; in revised form: 23 March 2009/ Accepted: 27 March 2009 /

Published: 30 March 2009

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**Abstract:** A new sesquiterpene polyol ester with a  $\beta$ -dihydroagarofuran skeleton, NW37 (**1**), and three known compounds NW13 (**2**), NW16 (**3**) and NW35 (**4**) were isolated by bioassay-guided fractionation from the highly polar MeOH extracts of the root bark of *Celastrus angulatus*. Their chemical structures were elucidated mainly by analyses of MS and NMR spectral data. The insecticidal activity of compound **1** against 4<sup>th</sup> instar *Mythimna separata* larvae with a  $KD_{50}$  value of 252.3  $\mu\text{g}\cdot\text{g}^{-1}$  was demonstrated.

**Keywords:** *Celastrus angulatus*;  $\beta$ -dihydroagarofuran sesquiterpene; Insecticidal activity.

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### 1. Introduction

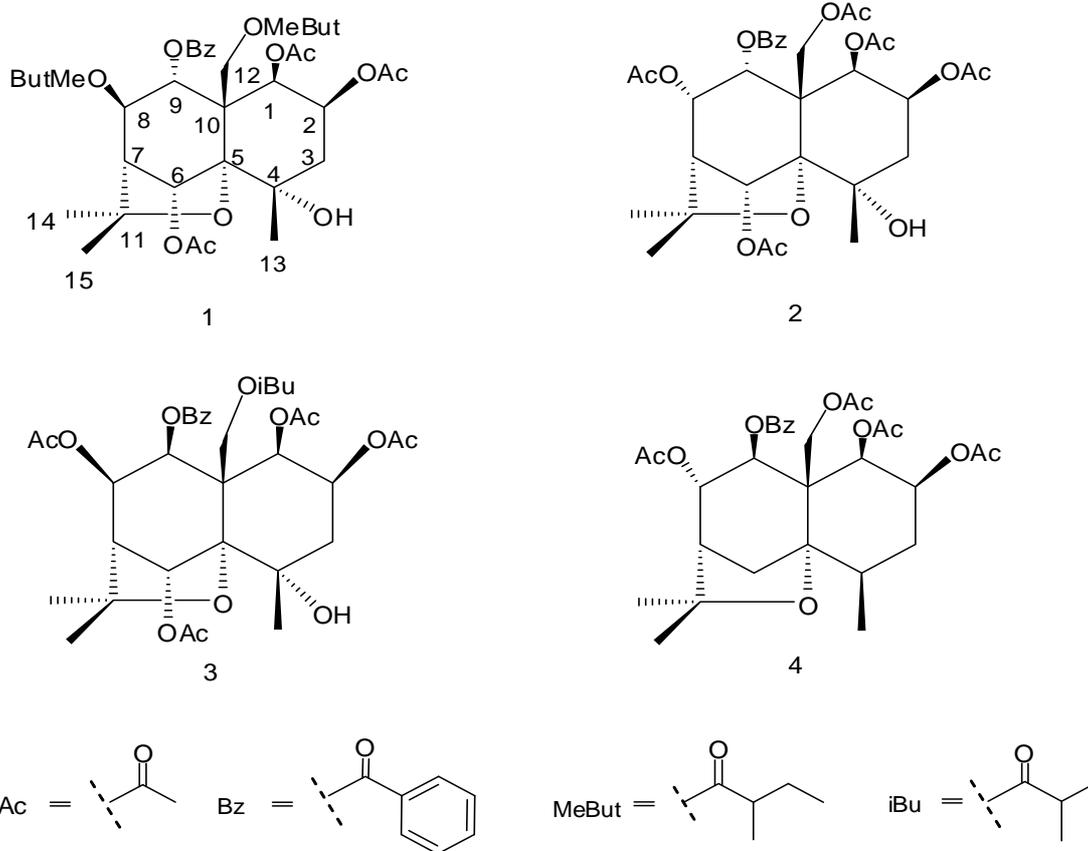
Various  $\beta$ -dihydroagarofuran sesquiterpene polyol esters and pyridine alkaloids, some of which exhibit insect antifeedant, insecticidal, antitumor, reversing multidrug resistance, anti-HIV, and immunosuppressive activities, have been obtained from the plants of the Celastraceae family [1-10]. *Celastrus angulatus*, a plant of the this family, is widely distributed in China and used for the treatment of rheumatism in traditional Chinese medicine and as an insecticide [11,12]. In our previous studies, some antifeedant, narcotic, and insecticidal ingredients were isolated from the toluene extracts of the root bark of *C. angulatus*. To obtain a sufficient number of compounds for QSAR research on their insecticidal activity against *Mythimna separata*, the chemical constituents from the root bark of

*C. angulatus* were re-investigated guided by activity-guided fractionation. These studies have led to the isolation of a novel sesquiterpene polyol ester NW37 (**1**). In this paper, the isolation, structure elucidation and insecticidal activity of compound **1** were presented.

## 2. Results and Discussion

Four sesquiterpene polyol esters **1-4** were isolated from the MeOH extracts of the root bark of *C. angulatus* by macroporous resin column chromatography and RP-HPLC, and their structures were elucidated on the basis of UV, HR-ESI-MS and NMR spectroscopic evidence. Compound **1**, a white powder, analyzed for C<sub>38</sub>H<sub>52</sub>O<sub>14</sub> by HR-ESI-MS ( $m/z$  750.3695 [M+NH<sub>4</sub>]<sup>+</sup>, calculated 750.3700), and NMR spectra data (Table 1). Its IR spectrum revealed characteristic ester absorptions at 1,741 cm<sup>-1</sup>, and a free hydroxyl absorption at 3,510 cm<sup>-1</sup>. The UV spectrum contained an aromatic moiety (232 and 275 nm). The NMR spectra suggested the presence of three acetate esters,  $\delta$  C 169.85 (CO), 169.60 (CO), 169.47 (CO), 21.59 (CH<sub>3</sub>), 21.28 (CH<sub>3</sub>), 20.53 (CH<sub>3</sub>),  $\delta$  H 2.10 (3H, s), 2.07 (3H, s), 1.46 (3H, s), one benzoate ester,  $\delta$  C 164.64 (CO), 133.96 (CH), 130.34 (2×CH), 128.60 (2×CH), 128.52 (C),  $\delta$  H 8.00 (2H, d,  $J=7.0$  Hz), 7.59 (1H, t,  $J=7.0$  Hz), 7.45 (2H, t,  $J=7.0$  Hz) and two  $\alpha$ -methylbutanoate esters,  $\delta$  C 176.68 (CO), 175.42 (CO), 41.28 (CH), 41.22 (CH), 26.65 (CH<sub>2</sub>), 26.58 (CH<sub>2</sub>), 16.68 (CH<sub>3</sub>), 16.49 (CH<sub>3</sub>), 11.82 (CH<sub>3</sub>), 11.67 (CH<sub>3</sub>),  $\delta$  H 2.59 (1H, m), 2.50 (1H, m), 1.80 (2H, m), 1.55 (2H, m), 1.25 (3H, d,  $J=2.0$  Hz), 1.23 (3H, d,  $J=2.0$  Hz), 0.96 (6H, m). The <sup>1</sup>H-NMR of **1** showed the presence of three methyl groups at  $\delta$  1.49 (3H, s, H-13), 1.65 (3H, s, H-14), 1.62 (3H, s, H-15). Based on the published literature [13-14], the <sup>1</sup>H-<sup>1</sup>H COSY spectrum signals at  $\delta$  5.62 (1H, d,  $J=3.5$  Hz, H-1), 5.56 (1H, dd,  $J=3.5$  Hz, 3.0, H-2), 6.25 (1H, s, H-6), 5.32 (1H, d,  $J=3.0$  Hz, H-8) and 5.68 (1H, s, H-9) can be assigned to five protons attached to carbon atoms bearing secondary ester groups, while signals at  $\delta$  4.87 (1H, d,  $J=10.0$  Hz, H-12a) and  $\delta$  4.83 (1H, d,  $J=10.0$  Hz, H-12b) can be assigned to the two protons attached to carbon atoms bearing primary ester groups.

The <sup>13</sup>C-NMR (DEPT) spectrum of the parent skeleton of **1** showed three methyls at  $\delta$  24.72, 25.83 and 29.76, one methylene at  $\delta$  42.27, one methylene attached to an oxygen function at  $\delta$  65.72, one methine at  $\delta$  53.35, five methines attached to an oxygen function at  $\delta$  71.09, 68.26, 75.58, 76.32 and 72.31, one quaternary carbon at  $\delta$  54.20, and three quaternary carbons attached to an oxygen function at  $\delta$  70.06, 83.69 and 91.64, whose chemical shifts were very similar to those of reported  $\beta$ -dihydroagarofurans. It was thus determined that compound **1** was a  $\beta$ -dihydroagarofuran sesquiterpene substituted with three acetate, one benzoate and two  $\alpha$ -methylbutanoate esters. The ester group distributions were determined from the HMBC spectrum, which showed cross-peaks between H-9 and the carbonyl at  $\delta$  164.64 of the benzoate ester, H-12, H-8 and the carbonyl at  $\delta$  176.68, 175.42 of the two  $\alpha$ -methylbutanoate esters, H-1, H-2, H-6 and the carbonyls at  $\delta$  169.85, 169.60, 169.47 of three acetate esters, respectively. In the molecular skeleton of  $\beta$ -dihydroagarofuran sesquiterpenes, H-1 and H-6 have axial stereochemistry. From the results of the NOESY spectrum of **1**, the correlation between H-6 and H-9 indicated the presence of H-9<sub>eq</sub> and the correlation between H-14 and H-8 indicated the presence of H-8<sub>eq</sub> (Figure 2). Therefore, compound **1** was identified as 1 $\beta$ ,2 $\beta$ ,6 $\alpha$ -triacetoxy-8 $\beta$ ,12-di-( $\alpha$ -methyl)butanoyl-9 $\alpha$ -benzoyloxy-4 $\alpha$ -hydroxy- $\beta$ -dihydroagarofuran.

**Figure 1.** The structures of compounds 1-4.

NW13(**2**), NW16(**3**) and NW35(**4**) were known compounds, and there were characterized as 1 $\beta$ ,2 $\beta$ ,6 $\alpha$ ,8 $\alpha$ ,12-pentaacetoxy-9 $\alpha$ -benzoyloxy-4 $\alpha$ -hydroxy- $\beta$ -dihydroagarofuran (**2**) [13], 1 $\beta$ ,2 $\beta$ ,6 $\alpha$ ,8 $\beta$ -tetraacetoxy-9 $\beta$ -benzoyloxy-12-isobutanoyloxy-4 $\alpha$ -hydroxy- $\beta$ -dihydroagarofuran (**3**) [14] and 1 $\beta$ ,2 $\beta$ ,8 $\alpha$ ,12-tetraacetoxy-9 $\beta$ -benzoyloxy- $\beta$ -dihydroagarofuran (Angulatueoid B, **4**) [15] on the basis of UV, IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectroscopic evidence.

**Table 1.** The NMR data of compound **1**. ( $\text{CDCl}_3$ ,  $^1\text{H}$ -NMR at 500 MHz,  $^{13}\text{C}$ -NMR at 125MHz, respectively)

No.	$\Delta C$ (DEPT)	$\delta\text{H}$ (J, Hz)	HMBC
1	71.09 CH	5.62 (1H, d, $J=3.5$ Hz)	C-2,C-10, C=O of Ac
2	68.26 CH	5.56 (1H, dd, $J=3.5$ Hz, $J=3.0$ Hz)	C-10, C=O of Ac
3	42.27 $\text{CH}_2$	2.24 (1H, m), 2.00 (1H, m)	C-1,C-2,C-4,C-5,C-13
4	70.06 C		
5	91.64 C		
6	75.58 CH	6.25 (1H,s)	C-5,C-7,C-8,C-10,C-11, C=O of Ac
7	53.35 CH	2.37 (1H, d, $J=3.0$ Hz)	C-5,C-6,C-8,C-9
8	76.32 CH	5.32 (1H, d, $J=3.0$ Hz)	C=O of MeBut
9	72.31 CH	5.68 (1H, s)	C-5,C-7,C-8,C-10,C-12, C=O of Bz

Table 1. Cont.

No.	$\Delta C$ (DEPT)	$\delta H$ (J, Hz)	HMBC
10	54.20 C		
11	83.69 C		
12	65.72 CH <sub>2</sub>	4.87 (1H,d, $J=10.0$ Hz) 4.83 (1H,d, $J=10.0$ Hz)	C-1,C-5,C-9,C-10, C=O of MeBut
13	24.72 CH <sub>3</sub>	1.49 (3H, s)	C-4, C-5
14	25.83 CH <sub>3</sub>	1.65 (3H, s)	C-7, C-11
15	29.76 CH <sub>3</sub>	1.62 (3H, s)	C-7, C-11
Ac	169.85 (CO), 21.59 (CH <sub>3</sub> )	2.10 (3H, s)	
Ac	169.60 (CO), 21.28 (CH <sub>3</sub> )	2.07 (3H, s)	
Ac	169.47 (CO), 20.53 (CH <sub>3</sub> )	1.46 (3H, s)	
MeBut	176.68 (CO) 41.28 (CH), 26.65 (CH <sub>2</sub> ), 11.82 (CH <sub>3</sub> ), 16.68 (CH <sub>3</sub> )	2.59 (1H, m), 1.80 (2H, m), 1.25 (3H, d, $J=2.0$ Hz), 0.96 (3H, m)	
MeBut	175.42 (CO) 41.22 (CH), 26.58 (CH <sub>2</sub> ), 11.67 (CH <sub>3</sub> ), 16.49 (CH <sub>3</sub> )	2.50 (1H, m), 1.55 (2H, m), 1.23 (3H, d, $J=2.0$ Hz), 0.96 (3H, m)	
Bz	164.64 (CO), 133.96 (CH), 130.34 (2×CH), 128.60 (2×CH), 128.52 (C)	8.00 (2H, d, $J=7.0$ Hz), 7.59 (1H, t, $J=7.0$ Hz), 7.45 (2H, t, $J=7.0$ Hz)	

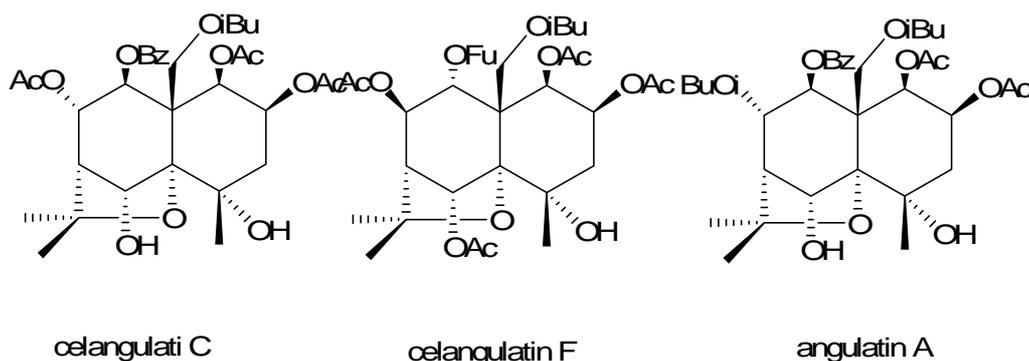
The insecticidal activities of compounds **1-4** against 4<sup>th</sup> instar larvae of *Mythimna separata* were tested by the leaf disc method (for the methodology see [13-14,16-17]). The result showed that the  $KD_{50}$  value for compound **1** was  $252.3 \mu\text{g}\cdot\text{g}^{-1}$ . The symptoms displayed by the *Mythimna separata* indicated that these compounds have stronger insecticidal but not narcotic or antifeedant activities. On comparison of the  $KD_{50}$  data of compounds **1-4** presented in Table 2 and other compounds isolated in our laboratory, such as celangulatin C ( $KD_{50}=280.4 \mu\text{g}\cdot\text{g}^{-1}$ ), celangulatin F ( $KD_{50}=201.5 \mu\text{g}\cdot\text{g}^{-1}$ ) and angulatin A ( $KD_{50}=300.9 \mu\text{g}\cdot\text{g}^{-1}$ ) (for structures see Figure 3) [18], it was very interesting to note that compound **4** exhibited weaker activities than compound **1-3** and other compounds. For the structure of these compounds, it is obvious that the stereochemistry and the type of the ester groups at C-1 and C-2 in these compounds are similar, and the differences between them are the substitution groups at C-8, C-9 and C-12. In addition, the protons of C-4 and C-6 of compound **4** were not substituted by hydroxyl or ester groups, which indicated that the C-4 and C-6 substituents have a positive effect on

the insecticidal activity. Moreover, these results suggested that the substitutes and stereochemistry of C-8, C-9, and C-12 play important roles in these compounds [13, 18-20].

**Table 2.** The  $KD_{50}$  data of 1~4 and other compounds.

Compounds	$KD_{50}$ ( $\mu\text{g}\cdot\text{g}^{-1}$ )
1	252.3
2	290.1
3	360.2
4	884.3
Celangulatin C	280.4
Celangulatin F	201.5
Angulatin A	300.9

**Figure 3.** The structures of celangulatin C, celangulatin F and angulatin A.



### 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 (USA). IR spectra were determined on an IR-450 instrument (KBr plate).  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , DEPT, COSY, HMQC, HMBC, and NOESY spectra were recorded on Bruker Avance 500 MHz NMR Spectrometer with  $\text{CDCl}_3$  as solvent and TMS as internal standard. HR-ESI-MS was obtained on a Bruker Apex II mass spectrometer. Finnigan LCQ Advantage MAX LC/MS, equipped with Surveyor DAD detector and Hypersil  $\text{ODS}_2$   $\text{C}_{18}$  column (4.6×250 mm, 5  $\mu\text{m}$ , Dalian Elite Analytical Instruments Co., Ltd., P.R. China), was used to analyse the samples. Compounds were purified with a Waters 600E HPLC apparatus equipped with a Hypersil  $\text{ODS}_2$   $\text{C}_{18}$  preparative column (20 × 250 mm, 10  $\mu\text{m}$ , Dalian Elite Analytical Instruments Co., Ltd., P.R. China), MeOH- $\text{H}_2\text{O}$  (55: 45) as eluent, UV detector set at 230 nm.

### 3.2. Plant material

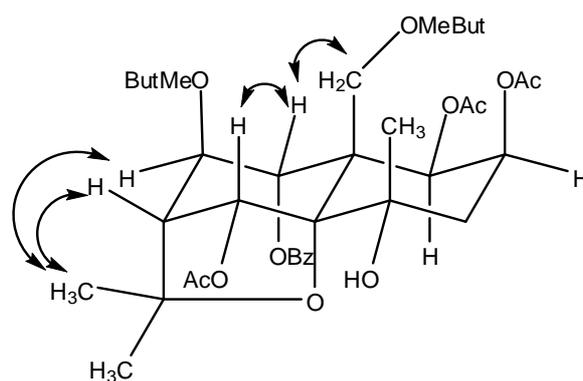
The root bark of *C. angulatus* was collected in Qinling mountain, Taibai County, Shaanxi Province, People's Republic of China, in October 2007, authenticated by Dr. Hua Yi of the College of Life Sciences, Northwest Agricultural & Forestry University, and dried in the shade (at room temperature). Voucher specimens (samples no. NWAU2007-A18) were deposited at the College of Plant Protection, Northwest Agricultural & Forestry University.

### 3.3. Extraction and isolation

The dried and pulverized root bark (2.0 kg) of *C. angulatus* was extracted four times with MeOH (6.0L) under reflux. The extracted material (120 g) was adsorbed in a D101 macroporous resin (Hebei Cangzhou Chemical Co., Ltd., P.R. China) column (5.0×150 cm) and eluted with MeOH-H<sub>2</sub>O (5:5, 6:4, 7:3), and 100 fractions of ca. 500 mL each were collected. After removal of the solvents under reduced pressure, fractions were analysed by LC/DAD/MS, and similar ones were combined. The insecticidal activity of every fraction was assayed. Then the fractions which containing unknown sesquiterpene polyol esters were selected for further purification by RP-HPLC column, affording four compounds: NW37 (**1**, 75 mg), NW13 (**2**, 78 mg), NW16 (**3**, 92mg) and NW35 (**4**, 35mg).

**Compound 1:** C<sub>38</sub>H<sub>52</sub>O<sub>14</sub>, white powder, -12.0° (CH<sub>3</sub>COCH<sub>3</sub>, c 1.20); IR  $\nu$ : 3510, 2926, 1741, 1632, 1380, 1232, 1060, 891, 712 cm<sup>-1</sup>; UV: 232, 275 nm; ESI-MS (MS/MS):  $m/z$  (%) 755 [M+Na]<sup>+</sup> (17), 695 [M+Na-AcOH]<sup>+</sup> (80), 653 [M+Na-MeBuOH]<sup>+</sup> (100), 633 [M+Na-BzOH]<sup>+</sup> (21), 593 [M+Na-AcOH-MeBuOH]<sup>+</sup> (12). <sup>1</sup>H- and <sup>13</sup>C-NMR (CDCl<sub>3</sub>) see Table 1. Major NOESY correlations Figure 2.

**Figure 2.** Major NOESY correlations in **1**.



### 3.4. Insecticidal activity

Toxic leaf discs of known area were treated with known amounts of the test samples dissolved in acetone (acetone and celangulin V were used as negative and positive control). The 4<sup>th</sup> instar larvae of *M. separata* were fed with the discs for 12 h (repeated 10 times for each 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<sub>50</sub> value) of the test sample [14].

### Acknowledgements

These projects were financed by the National 973 project (No. 2003CB114404) and the National Natural Science Foundation of China (No.30800729).

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*Sample Availability:* Samples of the compounds are available from the authors.

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