Sesquiterpene Esters from the Fruits of *Celastrus orbiculatus*

Yuanqiang Guo, Xian Li,† Jing Xu,† Dali Meng,† Yushan Li,† and Yasushi Ohizumi*
Department of Pharmaceutical Molecular Biology, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578
†*Research Department of Natural Medicine, Shenyang Pharmaceutical University, Shenyang 110016, P. R. China*

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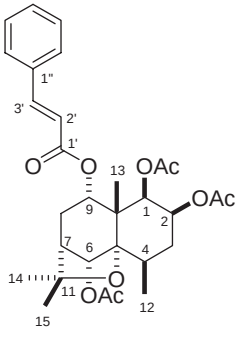
Two new β-dihydroagarofuran sesquiterpene esters, 1β,2β,6α-triacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**1**) and 1β,8β-diacetoxy-9β-cinnamoyloxy-2β-hexanoyloxy-β-dihydroagarofuran (**2**), and four known compounds (**3**–**6**) have been isolated from the fruits of *Celastrus orbiculatus* Thunb. Their structures were elucidated on the basis of spectroscopic data. In murine macrophage RAW264.7 cells, compounds **2** and **6** inhibited LPS-induced nitric oxide production with the IC_{50} values of 67.3 μM and 63.5 μM, respectively.

Celastrus orbiculatus is a medicinal plant widely distributed in China, which acts as a tranquilizer.[1] Some sesquiterpenes with antiinflammatory activities from *C. orbiculatus* have been reported previously.[2] During our survey of the active constituents responsible for antiinflammation from the fruits of *C. orbiculatus*, we have recently isolated several sesquiterpenes.[3] Continuing studies on bioactive compounds from the fruits resulted in the isolation of two new β-dihydroagarofuran sesquiterpene esters, 1β,2β,6α-triacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**1**) and 1β,8β-diacetoxy-9β-cinnamoyloxy-2β-hexanoyloxy-β-dihydroagarofuran (**2**), were isolated, along with four known compounds, 1β,6α,13-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**3**), 1β,2β,6α-triacetoxy-9α-benzoyloxy-β-dihydroagarofuran (**4**), 1β,2β-diacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran (**5**), and 1β,2β,8β-triacetoxy-9β-cinnamoyloxy-β-dihydroagarofuran (**6**). Furthermore, the effects of compounds **1**–**6** on lipopolysaccharide-induced nitric oxide (NO) production were examined in murine macrophage RAW264.7 cells. In the result, compounds **1**, **2**, **5**, and **6** inhibited LPS-induced NO production in RAW264.7 cells, with the IC_{50} values of 93.3, 67.3, 165.3, and 63.5 μM, respectively, where as such activity was not observed for **3** and **4**.



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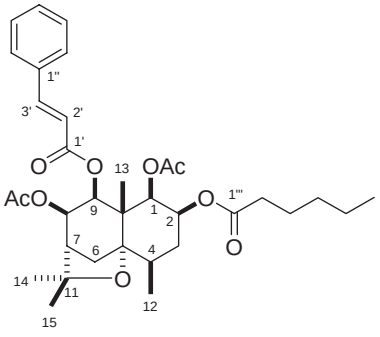

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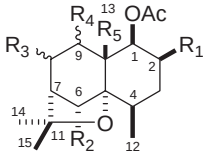

	R_1	R_2	R_3	R_4	R_5
3	H	OAc	H	α OBz	CH_2OAc
4	OAc	OAc	H	α OBz	CH_3
5	OAc	H	H	α OCin	CH_3
6	OAc	H	β OAc	β OCin	CH_3

The fruits (10 kg) of *C. orbiculatus* were extracted with 95% ethanol and partitioned successively with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The petroleum ether fraction (160 g) was subjected to column chromatography on Silica gel and PHPLC to provide compounds **1** (8 mg), **2** (4 mg), **3** (5 mg), **4** (4 mg), **5** (6 mg), and **6** (5 mg). The known compounds **3**, **4**, **5**, and **6** were identified by comparison of their physical and spectral data with those reported previously.[4–7]

Compound **1** was obtained as a white powder. The HRFABMS spectrum suggested a molecular formula of $C_{30}H_{38}O_9$ for **1**. The ^{13}C NMR spectrum revealed four methyl carbons [δ 18.5 (C-12), 20.6 (C-13), 25.8 (C-14), and 30.6 (C-15)], two methylene carbons [δ 31.0 (C-3) and 31.5 (C-8)], six methine carbons [δ 33.7 (C-4), 48.8 (C-7), 70.0 (C-2), 70.9 (C-1), 72.7 (C-9), and 79.1 (C-6)], and three quaternary carbons [δ 48.8 (C-7), 82.5 (C-11), and 89.4 (C-5)]. These spectral data in the ^{13}C and ^1H NMR spectra indicated the presence of a β-dihydroagarofuran sesquiterpene-type skeleton.[5,6,8] The carbonyl carbon signals of downfield and the methyl carbon signals of upfield (δ 169.6, 169.7, 169.7, 20.6, 21.3, and 21.3) in the ^{13}C NMR spectrum indicated that compound **1** contained three acetoxy groups; the aromatic carbon signals at δ 134.1–128.0, the carbonyl carbon signal at δ 166.3 and the two carbon signals at δ 117.9 (C-2′), 145.2 (C-3′) as well as the proton signals at δ 7.68, 6.45 (each 1H, d, J = 15.9 Hz), 7.37–7.59 (5H, m) showed the presence of one cinnamoyloxy group. The ester group distribution in **1** was determined from the HMBC spectrum, which showed cross peaks between H-1 [δ 5.58 (1H, s)] and the carbonyl (δ 169.7) of acetoxy, H-2 [δ 5.58 (1H, s)] and the carbonyl (δ 169.7) of acetoxy, H-6 [δ 5.37 (1H, s)] and the carbonyl (δ 169.6) of acetoxy, and H-9 [δ 4.74 (1H, d, J = 6.9 Hz)] and the carbonyl [δ 165.6 (C-1′)] of cinnamoyloxy, respectively. These results showed that the cinnamoyloxy was situated at C-9, while three acetoxy groups were at C-1, C-2, and C-6, respectively.

Generally, H-1 and H-6 have axial stereochemistry in this class of compounds.[8–10] In the NOESY spectrum (Figure 1), the correlations between H-1 and H-2, H-1 and H-3α, H-6 and H-12, H-6 and H-8β, H-6 and H-13, H-12 and H-13 indicated that two six-membered rings were trans-relationship with chair conformation, the other correlations between H-6 and H-7, H-7 and H-8β, but not H-6 and H-14 (H-15) suggested the furan ring nether. The correlations between H-12 and H-6 in the

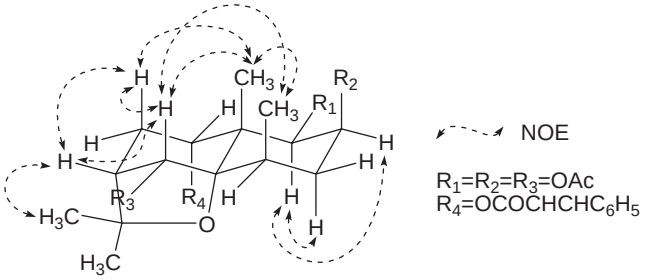

Figure 1. Partial correlations in NOESY spectrum of **1**.

NOESY spectrum indicated the axial methyl (C-12). The cross peaks between H-1 and H-2, H-13 and H-9, and H-6 and H-9, which suggests the orientations of equatorial H-2 and equatorial H-9. Thus the structure of **1** was elucidated to be 1β,2β,6α-triacetoxy-9α-cinnamoyloxy-β-dihydroagarofuran.[11]

The molecular formula of compound **2** was determined to be $C_{34}H_{46}O_9$ based on the HRESIMS spectrum. The UV, ^{1}H NMR, and ^{13}C NMR spectra showed the presence of two acetoxy groups, one cinnamoyloxy groups. In addition, one hexanoyloxy group was revealed from the NMR spectra.[12] The ^{1}H NMR and ^{13}C NMR spectra of **2** were very similar to those assigned to 1,2,8,9-tetrasubstituted β-dihydroagarofuran,[5,13] indicating that the position of the ester function was at C-1, C-2, C-8, and C-9. The HMBC spectrum showed cross peaks between H-1 [δ 5.53 (1H, d, $J = 3.6$ Hz)] and the carbonyl (δ 169.8) of acetoxy, H-2 [δ 5.56 (1H, dd, $J = 6.3$, 3.6 Hz)] and the carbonyl [δ 172.8 (C-1‴)] of hexanoyloxy, H-8 [δ 5.39 (1H, dd, $J = 6.0$, 3.0 Hz)] and the carbonyl (δ 169.8) of acetoxy, and H-9 [δ 5.08 (1H, d, $J = 6.0$ Hz)] and the carbonyl [δ 166.3 (C-1′)] of cinnamoyloxy, which means that acetoxy, hexanoyloxy, acetoxy, cinnamoyloxy groups were situated at C-1, C-2, C-8, and C-9, respectively. In the NOESY spectrum, cross peaks between H-1 and H-2, H-1 and H-9, and H-8 and H-9 suggested H-9 axial, H-2 equatorial, and H-8 equatorial orientations.[4,5,10,13] Thus compound **2** was identified as 1β,8β-diacetoxy-9β-cinnamoyloxy-2β-hexanoyloxy-β-dihydroagarofuran.[14]

The effects of compounds **1**–**6** on LPS-induced NO production were examined utilizing methodology previously reported.[3,15] The effects of compounds **1**–**6** were investigated on NO production in LPS-stimulated RAW264.7 cells with respect to aminoguanidine, an iNOS inhibitor. Compounds **2** and **6** possessed the inhibitory activity against LPS-induced NO production in RAW264.7 cells, with the IC_{50} values of 67.3 μM, 63.5 μM, respectively, which are roughly comparable to that of aminoguanidine (IC_{50} 18.2 μM). In contrast with **2** and **6**, compounds **1**, and **5** showed less activities (IC_{50} 93.3, and 165.3 μM), and compounds **3** and **4** were almost inactive. MTT assay showed that these compounds had no significant cytotoxicity in RAW264.7 cells at tested concentrations for the inhibitory effects of NO production (data not shown).

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References and Notes

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11 **1**: White powder (EtOAc), mp 177–179 °C; UV (MeOH) λ_{max} 279.4 nm; $[\alpha]^{25}_D$ +62.5° (MeOH, $c = 0.4$); IR ν_{max} (film) 1776, 1556, 1253, 1226 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.58 (1H, s, H-1), 5.58 (1H, s, H-2), 1.78 (1H, dd, $J = 13.6$, 3.4 Hz, H-3β), 2.32 (1H, ddd, $J = 13.6$, 7.8, 3.4 Hz, H-3α), 2.40 (1H, m, H-4), 5.37 (1H, s, H-6), 2.22 (1H, m, H-7), 2.42 (1H, m, H-8β), 2.13 (1H, dd, $J = 15.6$, 2.5 Hz, H-8α), 4.74 (1H, d, $J = 6.9$ Hz, H-9), 1.21 (3H, d, $J = 6.6$ Hz, H-12), 1.44 (3H, s, H-13), 1.39 (3H, s, H-14), 1.40 (3H, s, H-15), acetoxy [1.80, 2.04, 2.12 (each 3H, s, 1-, 2-, and 6-OCOC$\underline{H_3}$)], cinnamoyloxy [7.68 (1H, d, $J = 15.9$ Hz, H-3′), 6.36 (1H, d, $J = 15.9$ Hz, H-2′), 7.37–7.54 (5H, m, benzene ring)]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 70.9 (C-1), 70.0 (C-2), 31.0 (C-3), 33.7 (C-4), 89.4 (C-5), 79.1 (C-6), 48.8 (C-7), 31.5 (C-8), 72.7 (C-9), 49.7 (C-10), 82.5 (C-11), 18.5 (C-12), 20.6 (C-13), 25.8 (C-14), 30.6 (C-15), acetoxy [169.6, 20.6, 169.7, 21.3, 169.7, 21.3 (1-, 2-, and 6-O$\underline{C}$O$\underline{C}$H$_3$)], cinnamoyloxy [165.6 (C-1′), 117.7 (C-2′), 145.1 (C-3′), 134.1 (C-1″), 128.0 (2C, C-2″ and C-6″), 128.6 (2C, C-3″ and C-5″), 130.1 (C-4″)]; FABMS m/z 543 $[M + H]^+$; HRFABMS m/z 543.2590 $[M + H]^+$ (calcd for $C_{30}H_{39}O_9$, 543.2594).
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14 **2**: White powder (EtOAc), mp 194–196 °C; UV (MeOH) λ_{max}: 279.6 nm. $[\alpha]^{25}_D$ +5.0° (MeOH, $c = 0.40$). IR ν_{max} (film) 1550, 1453, 1279, 1234 cm^{-1}; ^{1}H NMR (300 MHz, in $CDCl_3$): 5.53 (1H, d, $J = 3.6$ Hz, H-1), 5.56 (1H, dd, $J = 6.3$, 3.6 Hz, H-2), 1.76 (1H, d, $J = 15.1$ Hz, H-3β), 2.38 (1H, ddd, $J = 15.1$, 6.3, 3.4 Hz, H-3α), 1.94 (1H, m, H-4), 2.15 (1H, m, H-6), 2.25 (1H, d, $J = 3.0$ Hz, H-7), 5.39 (1H, dd, $J = 6.0$, 3.0 Hz, H-8), 5.08 (1H, d, $J = 6.0$ Hz, H-9), 1.21 (3H, d, $J = 8.1$ Hz, H-12), 1.41 (3H, s, H-13), 1.55 (3H, s, H-14), 1.22 (3H, s, H-15), acetoxy [1.82, 2.15 (each 3H, s, 1- and 8-OCOC$\underline{H_3}$)]; hexanoyloxy [2.28 (2H, t, $J = 7.5$ Hz, H-2‴), 1.62 (2H, m, H-3‴), 1.45 (2H, m, H-4‴), 1.30 (2H, m, H-5‴), 0.89 (3H, t, $J = 6.6$ Hz, H-6‴); cinnamoyloxy [7.68 (1H, d, $J = 15.9$ Hz, H-3′), 6.45 (1H, d, $J = 15.9$ Hz, H-2′), 7.37–7.59 (5H, m, benzene ring)]. ^{13}C NMR (75 MHz, in $CDCl_3$): 70.6 (C-1), 70.0 (C-2), 31.1 (C-3), 39.1 (C-4), 86.7 (C-5), 35.8 (C-6), 48.4 (C-7), 70.4 (C-8), 72.2 (C-9), 47.4 (C-10), 82.3 (C-11), 19.0 (C-12), 19.8 (C-13), 24.8 (C-14), 31.1 (C-15), acetoxy [169.8, 20.6, 169.8, 20.8 (1- and 8-O$\underline{C}$O$\underline{C}$H$_3$)]; hexanoyloxy [172.8 (C-1‴), 34.7 (C-2‴), 24.6 (C-3‴), 31.1 (C-4‴), 22.2 (C-5‴), 13.8 (C-6‴)]; cinnamoyloxy [166.3 (C-1′), 117.9 (C-2′), 145.2 (C-3′), 134.4 (C-1″), 128.3 (2C, C-2″ and C-6″), 128.7 (2C, C-3″ and C-5″), 130.3 (C-4″)]; ESIMS m/z 599 $[M + H]^+$; HRESIMS: m/z 599.3227 $[M + H]^+$ (calcd for $C_{34}H_{47}O_9$, 599.3220).
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