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

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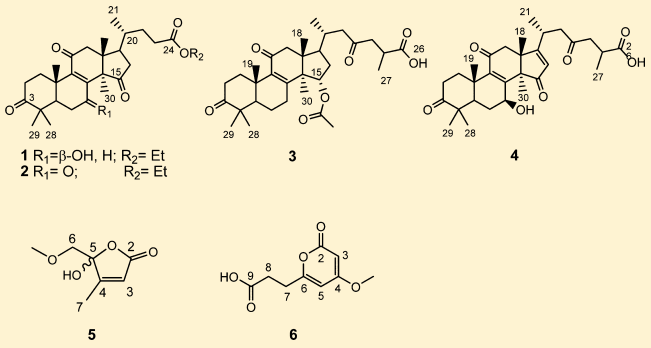
Supporting Information

ABSTRACT: Four new lanostanoids, ethyl lucidenate A (**1**), ethyl lucidenate F (**2**), 15-*O*-acetylganolucidate A (**3**), and 3,11,15,23-tetraoxo-27ξ-lanosta-8,16-dien-26-oic acid (**4**), and two new lactone derivatives, 5-hydroxy-5-(methoxymethyl)-4-methylfuran-2(5*H*)-one (**5**) and 3-(4-methoxy-2-oxo-2*H*-pyran-6-yl)propanoic acid (**6**), together with four known compounds, 11α-hydroxy-3,7-dioxolanost-8,24(*E*)-dien-26- oic acid (**7**), 3,7,11-trioxo-5α-lanosta-8,24(*E*)-dien-26-oic acid (**8**), methyl 3,7,11,12,15,23-hexaoxo-5α-lanost-8-en-26-oate (**9**), and ethyl 3,7,11,12,15,23-hexaoxo-5α-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 IC_{50} values $\leq$ 10 μM.



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-κB 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, 11α-hydroxy-3,7-dioxolanost-8,24(*E*)-dien-26-oic acid (**7**),[10] 3,7,11-trioxo-5α-lanosta-8,24(*E*)-dien-26-oic acid (**8**),[11] methyl 3,7,11,12,15,23-hexaoxo-5α-lanost-8-en-26-oate (**9**),[11] and ethyl 3,7,11,12,15,23-hexaoxo-5α-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).

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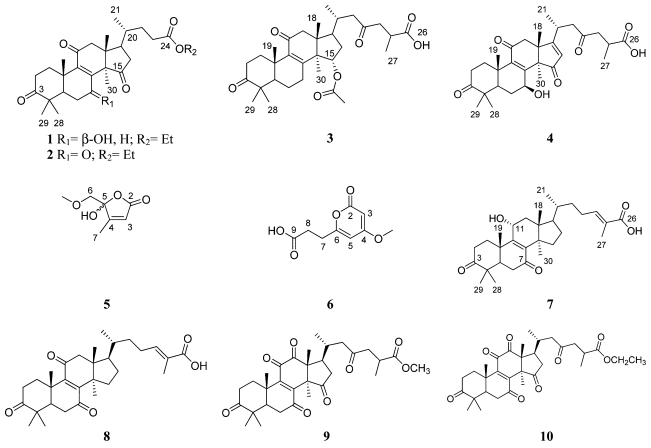

RESULTS AND DISCUSSION

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 ε 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 H_3-30. 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 A. 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 H_2-12, H_2-22, and H_2-23.

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-12β) and 2.42 (d, J = 17.0 Hz, H-12α)/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 NOSEY 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 H_3-18 (δ_H 0.93). Thus, compound **3** was elucidated as 15-*O*-acetylganolucidate 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),

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

	1		2		3		4	
	δ_H, (J_{Hz})[a]	δ_C[b]	δ_H, (J_{Hz})[c]	δ_C[d]	δ_H, (J_{Hz})[c]	δ_C[d]	δ_H, (J_{Hz})[c]	δ_C[d]
1	2.91 dt (13.6, 5.6)	35.6	2.86[e]	34.5	2.98 dt (13.7, 5.6)	35.0	2.92 m	35.6
	1.45 td (13.6, 5.6)		1.69[e]		1.58 m		1.55 td (14.0, 8.0)	
2	2.49 m	34.3	2.57 ddd (15.6, 9.6, 6.0)	33.8	2.50 m	34.1	2.46 m	34.2
	2.44 m		2.46 m		2.45 m			
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	27.2	2.67 dd (16.0, 13.6)	37.2	1.63 m	18.6	2.06 m	27.1
	1.62 td (13.2, 9.2)		2.43 dd (16.0, 7.6)		1.49 m		1.60 dd (15.0, 9.5)	
7	4.81 dd (9.2, 7.6)	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)	50.2	2.77 d (16.4)	48.8	2.69 d (17.0)	51.1	3.22 d (16.5)	51.4
	2.71 d (18.0)		2.74 d (16.4)		2.42 d (17.0)		2.55 d (16.5)	
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)	41.1	2.82 m	39.8	2.09 m	35.5	5.72 s	123.4
	2.12 dd (19.6, 9.6)		1.92 dd (18.4, 8.0)		1.69 m			
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	30.6	1.76 m	30.7	2.35 m	49.1	2.85 m	49.8
	1.39 m		1.32 m		2.21 m		2.61 dd (14.0, 4.2)	
23	2.35 dd (16, 7.6)	31.1	2.35 dd (16.0, 10.4)	31.2		208.0		206.2
	2.24 dd (16, 8.1)		2.20 dd (16.0, 8.0)					
24		173.4		173.4	2.43 dd (16.5, 8.0)	46.4	2.82 dd (17.2, 9.1)	45.3
					2.08 m		2.37 dd (17.2, 4.6)	
25					2.94 m	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
$OC\underline{H}_2CH_3$	4.10 q (7.1)	60.4	4.10 q (7.1)	60.4				
$OCH_2C\underline{H}_3$	1.22 t (7.1)	14.3	1.23 t (7.1)	14.2				
$-O\underline{C}OCH_3$						170.7		
$OCOC\underline{H}_3$					2.06 s	21.2		

[a]Measured in $CDCl_3$ at 400 MHz. [b]Measured in $CDCl_3$ at 100 MHz. [c]Measured in $CDCl_3$ at 500 MHz. [d]Measured in $CDCl_3$ at 125 MHz [e]Chemical 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 (H_3-30) (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 7β-hydroxy-3,11,15,23-tetraoxo-27ξ-lanosta-8,16-dien-26-oic acid.

Compound **5** was obtained as a pale yellow oil. The molecular formula was confirmed as $C_7H_{10}O_4$ by HRESIMS, which was deduced with an IHD of 3. The IR spectrum

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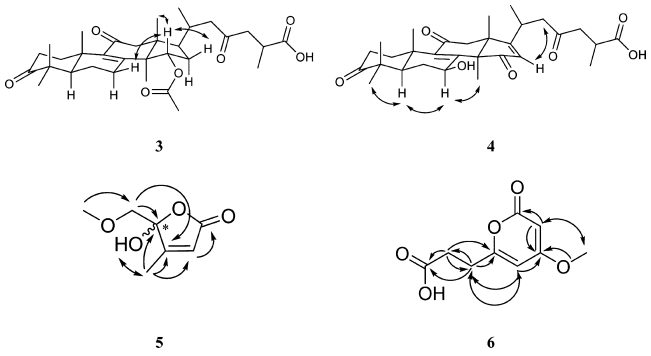

Figure 1. Selected HMBC (→) and NOESY (↔) correlations of compounds **3**, **4**, **5**, and **6**.

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 (δ_C 170.9), β,β-disubstituted conjugated olefinic carbons (δ_C 165.7 and 119.1), a dioxygenated quaternary carbon (δ_C 106.4), two carbon signals from a methoxymethylene group (δ_C 73.3 and 59.9), and a methyl carbon (δ_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)

	5		6	
position	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)	162.9	
		3.64 d (10.6)		
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 [δ_H 5.82 (1H, q, J = 1.6 Hz)], methyl protons [δ_H 2.03 (3H, d, J = 1.6 Hz)], and a methoxymethylene group [δ_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 δ_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 δ_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 δ_C 106.4 was assigned to C-5 according to the HMBC correlations with H_2-6 and H_3-7 (Figure 1). The remaining hydroxy group should be at C-5, which causes this hemiacetal carbon signal to shift to δ_C 106.4. The HMBC correlations [H-3 (δ_H 5.82)/C-2 (δ_C 170.9); H_3-7 (δ_H 2.03)/C-3 (δ_C119.1) and C-5 (δ_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 H_3-7 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(5*H*)-one.

Compound **6** was obtained as yellow needles. The molecular formula was confirmed as $C_9H_{10}O_5$ based on HREIMS and was deduced to have an IHD of 5. The IR absorption bands of **6** at 3300−2500, 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 [δ_H 2.72−2.75 (4H, m)], one methoxyl group [δ_H 3.77 (3H, s)], and two olefinic methines [δ_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 [δ_H 5.83 (H-5)/δ_C 171.2 (C-4), 162.9 (C-6), and 88.0 (C-3); δ_H 5.41 (H-3)/δ_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: δ_H 3.77 ($C\underline{H}_3O$)/δ_C 171.2 (C-4); δ_H 2.72 (H_2-8)/δ_C 162.9 (C-6); and δ_H 2.75 (H_2-7)/δ_C 175.5 (C-9). Thus, **6** was determined to be 3-(4-methoxy-2-oxo-2*H*-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 IC_{50} 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

[a]Quercetin was used as a positive control. Results are presented as mean ± SEM (n = 3). [b]Concentration necessary for 50% inhibition (IC_{50}).

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

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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. ^{1}H 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 H_2O 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−H_2O (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−H_2O (70:30) to yield **8** (2.4 mg), **9** (6.0 mg), and **10** (3.6 mg).

*Ethyl lucidenate A (**1**):* yellowish, amorphous solid; $[\alpha]_D^{25}$ +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}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; HRFABMS *m*/*z* 487.3072 $[M + H]^+$ (calcd for $C_{29}H_{43}O_6$, 487.3060).

*Ethyl lucidenate F (**2**):* yellowish, amorphous solid; $[\alpha]_D^{25}$ +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}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRFABMS *m*/*z* 485.2900 $[M + H]^+$ (calcd for $C_{29}H_{41}O_6$ 485.2903).

*15-O-Acetylganolucidate A (**3**):* yellowish, amorphous solid; $[\alpha]_D^{25}$ +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}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRFABMS *m*/*z* 543.3311 $[M + H]^+$ (calcd for $C_{32}H_{47}O_7$, 543.3322).

*7β-Hydroxy-3,11,15,23-tetraoxo-27ξ-lanosta-8,16-dien-26-oic acid (**4**):* yellowish crystal; mp 179−182 °C; $[\alpha]_D^{25}$ +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}; ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 1; HRFABMS *m*/*z*: 513.2857 $[M + H]^+$ (calcd for $C_{30}H_{41}O_7$, 513.2852).

*5-Hydroxy-3-(methoxymethyl)-4-methylfuran-2(5H)-one (**5**):* pale yellow oil; $[\alpha]_D^{25}$ ±0; UV (MeOH) λ_{max} (log ε) 215 (3.42) nm; IR (KBr) ν_{max} 3339, 2929, 1757, 1654, 937 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HRESIMS *m*/*z*: 159.0654 $[M + H]^+$ (calcd for $C_7H_{11}O_4$, 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}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; HREIMS *m*/*z* 198.0529 $[M]^+$ (calcd for $C_9H_{10}O_5$, 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 × 10^5 cells/mL and then incubated with or without LPS (1 μg/mL) in the absence or presence of material isolated from *Antrodia 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

^{1}H 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.

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