Celangulins II, III, and IV: New Insecticidal Sesquiterpenoids from Celastrus angulatus

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J. Nat. Prod., **1992**, 55 (9), 1294-1298• DOI:
10.1021/np50087a018 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 9, pp. 1294-1298, September 1992

CELANGULINS II, III, AND IV: NEW INSECTICIDAL SESQUITERPENOIDS FROM *CELASTRUS ANGULATUS*

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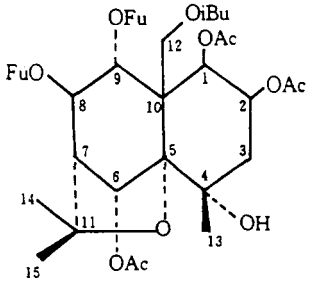
ABSTRACT.—Three new insecticidal sesquiterpene polyol esters were isolated from the root bark of *Celastrus angulatus*. Their structures were determined, mainly by nmr and ms, as 1β,2β,6α-triaacetoxy-12-isobutanoyloxy-8β,9α-di(β-furancarbonyloxy)-4α-hydroxy-β-dihydroagarofuran [**1**] (celangulin II), 1β,2β,6α,8β-tetraacetoxy-9α-benzoyloxy-12-isobutanoyloxy-4α-hydroxy-β-dihydroagarofuran [**2**] (celangulin III), and 1β,2β,6α,8β-tetraacetoxy-9β-benzoyloxy-12-isobutanoyloxy-4α-hydroxy-β-dihydroagarofuran [**3**] (celangulin IV).

The Chinese bittersweet, *Celastrus angulatus* Max. (Celastraceae), is a traditional insecticidal plant widely distributed over the Yellow River and Yangtze River basins of China. Results of bioactivity tests showed that the extract of the root bark of this plant exhibited antifeedant, narcotic, and insecticidal activity against several insect species (1). In our previous study, a new insect antifeedant, celangulin I, was isolated from the root bark of *C. angulatus* (2). Even though several other components were isolated subsequently by other researchers, no significant biological activity for these components was reported (3,4). Continued activity-guided fractionation has led to isolation of three new β-dihydroagarofuran compounds, celangulins II [**1**], III [**2**], and IV [**3**], from the root bark of *C. angulatus*. All three showed strong narcotic or insecticidal action against the armyworm (*Mythimna separata*). This paper presents the structure elucidation and bioassay of **1–3**.

RESULTS AND DISCUSSION

Compound **1** analyzed for $C_{35}H_{42}O_{16}$ by hrms. Its ir spectrum revealed absorptions for ester (ν 1747 cm^{-1}) and free hydroxy (ν 3560 cm^{-1}) groups. The eims exhibited peaks at *m/z* 659 $[M-OAc]^+$, *m/z* 607 $[M-C_5H_3O_3]^+$, and *m/z* 71 $[C_3H_7CO]^+$. The ^1H- and ^{13}C-nmr spectra suggested the presence of three acetate esters [δ_H 1.66, 2.08, and 2.13 (3 × 3H, 3 × s); δ_C 20.5, 21.0, and 21.4 (3 × Me), and 3 × 169.6 (3 × $-CO_2-$)], one isobutanoate ester [δ_H 1.07 and 1.15 (2 × 3H, 2 × d, $J=7$ Hz) and 2.59 (1H, m); δ_C 18.8 and 18.9 (2 × Me), 34.0 (CH), and 176.7 ($-CO_2-$)], two β-furancarboxylate esters [δ_H 8.21 and 8.02 (2 × 1H, 2 × dd, $J=0.8$, 1.5 Hz), 7.46, and 7.44 (2 × 1H, 2 × dd, $J=1.5$, 2Hz), and 6.85 and 6.74 (2 × 1H, 2 × dd, $J=0.8$, 2 Hz); δ_C 148.9, 148.7, 2 × 143.9, 109.8, and 109.7 (6 × CH), 118.8, and 118.1 (2 × quaternary carbons), and 161.3 and 160.6 (2 × $-CO_2-$)], and one free hydroxy group [δ_H 2.74 (1H, brs, disappeared when exchanged with D_2O)]. In addition, the ^{13}C-nmr and DEPT spectra suggested that the remaining parent consisted of fifteen carbons: three methyl, two methylene, six methine, and four quaternary carbons, whose chemical shifts (Table 2) together with the ^1H-nmr data (Table 1) were very similar to those assigned to the 1,2,4,6,8,9,12-heptasubstituted β-dihydroagarofuran skeleton (2,5). Therefore, **1** had substituents at C-1, C-2, C-4, C-6, C-8, C-9, and C-12.



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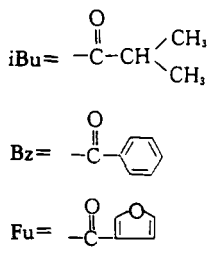

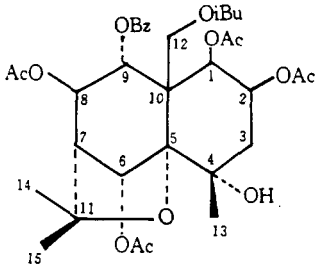

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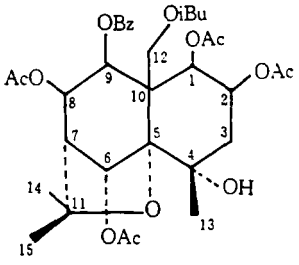

3

Generally, H-1, H-2, and H-6 in this class of compounds have axial, equatorial, and axial sterochemistry (2,6), respectively. The weak coupling ($J_{8,9} = 0$ Hz) between H-8 and H-9 suggested that both H-8 and H-9 had equatorial sterochemistry as, in this class of compounds, the angle between H-8 and H-9 is near 90° (6,7).

The free hydroxy group was assumed to be at C-4 because in all other known compounds of this class the tertiary 4-OH is not esterified (6). The ester group distribution was determined on the basis of ^{1}H-^{13}C long-range correlation (COLOC) spectrum (8),

TABLE 1. ^{1}H-nmr Data for the Sesquiterpenoid Protons of Compounds **1–3**.[a]

Hydrogen	Compound		
	1	**2**	**3**
H-1	5.53[b]	5.61 d (3.7)	5.48 d (3.8)
H-2	5.53[b]	5.54 m	5.33 m
H_{ax}-3	2.22 dd (6,14)	2.23 dd (6,14)	2.22 dd (5,15)
H_{eq}-3	1.88 dd (2,14)	1.89 dd (2,14)	1.95 dd (3,15)
H-6	6.36 s	6.33 s	6.81 s
H-7	2.47 d (3)	2.40 d (3)	2.44 d (3.5)
H-8	5.45 d (3)	5.27 d (3)	5.58 dd (3.5, 6)
H-9	5.75 s	5.64 s	5.62 × d (6)
H-12	4.78, 4.98 ABq (13)	4.66, 4.97 ABq (13)	4.70, 5.17 ABq (13.6)
Me-13	1.49 s	1.50 s	1.50 s
Me-14	1.68 s	1.65 s	1.65 s
Me-15	1.60 s	1.57 s	1.56 s

[a]Assignments of signals were made based on comparison with literature data (2) and on COLOC spectra.

[b]Signals appear as a multiplet.

TABLE 2. ^{13}C-nmr Chemical Shifts for the Sesquiterpenoid Carbons of Compounds **1–3**.[a]

Carbon	Compound		
	1	**2**	**3**
C-1[b]	70.3	68.0	67.9
C-2[b]	70.6	70.8	69.9
C-3	42.0	42.7	42.1
C-4	69.9	69.9	69.8
C-5	91.4	91.5	91.7
C-6[b]	75.6	72.5	72.0
C-7	53.6	53.2	53.4
C-8[b]	76.1	75.8	75.5
C-9[b]	68.0	76.7	75.9
C-10	54.4	54.4	53.1
C-11	83.4	83.3	82.5
C-12	65.0	65.6	60.8
Me-13	24.4	24.6	24.3
Me-14	25.5	25.7	24.4
Me-15	29.6	29.5	29.3

[a]Assignments of chemical shifts were made on the basis of DEPT and COLOC spectra and by comparison with the literature data (2).

[b]Data in same column may be exchangeable.

which showed the cross peaks between H-1 (δ 5.53) and H-2 (δ 5.53), and the carbonyls (δ 169.6) of two acetate esters, between H-6 (δ 6.36) and the carbonyl (δ 169.6) of third acetate ester, between H-8 (δ 5.45) and the carbonyl (δ 161.3) of one β-furancarboxylate ester, and between H-9 (5.75) and the carbonyl (δ 160.6) of a second β-furancarboxylate ester. These observations suggested three acetate esters at C-1, C-2, and C-6, and two β-furancarboxylate esters at C-8 and C-9. The remaining isobutanoate ester was located at C-12. Therefore, the structure of **1** is 1β,2β,6α-triacetoxy-12-isobutanoyloxy-8β,9α-di(β-furancarbonyloxy)-4α-hydroxy-β-dihydroagarofuran.

Compound **2** analyzed for $C_{34}H_{44}O_{14}$ by hrms. Its ir, mass, ^{1}H-nmr, and ^{13}C-nmr spectral data (see Experimental) suggested the presence of four acetate esters, one benzoate ester, one isobutanoate ester, and one free hydroxy group. The ^{1}H- and ^{13}C-nmr data for the parent were very similar to those of **1**, suggesting that **2** also contained the 1,2,4,6,8,9,12-heptasubstituted β-dihydroagarofuran skeleton, and that **2** had the same sterochemistry for H-8 and H-9 as **1**. As with **1**, the ester group distribution was determined from the ^{1}H-^{13}C long-range correlation spectrum, which showed cross peaks between H-1 (δ 5.61) and the carbonyl (δ 169.3) of one acetate ester, H-2 (δ 5.54) and the carbonyl (δ 169.6) of a second acetate ester, H-6 (δ 6.33) and the carbonyl (δ 169.5) of a third acetate ester, H-8 (δ 5.27) and the carbonyl (δ 169.6) of a fourth acetate ester, and H-9 (δ 5.64) and the carbonyl (δ 164.5) of a benzoate ester. This suggested four acetate esters at C-1, C-2, C-6, and C-8, and a benzoate ester at C-9. The remaining isobutanoate ester was located at C-12. Therefore, **2** was elucidated as 1β,2β,6α,8β-tetraacetoxy-9α-benzoyloxy-12-isobutanoyloxy-4α-hydroxy-β-dihydroagarofuran.

Compound **3** analyzed for $C_{34}H_{44}O_{14}$ by hrms. As with compound **2**, the ir, mass, ^{1}H-nmr, and ^{13}C-nmr spectral data suggested that **3** also contained four acetate esters, one benzoate ester, one isobutanoate ester, one free hydroxy group, and the 1,2,4,6,8,9,12-heptasubstituted β-dihydroagarofuran skeleton. These two com-

pounds were not same, because **2** did not have ^{1}H- and ^{13}C-nmr data identical with those of **3**. The spectra of **3**, however, similar to those of celangulin [**1**] (2).

As reported in our previous investigation (2), the coupling constant ($J_{8,9}$ = 6 Hz) between H-8 and H-9 suggested that H-8 was equatorial and that H-9 was axial. This was confirmed by an nOe difference experiment in which irradiation of H-14 (δ 1.65) caused enhancements of H-8 at δ 5.58 (9.3%) and H-9 at δ 5.62 (11%). The ester group distribution was also determined from the ^{1}H-^{13}C long-range correlation spectrum, which showed cross peaks between H-1 (δ 5.48), H-2 (δ 5.33), and H-6 (δ 6.81), and the carbonyls (δ 169.4) of three acetate esters, H-8 (δ 5.58) and the carbonyl (δ 169.9) of a fourth acetate ester, and H-9 (δ 5.62) and the carbonyl (δ 164.5) of a benzoate ester. This indicated four acetate esters at C-1, C-2, C-6, and C-8, and a benzoate ester at C-9. The remaining isobutanoate ester was located at C-12. Thus, **3** was elucidated as 1β,2β,6α,8β-tetraacetoxy-9β-benzoyloxy-12-isobutanoyloxy-4α-hydroxy-β-dihydroagarofuran.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr, ^{13}C-nmr, DEPT, nOe difference, and ^{1}H-^{13}C long-range COSY spectra were obtained on a Bruker AM-400 nmr spectrometer in $CDCl_3$ with TMS as internal standard. The data matrix for COLOC was 512 × 2K. Uv spectra in MeOH were obtained on a UV-240 spectrophotometer. Ir spectra were determined on an FT-170SX instrument (KBr). Rotation data were recorded on a J-20C instrument in $CHCl_3$. Eims and hrms were obtained on a VG ZAB-HS mass spectrometer operating at 70 eV. Hplc was carried out on a reversed-phase column (μ Bondapak C_{18}, 10μ, 30 × 0.78 cm i.d.) with MeOH/H_2O as eluent.

PLANT MATERIAL.—The root bark of *C. angulatus* was collected in Shaanxi Province (China), and authenticated by the faculty of the Department of Plant Protection (Northwestern Agricultural University). Voucher specimens were deposited at the Department of Plant Protection. A modified leaf-sandwich method of Campbell and Filmer (9) with the 5th instar larvae of *M. separata* was used for bioactivity-guided isolation.

EXTRACTION AND ISOLATION.—The dried and pulverized root bark (1 kg) of *C. angulatus* was extracted with petroleum ether under reflux for 3 h. The extracted material was re-extracted with petroleum ether two times. The extracts obtained were combined and concentrated to give a yellow semisolid residue (41.8 g). A portion (20 g) of this crude extract was chromatographed on a Si gel (200–300 mesh) column using EtOAc-petroleum ether (2:8→8:2) as eluent to give 145 fractions (each 50 ml), and then using EtOAc-MeOH (2:8) as eluent to give fractions 146–170 (each 50 ml). Fractions 71–96 were combined (4.6 g) and rechromatographed on Si gel (200–300 mesh) using dioxane-petroleum ether (25:75) to give 92 fractions (each 50 ml). Fractions 39–42 (760 mg) and 55–61 (850 mg) were subjected to reversed-phase hplc on a C_{18} column with MeOH-H_2O (57:43) as eluent to yield compounds **1** (28 mg), **2** (33 mg), and **3** (38 mg).

BIOASSAY.—Leaf discs of known area were treated with known amounts of the test samples dissolved in Me_2CO. The 5th instar larvae of *M. separata* were fed with the discs for 2 h. The areas eaten were measured under a binocular microscope by counting 1-mm squares exposed when the partially eaten disc was placed on a circle the exact size of the disc drawn on mm-ruled paper. 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) or dead larvae were recorded, and the toxicity was ascertained by estimating the median knock-down dose or median lethal dose of the test sample. The KD_{50} (the dose required to knock down 50% of the population) for **1** and **3** was 46 μg/g and 260 μg/g, respectively, and the LD_{50} (the dose required to kill 50% of the population) for **2** was 110 μg/g.

Compound **1**.—Compound **1** was obtained as an amorphous white powder: $[\alpha]^{18}D$ −28.2 (c = 0.865, $CHCl_3$); uv λ max nm (log ε) 245 (3.898); ir ν max cm^{-1} (KBr) 3560, 2980, 1747, 1576, 1510, 1370, 1312, 1234, 1162, 1081, 1037, 982, 945, 873, 751; eims *m/z* $[M-OAc]^+$ 659, $[M-$furancarbonyloxy$]^+$ 607, $[M-2\times OAc]^+$ 600, $[607-Me]^+$ 592, $[607-HOAc]^+$ 547, $[547-HOAc]^+$ 487, 244, 192, [furancarbonyl]$^+$ 95 (100%), 71; hrms *m/z* 607.2361 (calcd for $C_{30}H_{39}O_{13}$, 607.2379); ^{1}H nmr δ ppm 1.66, 2.08 and 2.13 (3 × 3H, 3 × s, 3 × Ac), 6.74 and 6.85 (2 × 1H, 2 × dd, J = 0.8, 2 Hz), 7.44 and 7.46 (2 × 1H, 2 × dd, J = 1.5, 2 Hz), 8.02 and 8.21 (2 × 1H, 2 × dd, J = 0.8, 1.5 Hz, 2 × β-furancarbonyl), 1.07 and 1.15 (2 × 3H, 2 × d, J = 7 Hz), 2.59 (1H, m, isobutanoyl), 2.74 (1H, brs, 4-OH); ^{13}C nmr δ ppm 20.5, 21.0, 21.4, 3 × 169.6 (3 × OAc), 109.7, 109.8, 118.1, 118.8, 148.7,

148.9, 2×143.9, 160.6 and 161.3 (2×β-furancarbonyloxy), 18.8, 18.9, 34.0, 176.7 (isobutanoyloxy); ^{1}H nmr (sesquiterpenoid portion) see Table 1; ^{13}C nmr (sesquiterpenoid portion) see Table 2.

Compound **2**.—Compound **2** was obtained as amorphous white powder: $[\alpha]^{18}D$ −2.8 (c = 0.750, $CHCl_3$); uv λ max nm (log ε) 208.5 (2.765), 237 (3.937), 274 (1.573), 281 (2.059); ir ν max cm^{-1} (KBr) 3560, 2979, 1746, 1570, 1252, 1150, 1090, 1028, 916, 758, 712; eims *m/z* $[M-Me]^+$ 661, $[M-OAc]^+$ 617, $[M-2\times HOAc]^+$ 556, [556 − ketene]$^+$ 514, 437, 244, 202, $[PhCO]^+$ 105 (100%), 71, 43; hrms *m/z* 556.2273 (calcd for $C_{30}OH_{36}O_{10}$, 556.2298); ^{1}H nmr δ ppm 1.47, 2.09, 2.12, and 2.20 (4×3H, 4×s, 4×Ac), 7.44 (2H, m), 7.60 (1H, m), 8.00 (2H, dt, J = 1.3, 8.3 Hz, PhCO), 1.29 and 1.31 (2×3H, 2×d, J = 7 Hz), 2.82 (1H, m, isobutanoyl), 2.75 (1H, brs, 4-OH); ^{13}C nmr δ ppm 20.3, 2×21.0, 21.4, 169.3, 169.5, 2×169.6 (4×OAc), 2×128.4, 129.5, 2×130.2, 133.8, 164.5 ($PhCO_2$), 19.0, 19.1, 34.1, 176.9 (isobutanoyloxy); ^{1}H nmr (sesquiterpenoid portion) see Table 1; ^{13}C nmr (sesquiterpenoid portion) see Table 2.

Compound **3**.—Compound **3** was obtained as an amorphous white powder: $[\alpha]^{18}D$ +7.6 (c = 0.893, $CHCl_3$); uv λ max nm (log ε) 240 (4.12), 273 (2.911), 281 (2.714); ir ν max cm^{-1} (KBr) 3562, 2977, 1742, 1570, 1229, 1150, 1078, 1027, 875, 759, 712; eims *m/z* $[M]^+$ 676, $[M-Me]^+$ 661, $[M-OAc]^+$ 617, [617 − OAc]$^+$ 557, [557 − ketene]$^+$ 515, [515 − OAc]$^+$ 456, 244, 202, $[PhCO]^+$ 105 (100%), 71, 43; hrms *m/z* 617.2568 (calcd for $C_{32}H_{41}O_{12}$, 617.2586); ^{1}H nmr δ ppm 1.50, 2.05, 2.12 and 2.13 (4×3H, 4×s, 4×Ac), 7.45 (2H, m), 7.58 (1H, m), 7.96 (2H, dt, J = 1.3, 8.5 Hz), 1.28 and 1.29 (2×3H, 2×d, J = 7 Hz), 2.93 (1H, m, isobutanoyl), 2.72 (1H, brs, 4-OH); ^{13}C nmr δ ppm 20.2, 20.8, 21.1, 21.4, 3×169.4, and 169.9 (4×OAc), 2×128.7, 129.3, 2×129.5, 133.5, 164.5 ($PhCO_2$), 19.1, 19.3, 33.9, 177.2 (isobutanoyloxy); ^{1}H nmr (sesquiterpenoid portion) see Table 1; ^{13}C nmr (sesquiterpenoid portion) see Table 2.

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Received 6 March 1992