

Anti-inflammatory Lanostanoids and Lactone Derivatives from *Antrodia camphorata*

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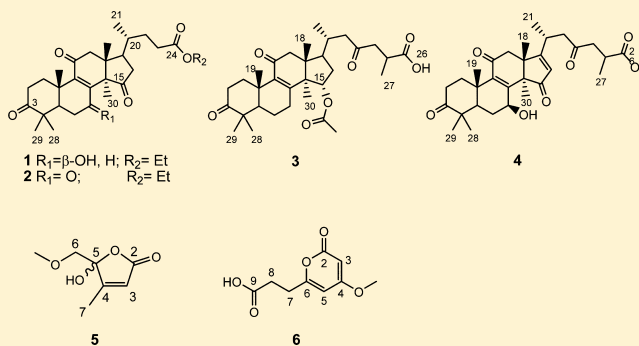
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Supporting Information

ABSTRACT: Four new lanostanoids, ethyl lucidenate A (**1**), ethyl lucidenate F (**2**), 15-*O*-acetylganolic acid (**3**), and 3,11,15,23-tetraoxo-27 ξ -lanosta-8,16-dien-26-oic acid (**4**), and two new lactone derivatives, 5-hydroxy-5-(methoxymethyl)-4-methylfuran-2(*5H*)-one (**5**) and 3-(4-methoxy-2-oxo-2H-pyran-6-yl)propanoic acid (**6**), together with four known compounds, 11 α -hydroxy-3,7-dioxolanost-8,24(*E*)-dien-26-oic acid (**7**), 3,7,11-trioxo-5 α -lanosta-8,24(*E*)-dien-26-oic acid (**8**), methyl 3,7,11,12,15,23-hexaoxo-5 α -lanost-8-en-26-oate (**9**), and ethyl 3,7,11,12,15,23-hexaoxo-5 α -lanost-8-en-26-oate (**10**), were characterized from *Antrodia camphorata*. The structures of these new compounds were determined by analysis of their spectroscopic data, including 1D and 2D NMR experiments. Ten components were evaluated for anti-inflammatory activity by examining their effect on LPS-iNOS-dependent NO production in murine macrophage (RAW 264.7) cells. Among them, compounds **1**, **3**, **7**, **8**, **9**, and **10** significantly suppressed the NO concentration in LPS-treated RAW 264.7 cells with IC₅₀ values $\leq 10 \mu\text{M}$.

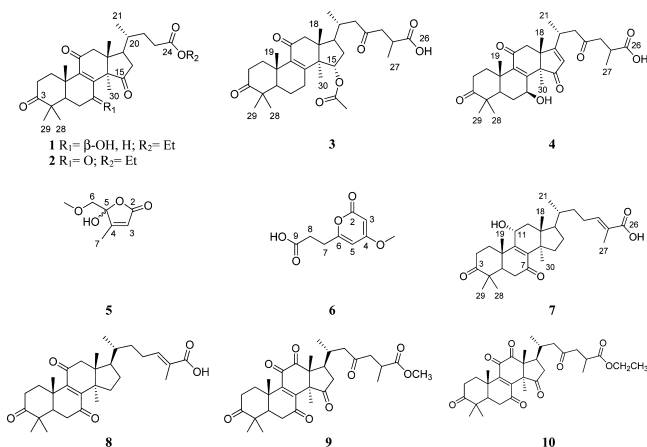


Many polypores are used for medicinal purposes in traditional Chinese medicine. *Antrodia camphorata*, known as “niu-chang-chih”, is restricted to the endemic tree *Cinnamomum kanehirai* Hay (Lauraceae) in Taiwan. Traditionally, the fungus has been used for the treatment of food and drug intoxication, diarrhea, abdominal pain, hypertension, and liver cancer.¹ Previous studies indicated that polysaccharides from *A. camphorata* inhibited endothelial tube formation.² Extracts of this fungus also showed anti-inflammatory potential by inhibiting LPS induction of cytokine, iNOS, and COX-2 expression by blocking NF- κ B activation.³ Chemical studies of the fruiting body of *A. camphorata* have led to reports of several components, such as lignans, phenyl derivatives, sesquiterpenes,⁴ steroids,^{5,6} and triterpenoids.^{7,8} In addition, some diterpenes from the fruiting body showed neuroprotection against damage by amyloid- β .⁹ In continuation of our interest in the bioactive components of this Chinese medicine, we isolated a series of lanostane triterpenes and lactone derivatives

from the EtOAc-soluble fraction by repeated chromatography. Herein, we report the isolation and structural elucidation of four new lanostane-type triterpenoids (**1**, **2**, **3**, and **4**) and two new lactone derivatives (**5** and **6**). In addition, these new compounds, together with four known compounds, 11 α -hydroxy-3,7-dioxolanost-8,24(*E*)-dien-26-oic acid (**7**),¹⁰ 3,7,11-trioxo-5 α -lanosta-8,24(*E*)-dien-26-oic acid (**8**),¹¹ methyl 3,7,11,12,15,23-hexaoxo-5 α -lanost-8-en-26-oate (**9**),¹¹ and ethyl 3,7,11,12,15,23-hexaoxo-5 α -lanost-8-en-26-oate (**10**),¹¹ were evaluated for their anti-inflammatory activity using LPS-induced iNOS-dependent NO production in murine macrophage cells (RAW 264.7).

Received: June 25, 2012

Published: March 21, 2013



RESULTS AND DISCUSSION

Compound **1** was obtained as a yellowish, amorphous solid by HPLC in the solvent system of *n*-hexane and ethyl acetate (30:70). The molecular formula $C_{29}H_{42}O_6$ was established by its HRFABMS data, representing an index of hydrogen deficiency (IHD) of 9. The maximum UV absorption band at 253 ($\log \epsilon$ 4.10) nm was characteristic of a trialkyl-substituted conjugated ketone. The IR absorption bands at 3453, 1745, 1728, 1705, and 1670 cm^{-1} indicated the presence of hydroxy, cyclopentanone, ester, cyclohexanone, and conjugated carbonyl groups. The ^1H NMR spectrum of **1** exhibited signals for a carbinol proton [δ_{H} 4.81 (dd, $J = 9.1, 7.6$ Hz)], five tertiary methyl protons (δ_{H} 1.30, 1.22, 1.09, 1.06, and 0.96), one secondary methyl proton [δ_{H} 0.95 (d, $J = 6.5$ Hz)], and one primary methyl proton of an ethyl ester [δ_{H} 1.22 (t, $J = 7.1$ Hz)] (see Table 1). The ^{13}C and DEPT experiments of **1** displayed the presence of 29 resonances, including four carbonyl carbons (δ_{C} 218.0, 216.7, 197.7, and 173.4), two olefinic carbons (δ_{C} 157.9 and 141.2), one oxymethine carbon (δ_{C} 66.3), one oxymethylene carbon (δ_{C} 60.4), and seven methyl signals (δ_{C} 27.0, 24.6, 20.7, 18.2, 18.0, 17.7, and 14.3) (see Table 1). From the ^{13}C NMR data, **1** was proposed to be a 25,26,27-trinorlanostane derivative. The conjugated carbonyl signal (δ_{C} 197.7) was assigned to C-11 by comparison with those of lucidenic acid **A**,¹² as well as signals for C-3 (δ_{C} 218.0), C-8 (δ_{C} 157.9), C-9 (δ_{C} 141.2), and C-15 (δ_{C} 216.7).¹² The oxymethine (δ_{H} 4.81; δ_{C} 66.3) was assigned to H-7 in α -axial orientation based on the large coupling constant ($J = 9.2, 7.6$ Hz) and the NOESY correlation with H₃-30. Differing from lucidenic acid **A**, **1** possessed one more set of NMR signals for one methylene [δ_{H} 4.10 (2H, q, $J = 7.1$ Hz); δ_{C} 60.4] and one methyl [δ_{H} 1.22 (3H, t, $J = 7.1$ Hz); δ_{C} 14.3], suggesting the presence of a terminal ethyl ester group. An additional difference is that the signal at δ_{C} 178.2 (–COOH) in lucidenic acid **A** is replaced with a higher field signal at δ_{C} 173.4 (–COOEt) in **1**. On the basis of the above spectral data, **1** was confirmed as ethyl lucidenate. The structure of **1** proposed herein was also reported in a Chinese patent,¹³ although there were some minor but clear differences in the ^1H NMR assignment of H₂-12, H₂-22, and H₂-23.

Compound **2**, a yellowish, amorphous solid, had a molecular formula of $C_{29}H_{40}O_6$ on the basis of its HRFABMS, ^{13}C NMR (see Table 1), and DEPT data. Its IR spectrum showed the presence of cyclopentanone (1746 cm^{-1}), cyclohexanone (1703 cm^{-1}), and a conjugated carbonyl group (1679 cm^{-1}), but no hydroxy group absorption band. The UV maximum absorption at 251 indicated the presence of a conjugated carbonyl group.

The ^1H and ^{13}C NMR and HSQC spectra of **2** showed the presence of five tertiary methyl groups (δ_{H} 1.62, 1.24, 1.11, 1.09, and 0.82), one secondary methyl group [δ_{H} 0.93 (d)], one ethyl ester group [δ_{H} 4.10 (q) and 1.23 (t)], four carbonyl groups (δ_{C} 215.5, 207.4, 199.5, and 199.4), two quaternary olefinic carbons (δ_{C} 149.7 and 146.8), and one ester carbonyl group (δ_{C} 173.4) in the molecule. These data implied that **2** is a 25,26,27-trinorlanostane-type compound with a terminal ethyl ester group similar to **1** (see Table 1). The only difference is the presence of a carbonyl group (δ_{C} 199.5) in **2** to replace an oxymethine group (δ_{H} 4.81; δ_{C} 66.3) in **1**. The small difference in ^{13}C NMR shifts between C-8 and C-9, similar to those of lucidenic acid **F**,¹² indicated the oxo group is located at C-7. Comparison of the ^1H and ^{13}C NMR data with those of lucidenic acid **F**¹² indicated that **2** is the ethyl ester of lucidenic acid **F**.

Compound **3** was obtained as a yellowish, amorphous solid and had the molecular formula $C_{32}H_{46}O_7$ based on the HRFABMS data, acquiring an IHD of 10. The UV and IR spectra of **3** indicated the presence of α,β -unsaturated carbonyl (UV: λ_{max} 245 nm; IR: 1662 cm^{-1}), carboxylic acid (3300–2500 cm^{-1}), ester (1730 cm^{-1}), and isolated ketone groups (1712 cm^{-1}), but no absorption for hydroxy groups. The ^1H NMR spectrum of **3** showed the presence of seven methyl groups [δ_{H} 1.22 (s), 1.20 (s), 1.18 (d), 1.09 (s), 1.04 (s), 0.92 (s), and 0.83 (d)], an oxymethine [δ_{H} 5.15 (dd, $J = 9.4, 5.6$ Hz)], and an acetyl group [δ_{H} 2.06 (s)] (see Table 1). The ^{13}C NMR and HSQC experiments of **3** displayed the presence of 11 quaternary carbons, including three ketone signals (δ_{C} 218.0, 208.0, and 197.9), a carboxylic acid signal (δ_{C} 180.4), two quaternary olefinic carbons (δ_{C} 162.0 and 138.7), an acetyl with two carbon signals (δ_{C} 21.2 and 170.7), and five methine, eight methylene, and eight methyl carbon signals in the molecule (see Table 1). Accordingly, **3** was suggested to be a lanostane. The HMBC correlations [δ_{H} 1.18 (H-27)/ δ_{C} 180.4 (C-26), 34.4 (C-25); δ_{H} 2.94 (H-25)/ δ_{C} 208.0 (C-23), 180.4 (C-26); and δ_{H} 0.83 (H-21)/ δ_{C} 49.1 (C-22), 32.4 (C-20)] indicated the presence of an aliphatic side chain with a carbonyl group at C-23 and a terminal carboxylic acid at C-26. Other HMBC correlations [δ_{H} 1.04 (H-29)/ δ_{C} 218.0 (C-3), 51.5 (C-5), and 20.2 (C-28); δ_{H} 1.09 (H-28)/ δ_{C} 218.0 (C-3) and 27.7 (C-29); δ_{H} 2.69 (d, $J = 17.0$ Hz H-12 β) and 2.42 (d, $J = 17.0$ Hz, H-12 α)/ δ_{C} 197.9 (C-11), 46.3 (C-13), and 17.2 (C-18)] (Figure 1) indicated the location of carbonyl groups at C-3 and C-11. According to the HMBC correlations and other 2D NMR spectra results including NOESY and ^1H – ^1H COSY, **3** closely resembled ganolucidic acid **A**,¹² with an additional acetoxyl group. The oxymethine signal (δ_{H} 5.15) expressed a downfield shift due to the connecting acetyl group. It was assigned to C-15 due to the key HMBC correlations [δ_{H} 5.15 (H-15)/ δ_{C} 170.7 (acetyl carbonyl), 162.0 (C-8), 52.7 (C-14), 35.5 (C-16), and 20.5 (C-30)]. In addition, H-15 was determined to be in β -orientation based on the NOESY correlation (Figure 1) with H₃-18 (δ_{H} 0.93). Thus, compound **3** was elucidated as 15-*O*-acetyl ganolucidate **A**.

Compound **4**, a yellowish crystal, has a molecular formula of $C_{30}H_{40}O_7$ by HRFABMS and supported by ^{13}C NMR data. The UV and IR spectra indicated the presence of hydroxy (3433 cm^{-1}), carboxylic acid (3300–2500 cm^{-1}), conjugated cyclohexenone (1695 cm^{-1}), cyclopentenone (1740 cm^{-1}), and isolated ketone and carboxyl acid carbonyl (1720, 1712 cm^{-1}) groups. The ^1H and ^{13}C NMR (Table 1) and HSQC spectra of **4** showed seven methyl groups [δ_{H} 1.43 (s), 1.21 (s), 1.20 (d),

Table 1. ^1H and ^{13}C NMR Data for Lanostane-Type Compounds 1–4

	1		2		3		4	
	$\delta_{\text{H}} (J_{\text{Hz}})^a$	δ_{C}^b	$\delta_{\text{H}} (J_{\text{Hz}})^c$	δ_{C}^d	$\delta_{\text{H}} (J_{\text{Hz}})^c$	δ_{C}^d	$\delta_{\text{H}} (J_{\text{Hz}})^c$	δ_{C}^d
1	2.91 dt (13.6, 5.6) 1.45 td (13.6, 5.6)	35.6	2.86 ^e 1.69 ^e	34.5	2.98 dt (13.7, 5.6) 1.58 m	35.0	2.92 m 1.55 td (14.0, 8.0)	35.6
2	2.49 m 2.44 m	34.3	2.57 ddd (15.6, 9.6, 6.0) 2.46 m	33.8	2.50 m 2.45 m	34.1	2.46 m	34.2
3		218.0		215.5		218.0		217.3
4		46.8		46.9		46.9		46.7
5	1.53 m	48.8	2.29 dd (13.6, 7.6)	50.8	1.62 m	51.5	1.66 m	48.7
6	2.08 m 1.62 td (13.2, 9.2) 4.81 dd (9.2, 7.6)	27.2	2.67 dd (16.0, 13.6) 2.43 dd (16.0, 7.6)	37.2	1.63 m 1.49 m	18.6	2.06 m 1.60 dd (15.0, 9.5)	27.1
7		66.3		199.5	2.24 m	29.0	4.81 dd (9.5, 7.5)	66.5
8		157.9		149.7		162.0		158.5
9		141.2		146.8		138.7		140.6
10		38.2		39.2		37.1		38.3
11		197.7		199.4		197.9		198.0
12	2.74 d (18.0) 2.71 d (18.0)	50.2	2.77 d (16.4) 2.74 d (16.4)	48.8	2.69 d (17.0) 2.42 d (17.0)	51.1	3.22 d (16.5) 2.55 d (16.5)	51.4
13		44.9		43.8		46.3		44.0
14		59.4		57.1		52.7		58.7
15		216.7		207.4	5.15 dd (9.4, 5.6)	75.1		210.8
16	2.77 dd (19.6, 8.2) 2.12 dd (19.6, 9.6)	41.1	2.82 m 1.92 dd (18.4, 8.0)	39.8	2.09 m 1.69 m	35.5	5.72 s	123.4
17	1.99 dt (9.6, 8.2)	46.3	2.10 td (9.6, 8.0)	45.0	1.89 m	48.4		189.1
18	0.96 s	17.7	0.82 s	15.9	0.92 s	17.2	1.17 s	23.9
19	1.22 s	18.2	1.24 s	18.6	1.20 s	18.9	1.21 s	18.5
20	1.52 m	35.2	1.48 m	35.3	1.96 m	32.4	2.87 m	28.3
21	0.95 d (6.5)	18.0	0.93 d (6.4)	18.2	0.83 d (6.1)	19.1	1.20 d (6.3)	21.1
22	1.75 m 1.39 m	30.6	1.76 m 1.32 m	30.7	2.35 m 2.21 m	49.1	2.85 m 2.61 dd (14.0, 4.2)	49.8
23	2.35 dd (16, 7.6) 2.24 dd (16, 8.1)	31.1	2.35 dd (16.0, 10.4) 2.20 dd (16.0, 8.0)	31.2		208.0		206.2
24		173.4		173.4	2.43 dd (16.5, 8.0) 2.08 m 2.94 m	46.4	2.82 dd (17.2, 9.1) 2.37 dd (17.2, 4.6)	45.3
25						34.4	2.94 m	34.7
26						180.4		180.2
27					1.18 d (7.2)	16.8	1.19 d (6.9)	16.9
28	1.09 s	20.7	1.09 s	20.2	1.09 s	20.2	1.07 s	20.5
29	1.06 s	27.0	1.11 s	27.6	1.04 s	27.7	1.11 s	27.4
30	1.30 s	24.6	1.62 s	20.8	1.22 s	20.5	1.43 s	31.4
OCH ₂ CH ₃	4.10 q (7.1)	60.4	4.10 q (7.1)	60.4				
OCH ₂ CH ₃	1.22 t (7.1)	14.3	1.23 t (7.1)	14.2				
-OCOCH ₃						170.7		
OCOCH ₃					2.06 s	21.2		

^aMeasured in CDCl₃ at 400 MHz. ^bMeasured in CDCl₃ at 100 MHz. ^cMeasured in CDCl₃ at 500 MHz. ^dMeasured in CDCl₃ at 125 MHz. ^eChemical shift overlapping.

1.19 (d), 1.17 (s), 1.11 (s), and 1.07 (s)], four ketone carbon signals [δ_{C} 217.3, 210.8, 206.2, and 198.0], a tetrasubstituted olefinic carbon [δ_{C} 158.5 and 140.6], a trisubstituted olefinic carbon [δ_{C} 189.1 and 123.4], and a carboxylic acid group [δ_{C} 180.2] in the molecule, indicating that **4** was a lanostane. The HMBC correlations of δ_{H} 1.19 (H-27)/ δ_{C} 180.2 (C-26) and 34.7 (C-25); δ_{H} 2.94 (H-25)/ δ_{C} 206.2 (C-23) and 180.2 (C-26) suggested the presence of an aliphatic side chain with a carbonyl group at C-23 and a terminal carboxylic acid at C-26, as found in **3**. The signal [δ_{H} 4.81 (dd, $J = 9.5, 7.5$ Hz); δ_{C} 66.5] was assigned to H-7 in α -axial orientation due to its lower chemical shift, larger coupling constants, and the NOESY correlations with δ_{H} 1.66 (H-5) and 1.43 (H₃-30) (Figure 1).

By comparing the NMR data, it was suggested that the A, B, C, and D rings and substitution pattern in **1** are the same as those in **4**. Although only one maximum UV absorption is present at 244 nm, another conjugated cyclopentenone functionality was discerned from the signals at δ_{H} 5.72 (s, H-16) and δ_{C} 210.8 (C-15), 189.1 (C-17), and 123.4 (C-16) and its HMBC correlations [δ_{H} 5.72/ δ_{C} 210.8 (C-15), 189.1 (C-17), 58.7 (C-14), 44.0 (C-13), and 28.3 (C-20)]. Thus, compound **4** was determined to be 7 β -hydroxy-3,11,15,23-tetraoxo-27 ξ -lanosta-8,16-dien-26-oic acid.

Compound **5** was obtained as a pale yellow oil. The molecular formula was confirmed as C₇H₁₀O₄ by HRESIMS, which was deduced with an IHD of 3. The IR spectrum

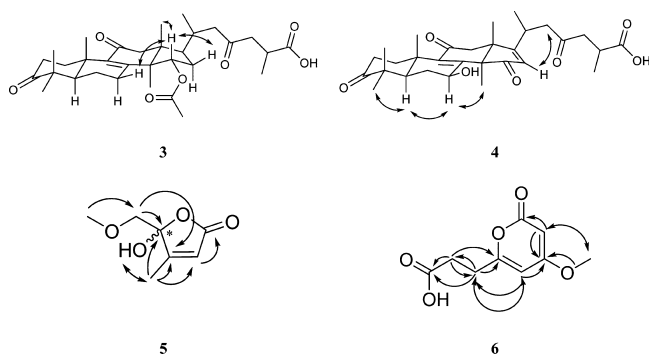


Figure 1. Selected HMBC (→) and NOESY (↔) correlations of compounds 3, 4, 5, and 6.

supported the presence of a hydroxy group (3339 cm^{-1}) and an α,β -unsaturated butenolide (1757 cm^{-1}).¹⁴ The ^{13}C NMR and HMQC spectra of **5** showed the presence of an ester carbonyl carbon ($\delta_{\text{C}} 170.9$), β,β -disubstituted conjugated olefinic carbons ($\delta_{\text{C}} 165.7$ and 119.1), a dioxygenated quaternary carbon ($\delta_{\text{C}} 106.4$), two carbon signals from a methoxymethylene group ($\delta_{\text{C}} 73.3$ and 59.9), and a methyl carbon ($\delta_{\text{C}} 12.8$) (see Table 2). The ^1H NMR spectrum of **5** contained

Table 2. ^{13}C and ^1H NMR Data for Compounds **5** and **6** (100 and 400 MHz in CDCl_3 , J in Hz)

position	5		6	
	δ_{C}	δ_{H}	δ_{C}	δ_{H}
2	170.9		164.8	
3	119.1	5.82 q (1.6)	88.0	5.41 d (2.4)
4	165.7		171.2	
5	106.4		100.6	5.83 d (2.4)
6	73.3	3.57 d (10.6) 3.64 d (10.6)	162.9	
7	12.8	2.03 d (1.6)	28.5	2.75 m
8			30.4	2.72 m
9			175.5	
4-OCH ₃			55.9	3.77 s
6-OCH ₃	59.9	3.40 s		

signals for an olefinic proton [$\delta_{\text{H}} 5.82$ (1H, q, $J = 1.6$ Hz)], methyl protons [$\delta_{\text{H}} 2.03$ (3H, d, $J = 1.6$ Hz)], and a methoxymethylene group [$\delta_{\text{H}} 3.40$ (3H, s), 3.64 and 3.57 (1H each, d, $J = 10.6$ Hz)] (see Table 2). The IR absorption band at 1757 cm^{-1} , as well as the UV maximum absorption band at 215 nm and the olefinic proton at $\delta_{\text{H}} 5.82$, supported the presence of butenolide with a methyl group attached at the β -position. The nonequivalent signals of the methylene group at $\delta_{\text{H}} 3.64$ and 3.57 displayed a clear germinal coupling, implying that it is located at a quaternary chiral carbon (C-5). One dioxygenated carbon at $\delta_{\text{C}} 106.4$ was assigned to C-5 according to the HMBC correlations with H₂-6 and H₃-7 (Figure 1). The remaining hydroxy group should be at C-5, which causes this hemiacetal carbon signal to shift to $\delta_{\text{C}} 106.4$. The HMBC correlations [H-3 ($\delta_{\text{H}} 5.82$)/C-2 ($\delta_{\text{C}} 170.9$); H₃-7 ($\delta_{\text{H}} 2.03$)/C-3 ($\delta_{\text{C}} 119.1$) and C-5 ($\delta_{\text{C}} 106.4$)] further confirmed the presence of a β -methylbutenolide moiety, which was verified by the NOESY correlation and the long-range allylic coupling ($J = 1.6$ Hz) between H₃-7 and H-3. However, the configuration of C-5 was proposed as a racemic mixture because of the [α]

value, which was near zero. Thus, **5** was determined to be 5-hydroxy-5-(methoxymethyl)-4-methylfuran-2(*SH*)-one.

Compound **6** was obtained as yellow needles. The molecular formula was confirmed as $\text{C}_9\text{H}_{10}\text{O}_5$ based on HREIMS and was deduced to have an IHD of 5. The IR absorption bands of **6** at $3300\text{--}2500$, 1730 , and 1705 cm^{-1} indicated that this compound contains carboxylic acid and ester groups, respectively. The ^{13}C NMR and HMQC spectra of **6** showed nine ^{13}C resonances: two carbonyl carbons (including one ester and one carboxylic acid), four olefinic carbons (two CH and two C), two methylene carbons, and one methoxyl group (see Table 2). The ^1H NMR spectrum of **6** showed the presence of two methylenes [$\delta_{\text{H}} 2.72\text{--}2.75$ (4H, m)], one methoxyl group [$\delta_{\text{H}} 3.77$ (3H, s)], and two olefinic methines [$\delta_{\text{H}} 5.41$ (1H, d, H-3), 5.83 (1H, d, H-5)] with a mutual *W*-form coupling ($J = 2.4$ Hz), which is typical for a disubstituted 2-pyrone (see Table 2). The HMBC correlations [$\delta_{\text{H}} 5.83$ (H-5)/ $\delta_{\text{C}} 171.2$ (C-4), 162.9 (C-6), and 88.0 (C-3); $\delta_{\text{H}} 5.41$ (H-3)/ $\delta_{\text{C}} 171.2$ (C-4), 164.8 (C-2), and 100.6 (C-5)] confirmed the presence of the disubstituted 2-pyrone moiety. A methoxyl and a propanoic acid were located at C-4 and C-6, respectively, based on the following HMBC correlations: $\delta_{\text{H}} 3.77$ (CH₃O)/ $\delta_{\text{C}} 171.2$ (C-4); $\delta_{\text{H}} 2.72$ (H₂-8)/ $\delta_{\text{C}} 162.9$ (C-6); and $\delta_{\text{H}} 2.75$ (H₂-7)/ $\delta_{\text{C}} 175.5$ (C-9). Thus, **6** was determined to be 3-(4-methoxy-2-oxo-2H-pyran-6-yl)propanoic acid.

The anti-inflammatory activities of compounds **1–6**, together with those of known compounds **7–10**, were evaluated by examining their inhibitory effects on lipopolysaccharide (LPS)-induced inducible nitric oxide synthase (iNOS)-dependent NO production in the murine macrophage cell line RAW 264.7. Among them, compounds **1**, **3**, **7**, **8**, **9**, and **10** showed significant inhibitory effects with IC₅₀ values of 10.8, 4.9, 5.0, 5.0, 8.9, and 6.2 μM , respectively (Table 3). We did not observe

Table 3. Effects of Compounds Isolated from *A. camphorata* on the Suppression of NO Concentration in LPS-Treated RAW 264.7 Cells^a

	IC ₅₀ (μM) ^b
1	10.8 ± 0.4
2	>20
3	4.9 ± 0.2
4	>20
5	>20
6	>20
7	5.0 ± 0.3
8	5.0 ± 0.4
9	8.9 ± 0.7
10	6.2 ± 0.5
quercetin	16.4 ± 0.8

^aQuercetin was used as a positive control. Results are presented as mean ± SEM ($n = 3$). ^bConcentration necessary for 50% inhibition (IC₅₀).

cytotoxicity of these compounds toward LPS-treated RAW 264.7 cells at a dose of 20 $\mu\text{g}/\text{mL}$ (Supporting Information Table S1), and the IC₅₀ values of ethyl lucidenate A against various cancer cells were about 35–50 $\mu\text{g}/\text{mL}$ in 24 h,¹³ implying that these compounds could potentially be developed as anti-inflammatory drugs for human use.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined on a Yanaco MP-53 micromelting point apparatus without correlation. Specific rotations were recorded on a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a Perkin-Elmer 983 G spectrometer. UV spectra were taken on a Hitachi UV-3210 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Varian Unity Plus-400 and Bruker DMX 500 MHz FT-NMR spectrometers. HREIMS were measured with a JEOL SX-102A mass spectrometer. HRFABMS were measured with a JEOL JMS-H110 mass spectrometer. Extracts were chromatographed on silica gel (Merck 70–230 mesh, 230–400 mesh) and purified on a semipreparative normal-phase HPLC column [250 × 10 mm, Licrosorb Si 60 (7 μm)] carried out with a LCD Refracto Monitor III.

Fungal Material. A mixture of mycelia and fruiting bodies of cultured *A. camphorata* was identified and provided by Well Shine Biotechnology Development, Taipei, Taiwan. A voucher specimen (No. CMU-AC200706) was deposited at the School of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, Taiwan.

Extraction and Isolation. Dried fruiting bodies of *A. camphorata* (3.0 kg) were extracted with MeOH (12 L) at room temperature (5 days twice). After evaporation, the residue of the MeOH extract was mixed with H₂O to bring the total volume to 1 L. This phase was extracted with EtOAc (three times), and the combined organic phase was evaporated to give a black syrup (150 g). This EtOAc layer was chromatographed on silica gel eluting with hexane and EtOAc solutions. The fraction eluted with *n*-hexane–EtOAc (6:4, v/v) was separated by Sephadex LH-20 CC (10 × 70 cm) eluting with MeOH and further purified by semipreparative HPLC using a preparative silica gel column with a mixture of acetone–hexane as eluent to give pure **2** (8.5 mg), **1** (10.4 mg), **7** (8.3 mg), **3** (4.6 mg), **4** (5.2 mg), **5** (14.6 mg), and **6** (6.8 mg). The fraction eluted with *n*-hexane–EtOAc (5:1, v/v) was subjected to Sephadex LH-20 CC (10 × 70 cm) eluting with MeOH and semipreparative reversed-phase HPLC eluting with isocratic MeOH–H₂O (80:20) to yield **7** (3.8 mg). The fraction eluted with *n*-hexane–EtOAc (7:3, v/v) was chromatographed using semipreparative reversed-phase HPLC with isocratic MeOH–H₂O (70:30) to yield **8** (2.4 mg), **9** (6.0 mg), and **10** (3.6 mg).

Ethyl lucidenate A (1): yellowish, amorphous solid; $[\alpha]_{\text{D}}^{25} +13.2$ (c 0.164 MeOH); UV (MeOH) λ_{max} (log ϵ) 253 (4.10) nm; IR (KBr) ν_{max} 3453, 2974, 1745, 1728, 1705, 1670 cm⁻¹; ^1H NMR (CDCl₃, 400 MHz) and ^{13}C NMR (CDCl₃, 100 MHz), see Table 1; HRFABMS m/z 487.3072 [M + H]⁺ (calcd for C₂₉H₄₃O₆, 487.3060).

Ethyl lucidenate F (2): yellowish, amorphous solid; $[\alpha]_{\text{D}}^{25} +11.3$ (c 0.08, MeOH); mp 86–89 °C; UV (MeOH) λ_{max} (log ϵ) 251 (3.99) nm; IR (KBr) ν_{max} 2976, 1746, 1703, 1679 cm⁻¹; ^1H NMR (CDCl₃, 500 MHz) and ^{13}C NMR (CDCl₃, 125 MHz), see Table 1; HRFABMS m/z 485.2900 [M + H]⁺ (calcd for C₂₉H₄₁O₆, 485.2903).

15-O-Acetylganolucidate A (3): yellowish, amorphous solid; $[\alpha]_{\text{D}}^{25} +15.01$ (c 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (4.05) nm; IR (KBr) ν_{max} 3300–2500, 2977, 1730, 1712, 1662 cm⁻¹; ^1H NMR (CDCl₃, 500 MHz) and ^{13}C NMR (CDCl₃, 125 MHz), see Table 1; HRFABMS m/z 543.3311 [M + H]⁺ (calcd for C₃₂H₄₇O₇, 543.3322).

7β-Hydroxy-3,11,15,23-tetraoxo-27ξ-lanosta-8,16-dien-26-oic acid (4): yellowish crystal; mp 179–182 °C; $[\alpha]_{\text{D}}^{25} +19.62$ (c 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 244 (4.14) nm; IR (KBr) ν_{max} 3433, 3300–2500, 2974, 1740, 1720, 1712, 1695 cm⁻¹; ^1H NMR (CDCl₃, 500 MHz) and ^{13}C NMR (CDCl₃, 125 MHz), see Table 1; HRFABMS m/z : 513.2857 [M + H]⁺ (calcd for C₃₀H₄₁O₇, 513.2852).

5-Hydroxy-3-(methoxymethyl)-4-methylfuran-2(5H)-one (5): pale yellow oil; $[\alpha]_{\text{D}}^{25} \pm 0$; UV (MeOH) λ_{max} (log ϵ) 215 (3.42) nm; IR (KBr) ν_{max} 3339, 2929, 1757, 1654, 937 cm⁻¹; ^1H NMR (CDCl₃, 400 MHz) and ^{13}C NMR (CDCl₃, 100 MHz), see Table 2; HRESIMS m/z : 159.0654 [M + H]⁺ (calcd for C₇H₁₁O₄, 159.0657).

3-(4-Methoxy-2-oxo-2H-pyran-6-yl)propanoic acid (6): yellow needles; mp 128–130 °C; UV (MeOH) λ_{max} (log ϵ) 239 (4.21), 277 (4.37) nm; IR (KBr) ν_{max} 3300–2500, 2924, 1730, 1705, 1567, 1250 cm⁻¹; ^1H NMR (CDCl₃, 400 MHz) and ^{13}C NMR (CDCl₃, 100

MHz), see Table 2; HREIMS m/z 198.0529 [M]⁺ (calcd for C₉H₁₀O₅, 198.0528).

Detection of Nitric Oxide Expression by Griess Reaction. RAW 264.7 cells were seeded in a 24-well plate at a density of 2 × 10⁵ cells/mL and then incubated with or without LPS (1 μg/mL) in the absence or presence of material isolated from *Antrrodia camphorata* for 24 h. Effects of these isolates on NO production were measured indirectly by analysis of nitrite levels using the Griess reaction.¹⁵ Quercetin was used as a positive control.¹⁶

ASSOCIATED CONTENT

Supporting Information

^1H and ^{13}C NMR spectra of triterpenoids **1–4** and lactone derivatives **5** and **6**, and their cytotoxicity data are available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We are grateful to the National Center for High-performance Computing for computer time and facilities (Hsin-Chu) and the Proteomics Research Core Laboratory, Office of Research and Development, at China Medical University for ESIMS measurements. This work was supported by grants from the National Science Council of the Republic of China, China Medical University (CMU97-CT-02), and in part by Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH101-TD-B-111-004) to Y.-H. K.

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