Notes

Sesquiterpene Polyol Esters from *Celastrus flagellaris*

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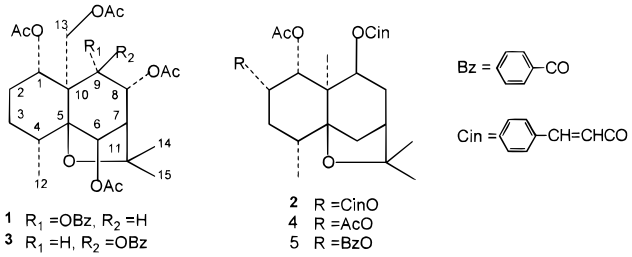
Two new β-dihydroagarofuran sesquiterpene polyol esters named celastrine A (**1**) and celastrine B (**2**), as well as three known sesquiterpenes (**3**−**5**), were isolated from the seed oil of *Celastrus flagellaris*. Their structures were elucidated as 1α,6β,8α,13-tetraacetoxy-9α-(benzoyloxy)-β-dihydroagarofuran (**1**) and 1α-acetoxy-2α,9β-bis(cinnamoyloxy)-β-dihydroagarofuran (**2**) on the basis of spectroscopic evidence.

Some species of the Celastraceae family, such as *Celastrus angulatus*, are widely distributed and used agriculturally in China as natural insecticides. Various β-dihydroagarofuran sesquiterpene polyol esters and alkaloids have been isolated from the members of the Celastraceae;[1] some of them exhibited insect antifeedant effect and antitumor activity.[2,3] We have reported two new sesquiterpenoids isolated from the seed oil of *C. orbiculatus*.[4] Recently, in our continuing pursuit of the further studies of chemical constituents of the Celastraceae, we have investigated the chemical constituents of *C. flagellaris*. Two new sesquiterpene polyol esters (**1** and **2**) and three known compounds with β-dihydroagarofuran skeletons were isolated from the MeOH extract of the seed oil. In this paper, we mainly report the isolation and structure elucidation of the new sesquiterpene esters named celastrines A (**1**) and B (**2**). A preliminary test of the Me_2CO solutions of compounds **1** and **3** (500 mg/L) against *Mythimn separata* exhibited 93.0% and 78.6% antifeedant activity, respectively.

The MeOH extract of the seed oil of *C. flagellaris* yielded compounds **1**−**5** after extensive purification by column chromatography on Si gel. Celastrine A (**1**), amorphous powder, analyzed for $C_{30}H_{38}O_{11}$ by HRMS. Its IR spectrum revealed characteristic ester absorption at 1740 and 1722 cm^{-1}. The ^1H NMR and ^{13}C NMR indicated four acetate esters [^1H NMR δ 1.47s, 2.04s, 2.13s, 2.29s (4 × 3H); ^{13}C NMR δ 20.54, 20.77, 21.09, 21.28, 169.58, 169.71, 169.71, 170.34 (4 × Ac)] and one benzoate ester [^1H NMR δ 7.45 dd (2H), 7.57 t (1H), 8.03 dd (2H); ^{13}C NMR δ 128.54, 129.55, 130.02, 133.24, 164.66], which were confirmed by fragmentation ions at m/z 515$[M + H - HOAc]^+$, 453$[M + H - PhCO_2H]^+$, 105$[PhCO]^+$, and 43$[Ac]^+$ in the mass spectrum.

The ^1H-NMR spectrum of compound **1** showed the presence of two tertiary methyl groups and one secondary methyl group. The signals observed at δ 5.41 (1H, dd, J = 11.8, 4.8), δ 5.59 (1H, dd, J = 5.8, 4.1), δ 5.66 (1H, d, J = 5.8), δ 6.67 (1H, s) were assigned to the four protons attached to carbon atoms bearing secondary ester groups, and signals at δ 4.50(1H, d, J = 13.2) and 5.02(1H, d, J = 13.2) were assigned to the two protons attached to the carbon atom bearing the primary ester group. The ^{13}C-NMR spectrum of the parent skeleton



1 R_1 =OBz, R_2 =H
3 R_1 =H, R_2 =OBz

2 R =CinO
4 R =AcO
5 R =BzO

of **1** showed three methyls, two methylenes, one methylene attached to an oxygen function, two methines, four methines attached to an oxygen function, one quaternary carbon, and two quaternary carbons attached to an oxygen function, which was very similar to that of **3**[5] and its analogues.[6] It was determined that compound **1** was esterified at C-1, C-6, C-8, C-9, and C-13.

From the $^1H-^1H$ COSY spectrum of compound **1**, the double peaks at δ 4.50 and 5.02, the double double peak at δ 5.41, the double double peak at δ 5.59, and the double peak at δ 5.66 were assigned to H-13, H-1, H-8, and H-9, respectively. The single peak at δ 6.67 was assigned to H-6 because the dihedral angles of H-6 and H-7 were near 90° in similar compounds containing an ester group at C-6.[1,5,6] The remaining ^1H signals were assigned as shown in Table 1. The ^{13}C-NMR signals were assigned on the basis of $^{13}C-^1H$ COSY and are given in Table 1. In the $^1H-^{13}C$ long-range correlation spectrum (HMBC[7]), the carbonyl carbon signal at δ 169.58 showed long-range correlation with the proton signal at δ 6.67, the carbonyl carbon signal at δ 169.71 showed long-range correlation with proton signals at δ 5.59 and 5.41, the carbonyl carbon signal at δ 170.34 showed long-range correlation with proton signals at δ 4.50 and 5.02, and the carbonyl carbon signal at δ 164.66 showed long-range correlation with proton signals at δ 5.66 and 8.03. These results clearly indicated that the four acetyl and benzoyl esters should be C-1, C-6, C-8, C-13, and C-9, respectively. The orientation of H-1 was axial because of the coupling constants of H-1 and H-2α, H-2β. The orientation of H-8 and H-9 was confirmed to be equatorial and axial, respectively, because of the correlation between H-13 and benzoyl proton (δ 8.03), H-1 and H-9, H-9 and H-8 in the NOESY spectrum. Thus, the structure of celastrine A (**1**) was

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Table 1. ^{1}H-NMR and ^{13}C-NMR Spectral Data of Compounds **1** and **2** in $CDCl_3$ (500 MHz)

H	1	2	C	1	2
1	5.41 dd (11.8, 4.8)	5.62 d (3.4)	1	79.25	71.05
2	1.60 m, 1.72 m	5.68 dd (3.4, 6.6)	2	23.01	71.10
3	1.50 m, 2.25 m		3	26.31	31.09
4	2.26 m		4	33.27	39.41
			5	90.68	87.19
6	6.67 s		6	74.74	35.99
7	2.43 d (4.1)		7	52.97	43.70
8	5.59 dd (5.8, 4.1)		8	70.25	31.02
9	5.66 d (5.8)	4.78 d (6.6)	9	72.18	73.48
			10	50.85	47.06
			11	81.01	82.12
12	1.02 d (7.5)	1.31 d (8.0)	12	15.05	19.27
13	4.50 d, 5.02 d (13.2)	1.22 s	13	60.29	20.63
14	1.58 s	1.39 s	14	24.39	24.14
15	1.43 s	1.42 s	15	30.32	30.19

elucidated as 1α,6β,8α,13-tetraacetoxy-9α-(benzolyoxy)-β-dihydroagarofuran. It is the epimer of **3** at C-9.

Celastrine B (**2**), amorphous powder, analyzed for $C_{35}H_{40}O_7$ by HRMS. Its IR spectrum showed characteristic ester absorption at 1740 and 1710 cm^{-1} and double-bond absorption at 1638 cm^{-1}. The UV, MS, ^{1}H-NMR, and ^{13}C-NMR spectra indicated that **2** contained one acetate and two cinnamate esters. In addition, the ^{13}C-NMR chemical shifts (Table 1) of its parent skeleton suggested, as compared with those of **5**,[8] that it was a 1,2,9-trisubstituted β-dihydroagarofuran.[8,9] Three ^{1}H-NMR signals [δ 5.62 (1H, d, J = 3.4, H-1); 5.68 (1H, dd, J = 3.4, 6.6, H-2); 4.78 (1H d, J = 6.6, H-9)] of **2** were similar to those [δ 5.68 (1H, d, J = 3.4, H-1); 5.83 (1H, m, H-2); 4.78 (1H, d, J = 6.4 , H-9)] of **5** in terms of coupling pattern and coupling constants, which suggested that the stereochemistry of **2** was the same as **5**.

Comparison of ^{1}H-NMR chemical shifts of **2** with those of **5** indicated that the cinnamate ester was situated at C-9 due to the same chemical shifts of H-9, and that the other cinnamate ester was situated at C-2 as indicated by the more upfield shifts of H-1 and H-2. The acetate ester was situated at C-1, which was confirmed by the cross peaks of the carbonyl carbon at δ 169.93 and 5.02, 166.12, and 5.68, 4.78 in the HMBC spectrum. As a result, **2** was determined to be 1α-acetoxy-2α,9β-bis(cinnamoyloxy)-β-dihydroagarofuran. This is the first report of two cinnamate esters in the same molecule of a β-dihydroagarofuran sesquiterpene.

Compounds **3**−**5** were identified, respectively, as Ejap-3,[5] 1α,2α-diacetoxy-9β-cinnamoyloxy-β-dihydroagarofuran, and celaforlin B-3[8] by careful comparison of UV, IR, MS, and NMR data.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR, ^{1}H−^{1}H COSY, ^{13}C−^{1}H COSY, HMBC, and NOESY spectra were recorded on a Bruker AM-500 NMR spectrometer with TMS as internal standard and $CDCl_3$ as solvent. Assignments of ^{13}C-NMR chemical shifts were made with the aid of DEPT spectra. UV spectra in MeOH were obtained on a Beckmann DU-8B spectrometer. IR spectra (KBr plate) were determined on Nicolet 5DX spectrometer. EIMS were obtained on VG ZAB-HS and MAT 4510 mass spectrometers, operating at 70 eV. Liquid chromatography was carried out on a Si gel column. Components were detected with a UV lamp. CD was recorded on JASCO J-500C instruments.

Plant Material. Voucher specimens of *C. flagellaris* are deposited at the Botanical System Group of China Agricultural University. Plant material was collected from Jian City, Jilin Province (China) in 1993.

Extraction and Isolation. Air-dried and pulverized seed (420 g) of *C. flagellaris* was extracted with petroleum ether at room temperature for a week. The solvent was evaporated under reduced pressure, yielding an oil (80.5 g), which was partitioned with petroleum ether−MeOH−H_2O (10:10:1). The MeOH layer was concentrated to give a crude extract (21.3 g); a partion (20 g) of this crude extract was chromatographed on a Si gel column with AcOEt−petroleum ether (1:5, 1:4, ...3:1) as eluent to give six fractions. The fractions of intermediate polarity were repeatedly subjected to lower pressure Si gel column chromatography Me_2CO−petroleum ether to yield compounds **1** (70 mg), **2** (15 mg), **3** (129 mg), **4** (20 mg), and **5** (21 mg).

Celastrine A (1): amorphous powder; CD $\Delta\epsilon = +3.8$ (226 nm); UV (MeOH) λ_{max} 231, 275, 282 nm. IR ν_{max} 2950, 2900, 1740, 1722, 1580, 1450, 1370, 1280, 1250, 1090, 1010, 865, 710 cm^{-1}, EIMS m/z 574 $[M]^+$, 559 $[M - Me]^+$, 532 $[M + H - HOAc]^+$, 472, 453 $[M + H - PhCO_2H]^+$, 410, 354, 309, 290, 232, 220, 190, 105 $[PhCO]^+$, 77, 43 $[Ac]^+$; HRMS m/z 574.2408, calcd for $C_{30}H_{38}O_{11}$ 574.2414.

Celastrine B (2): amorphous powder; UV (MeOH) λ_{max} 218, 223, 278 nm; IR ν_{max} 2940, 2900, 1740, 1710, 1638, 1580, 1450, 1380, 1360, 1310, 1280, 1235, 1160, 1010, 890, 760 cm^{-1}; ^{1}H NMR δ 1.81 s (3H), 6.38 d, 6.40 d (2H, J = 16.4), 7.63 d, 7.68 d (2H, J = 16.4), 7.37−7.55 m (10H); ^{13}C NMR δ 20.00, 169.93 (Ac), 118.39, 118.41, 127.95, 128.14, 128.73, 130.04, 130.19, 134.29, 134.48, 144.57, 144.91, 166.12, 166.12 (2 × PhCH-CHCO); EIMS m/z: 572 $[M]^+$, 441 $[M - PhCHCHCO]^+$, 424 $[M - PhCHCHCO_2H]^+$, 381 $[441 - HOAc]^+$, 293 $[424 - PhCHCHCO]^+$, 233 $[293 - HOAc]^+$, 217 $[M - 2 \times PhCHCHCO_2H - HOAc]^+$, 131 $[PhCHCHCO]^+$, 43 $[Ac]^+$; HRMS m/z 441.2271, calcd for $C_{26}H_{33}O_6$ 441.2277.

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