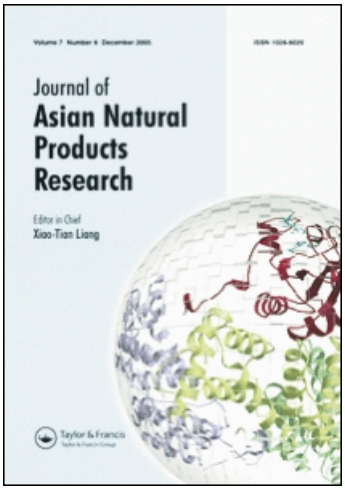

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A new sesquiterpene ester inhibiting no production from the fruits of *Celastrus orbiculatus*

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A new β-dihydroagarofuran sesquiterpene ester, 1β,2β,6α, 13-tetraacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**1**), and the known compound 1β,6α,13-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**2**), have been isolated from the fruits of *Celastrus orbiculatus* Thunb. Their structures have been elucidated on the basis of spectroscopic methods. Compound **1** shows moderate activity of inhibiting LPS-induced nitric oxide production in murine macrophage RAW264.7 cells, with an IC_{50} of 55.4 μM.

Keywords: 1β,2β,6α,13-Tetraacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran; Sesquiterpene ester; *Celastrus orbiculatus*

1. Introduction

Celastrus orbiculatus, a medicinal plant widely distributed in China, has activity in tranquilization [1]. Some sesquiterpenes with anti-inflammatory activities from *Celastrus orbiculatus* have been reported [2]. We have previously reported a new β-dihydroagarofuran sesquiterpene ester [3]. In our extended research, two β-dihydroagarofuran sesquiterpene esters have been obtained. We report here the structural elucidation of these sesquiterpene esters and their activities in inhibiting NO production.

2. Results and discussion

Compound **1** was isolated as white powder, mp 204–206°C. Its UV spectrum shows a maximum absorption at 284.0 nm. The peak at *m/z* 601 $[M+H]^+$ in the ESI-MS spectrum, along with the data in HRMS spectrum, suggest a molecular formula of $C_{32}H_{40}O_{11}$ for **1**. The ^{1}H NMR spectrum exhibits seven methyl signals, at δ 1.43 (3H, s, H-15), 1.18 (3H, d, $J = 7.2$ Hz, H-12), 1.41 (3H, s, H-14), 2.24 (3H, s), 2.10 (3H, s), 2.10 (3H, s), 1.79

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(3H, s). The ^{13}C NMR spectrum revealed three methyl signals at δ 17.6 (C-12), 25.7 (C-14), 30.2 (C-15), three methylene carbon signals at δ 30.7 (C-3), 34.6 (C-8), 65.3 (C-13), six methine signals at δ 69.1 (C-1), 69.5 (C-2), 33.0 (C-4), 71.1 (C-6), 48.7 (C-7), 78.0 (C-9), and three quaternary carbon signals at δ 89.1 (C-5), 53.1 (C-10), 82.5 (C-11). These spectral data indicate the presence of a β-dihydroagarofuran sesquiterpene-type skeleton [4,5].

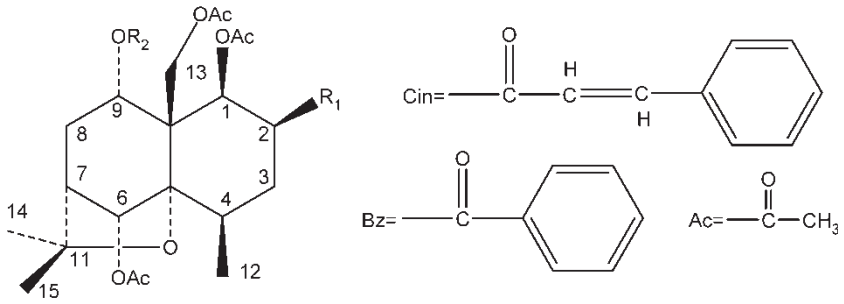
The five carbon signals at δ 69.1 (C-1), 69.5 (C-2), 71.1 (C-6), 78.0 (C-9), 65.3 (C-13) in the ^{13}C NMR spectrum indicate the presence of five oxygenated carbons. According to the literatures [4–6], the carbonyl carbon signals at δ 170.4, 169.9, 169.9, 169.5 in the ^{13}C NMR spectrum show that compound **1** contains four acetoxy groups; the aromatic carbon signals at δ 134.2–128.2, the carbonyl carbon signal at δ 165.6 and the two carbon signals at δ 117.5, 145.8, as well as the proton signals at δ 7.68, 6.36 (each 1H, d, J = 15.9 Hz), 7.43–8.07 (5H, m), reveal the presence of a cinnamoyloxy group.

In the HMQC spectrum, correlation between the following signals is compatible with above conclusions, δ 5.73 (1H, d, J = 3.3 Hz, H-1) with δ 69.1(C-1), 5.55 (1H, dd, J = 3.3, 3.1 Hz, H-2) with 69.5 (C-2), 5.95 (1H, s, H-6) with 71.1 (C-6), 5.18 (1H, d, J = 7.2 Hz, H-9) with 78.0 (C-9), and 4.32, 5.04 (each 1H, d, J = 12.6 Hz, H-13) with 65.3 (C-13).

In the HMBC spectrum, the proton signal at δ 5.73 (1H, d, J = 3.3 Hz, H-1) shows long-range correlations with the carbon signals at δ 69.5 (C-2), 30.7 (C-3), 89.1 (C-5), 53.1 (C-10), 65.3 (C-13) and 169.5. Long-range correlations also occur between signals at δ 5.55 (1H, d, J = 3.3, 3.1 Hz, H-2) with δ 69.1 (C-1), 30.7 (C-3), 33.0 (C-4), 53.1 (C-10), 169.9; δ 5.95 (1H, s, H-6) with δ 33.0 (C-4), 89.1 (C-5), 53.1 (C-10), 30.7 (C-3), 34.6 (C-8), 48.7 (C-7), 82.5 (C-11), 169.9; and δ 4.32, 5.04 (each 1H, d, J = 12.6 Hz, H-13) with δ 69.1 (C-1), 53.1 (C-10), 78.0 (C-9), 89.1 (C-5), 170.4; hence the four acetoxy groups should be situated at C-1, C-2, C-6 and C-13 respectively. The proton signal at δ 5.18 (1H, d, J = 7.2 Hz, H-9) shows long-range correlations with the carbon signals at δ 34.6 (C-8), 48.7 (C-7), 53.1 (C-10), 65.3 (C-13), 69.1 (C-1), 89.1 (C-5), 165.6, and, therefore, one cinnamoyloxy group is linked at C-9.

In this class of compounds, H-1 and H-6 generally have the axial configuration [7]. The coupling constants of the protons between H-1 and H-2, $J_{1,2}$ = 3.3 Hz, suggest that H-2 is equatorial. H-9 has equatorial stereochemistry by comparison of coupling constants of the protons ($J_{8,9}$ = 7.2 Hz) with those in the literature [4–7]. Thus, compound **1** is identified as 1β,2β,6α,13-tetraacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran.

Compound **2** was identified by comparison of its physical and spectral data with that in the literature [8]



(1) R_1 = OAc, R_2 = Cin; (2) R_1 = H, R_2 = Bz

Compounds **1** and **2** were examined for their dose-response effects on LPS-induced NO production. Excessive production of NO, which is formed by iNOS in macrophages and endothelial cells, is responsible for the inflammatory response and implicated in the pathogenesis of several inflammatory diseases such as septic shock, rheumatoid arthritis, graft rejection, and diabetes [9]. Compounds **1** and **2** were tested for their effect on NO production in LPS-stimulated RAW264.7 cells with respect to aminoguanidine, an iNOS inhibitor. Compound **1** inhibited LPS-induced NO production in the RAW264.7 cells dose-dependently with an IC_{50} of 55.4 μM; the data are comparable to that of aminoguanidine (IC_{50} 18.2 μM). Compound **2** was nearly inactive (IC_{50}s > 300 μM). The cell viability measured by MTT assay showed that **1** and **2** had no significant cytotoxicity to the RAW264.7 cells at their effective concentrations for the inhibition of NO production (data not shown).

3. Experimental

3.1 General experimental procedures

Melting points were measured on a Yamaco-hot-stage and are uncorrected. All NMR spectra were recorded on a Bruker-ARX-300 spectrometer, using TMS internal standard. The UV spectrum was recorded on a Shimadzu UV-260 UV-Vis spectrometer. ESI-MS was performed on a VG-70SE mass spectrometer. The optical rotation was measured on a Perkin-Elmer 241 polarimeter. Silica gel for chromatography was produced by Qingdao Ocean Chemical Group Co. of China. The HPLC system used a Shimadzu CTO-6A equipped with a UV detector, Shimadzu SPD-6A (Shimadzu Shim-pack PREP-ODS, i.d. 2.5×21.6 cm). Fetal bovine serum, media, and supplement materials for cell culture were purchased from GIBCO-BRL (Gaithersberg, MD).

3.2 Plant material

The plant material was collected in Shenyang city, Liaoning Province, and was identified by Professor Yunzheng Guo (Shenyang Pharmaceutical University, China). A voucher specimen (no. 200115) has been deposited in the Herbarium of the Research Department of Natural Medicine, Shenyang Pharmaceutical University, China.

3.3 Extraction and isolation

Dried fruits (10 kg) of *Celastrus orbiculatus* were extracted with 95% ethanol. The extract was concentrated and was then partitioned with light petroleum, chloroform, EtOAc and n-BuOH successively. The light petroleum partition (160 g) was subjected to column chromatography on silica gel (200-300 mesh), eluting with light petroleum–acetone (100:0–1:1), to provide 7 fractions. Column chromatography on PHPLC of fraction 3 yielded compound **2** (5 mg, 53 min) using MeOH–H_2O (80:20) as eluent; fraction 6 was subjected to column chromatography on PHPLC, using MeOH–H_2O (72:28) as eluent, to yield compound **1** (6 mg, 67 min).

Compound **1**: white powder (EtOAc), mp 204–206°C. UV λ_{max} (MeOH): 284.0 nm. $[\alpha]_D = -254$ (MeOH $c = 0.7$). ESI-MS: *m/z* 601 $[M + H]^+$; HRMS: *m/z* 601.2655 $[M + H]^+$ (calcd. for $C_{32}H_{41}O_{11}$, 601.2649). ^1H (300 MHz, in $CDCl_3$) and ^{13}C (75 MHz, in $CDCl_3$) NMR data are given in table 1.

Table 1. NMR data for compound **1** in $CDCl_3$ (δ/ppm).

No	δ_C *(ppm)*	δ_H *(ppm)*	*HMBC*
1[a]	69.1	5.73 (1H, d, $J = 3.3$ Hz)	C-2, C-3, C-5, C-10, C-13
2[a]	69.5	5.55 (1H, dd, $J = 3.3$, 3.1 Hz)	C-1, C-3, C-4, C-10
3	30.7	1.79 (1H, d, $J = 14.0$ Hz) 2.41 (1H, m)	C-2, C-5, C-1, C-4
4	33.0	[b]	
5	89.1		
6[a]	71.1	5.95 (1H, s)	C-4, C-5, C-7, C-8, C-10, C-11
7	48.7	[b]	
8	34.6	[b]	
9[a]	78.0	5.18 (1H, d, $J = 7.2$ Hz)	C-8, C-7, C-10, C-13, C-1, C-5
10	53.1		
11	82.5		
12	17.6	1.18 (3H, d, $J = 7.2$ Hz)	C-5, C-3, C-4
13	65.3	4.32, 5.04 (each 1H, d, $J = 12.6$ Hz)	C-1, C-10, C-9, C-5
14	25.7	1.41 (3H, s)	C-15, C-11, C-7
15	30.2	1.43 (3H, s)	C-14, C-11, C-7

^{1}H NMR ($CDCl_3$, 300M Hz): acetoxy [2.24, 2.10, 2.10, 1.79 (each 3H, s)]; cinnamoyloxy [6.36, 7.68 (each 1H, d, $J = 15.9$ Hz), 7.43–8.07 (5H, m)], ^{13}C NMR ($CDCl_3$, 75M Hz): acetoxy (20.6, 21.2, 21.2, 21.2, 170.4, 169.9, 169.9, 169.5), cinnamoyloxy (165.6, 145.8, 117.5, 134.2, 130.4, 128.8, 128.2).
[a] Signals of H-1, H-2, H-6, H-9 and H-13 also correlate with carbonyl carbon signals at δ 169.5, 169.9, 169.9, 165.6 and 170.4, respectively.
[b] Signals overlapped. All signals assigned by ^{1}H and ^{13}C NMR, HMQC, HMBC.

3.4 Determination of nitric oxide production

RAW264.7 cells were transferred in 96-well plates at a density of 1×10^5 cells well^{-1}. After 3 h incubation, the cells were stimulated with LPS (1 μg mL^{-1}) for 24 h in the absence or presence of the compounds tested. As a parameter of NO synthesis, nitrite concentration was measured in the supernatant of RAW264.7 cells by the Griess reaction [10]. Briefly, 100 μL of cell culture supernatant was reacted with 100 μL of Griess reagent [1:1 mixture of 0.1% *N*-(1-naphthyl)ethylenediamine in H_2O and 1% sulfanilamide in 5% phosphoric acid] in a 96-well plate, and the absorbance was read with a microplate reader (Molecular Devices Co., Menlo park, CA) at 570 nm. The nitrite concentration in the supernatants was calculated by comparison with a sodium nitrite standard curve.

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