Absolute Stereostructures of Olibanumols A, B, C, H, I, and J from Olibanum, Gum-Resin of *Boswellia carterii*, and Inhibitors of Nitric Oxide Production in Lipopolysaccharide-Activated Mouse Peritoneal Macrophages

Masayuki Yoshikawa,*[a] Toshio Morikawa,[a,b] Hideo Ominami,[a] and Hisashi Matsuda[a]

[a] *Kyoto Pharmaceutical University; Misasagi, Yamashina-ku, Kyoto 607–8412, Japan: and* [b] *Pharmaceutical Research and Technology Institute, Kinki University; 3–4–1 Kowakae, Higashi-Osaka, Osaka 577–8502, Japan.*
Received April 28, 2009; accepted June 23, 2009; published online July 3, 2009

Three new monoterpenes, olibanumols A (1), B (2), and C (3), and three new triterpenes, olibanumols H (4), I (5), and J (6), were isolated from olibanum, the exuded gum-resin from *Boswellia carterii* Birdw. Their structures including the absolute configuration were determined by chemical and physicochemical evidence. Among the constituents, olibanumols A (1), H (4), and I (5), and isofouquierol (12) exhibited nitric oxide production inhibitory activity in lipopolysaccharide-activated mouse peritoneal macrophages.

Key words olibanumol; olibanum; *Boswellia carterii*; monoterpene; triterpene; nitric oxide production inhibitory activity

Olibanum (Frankincense), which is the gum-resin exuded by incision from *Boswellia* species (Burseraceae), has been used as an incense since ancient times.[1,2] In recent years, it was important in the preparation of cosmetics and perfumes. On the other hand, olibanum is known to show analgesic, anti-inflammatory, sedative, anti-hyperlipidemic, and antibacterial activities in Unani (Islamic) and Chinese traditional medicines.[1—7] As the chemical constituents of this natural medicine, many terpenoids have been reported.[2,8—16]

A similar natural medicine, guggul-gum resin (the resin of *Balsamodendron mukul*), has been used as anti-inflammatory, anti-bacterial, anti-coagulant, and anti-atherosclerosis agents in Indian and Arabian countries. Previously, we reported that the aqueous methanolic extract from the resin of *B. mukul* showed potent anti-inflammatory effect on adjuvant-induced air-pouch granuloma in mice and polypodane-type triterpenes called myrrhanol A and myrrhanone A were isolated as the active contsitutents.[17,18] Furthermore, the extract was found to exhibit an inhibitory effect on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated mouse peritoneal macrophages and then new polypodane-type triterpenes, myrrhanol B, myrrhanones B and A acetate, and an octanordammarane-type triterpene, epimansumbinol, were isolated as the active constituents.[19] As an extention of those studies on perfumery, we examined the chemical constituents of olibanum, the gum-resin from *Boswellia carterii* and isolated three new monoterpenes, olibanumols A (**1**), B (**2**), and C (**3**), and three new triterpenes, olibanumols H (**4**), I (**5**), and J (**6**) together with seven known terpenoids. This paper deals with the isolation and structure elucidation of olibanumols (**1**—**6**) as well as the inhibitory effects of the isolated compounds on NO production.[20]

The gum-resin from *B. carterii* collected in Yemen and purchased at Cairo of Egypt was extracted with 80% aqueous acetone at room temperature. The aqueous acetone extract (78.9% from the natural medicine) was subjected to normal- and reversed-phase silica gel column chromatographies and finally HPLC to furnish olibanumols A (**1**, 0.037%), B (**2**, 0.026%), C (**3**, 0.0089%), H (**4**, 0.015%), I (**5**, 0.00074%), and J (**6**, 0.0031%) together with 3,6-dihydroxy-*p*-menth-1-ene[21] (**7**, 0.021%), *p*-menth-1-en-4α,6β-diol[22] (**8**, 0.018%), (−)-*trans*-sobrerol[23] (**9**, 0.010%), *p*-menth-4-en-1,2-diol[24] (**10**, 0.014%), *p*-menth-5-en-1,2-diol[25] (**11**, 0.043%), isofouquierol[26] (**12**, 0.0023%), and epilupeol[27] (**13**, 37.7%).

Absolute Stereostructures of Olibanumols A (1), B (2), and C (3) Olibanumol A (**1**) was obtained as a colorless oil with positive optical rotation ($[\alpha]_D^{28}$ +7.6° in MeOH). The molecular formula of **1** was determined to be $C_{10}H_{18}O_3$ from the molecular ion peak at *m/z* 186 (M^+) and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands at 3426 cm^{-1} assignable to hydroxyl group. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[28] showed signals assignable to two secondary methyls [δ 1.00, 1.06 (3H each, both d, J=6.6 Hz, 9, 10-H_3)], a tertiary methyl [δ 1.35 (3H, s, 7-H_3)], three methines bearing an oxygen function [δ 3.06 (1H, d, J=4.0 Hz, 2-H), 3.40 (1H, dd-like, 3-H), 3.62 (1H, dd-like, 6-H)], a methylene [δ 1.52 (1H, m, 5β-H), 1.95 (1H, m, 5α-H)], and two methines [δ 1.75 (1H, m, 8-H), 1.87 (1H, m, 4-H)] together with a quaternary carbon bearing an oxygen function [δ_C 69.8 (1-C)]. The *p*-menthane-type monoterpene structure of **1** was constructed on the basis of the ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY) and heteronuclear multiple bond correlation (HMBC) experiments as shown in Fig. 1. Thus, ^{1}H–^{1}H COSY experiment indicated the presence of partial structure in bold line. In the HMBC experiment on **1**, long-range correlations were observed between the following protons and carbons: 7-H_3 and 1,2,6-C; 2,6-H and 1-C; 9,10-H_3 and 4,8-C. The relative stereostructure of **1** was characterized by a nuclear Overhauser experiment spectrometry (NOESY), which showed the NOE corralations between the following proton pairs: 7-H_3 and 2, 6-H; 2-H and 3-H; 4-H and 5α-H; 5β-H and 6-H (Fig. 2). Finally, the absolute stereostructure of **1** was determined by the application of modified Mosher's method.[29] Namely, treatment of **1** with (*R*)- or (*S*)-2-methoxy-2-trifluoromethylphenylacetic acid (MTPA) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) and 4-dimethylaminopyridine (4-DMAP) gave the 6-mono-(*R*)-MTPA ester (**1a**) and 6-mono-(*S*)-MTPA ester (**1b**), respec-

* To whom correspondence should be addressed. e-mail: myoshika@mb.kyoto-phu.ac.jp

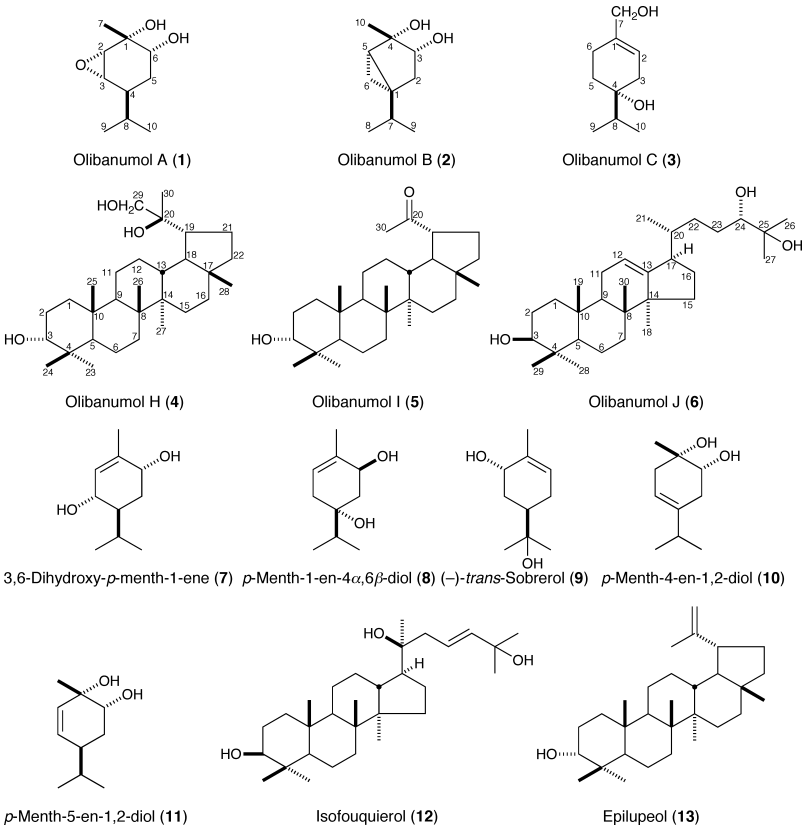

Chart 1

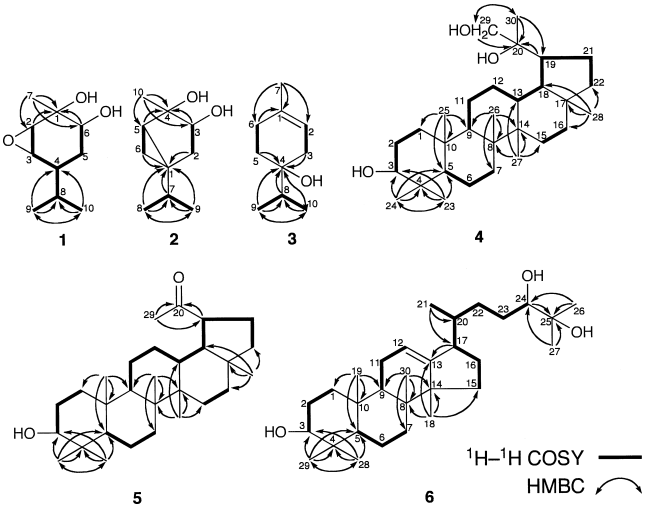

Fig. 1. ^{1}H–^{1}H COSY and HMBC Experiments of **1**—**6**

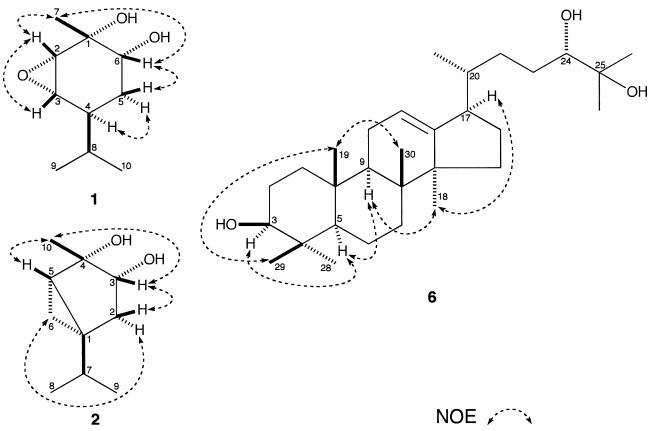

Fig. 2. NOESY Experiments of **1**, **2**, and **6**

tively, which showed an acylation shift at the 6-position [**1a**, **1b**: δ 4.87 (6-H)]. As shown in Fig. 3, the proton signals due to the 2 and 7-positions in **1b** were observed at higher fields as compared with those of **1a** ($\Delta\delta$: negative), while the signals due to the 4, 5, and 8—10-positions of **1b** were observed at lower fields as compared with those of **1a** ($\Delta\delta$: positive), so that the absolute configuration at the 6-position was clarified to be *R* orientation. Consequently, the absolute stereostructure of olibanumol A (**1**) was determined as shown.

Olibanumol B (**2**) was also obtained as a colorless oil with negative optical rotation ($[\alpha]_D^{24}$ −72.7° in MeOH) and

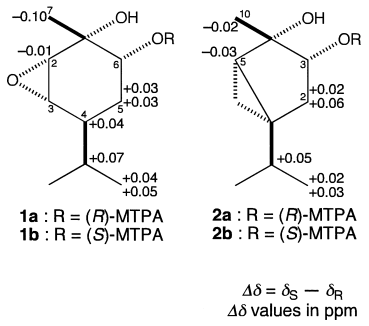

Fig. 3

Table 1. ^{13}C-NMR Data of Olibanumols A (**1**), B (**2**), and C (**3**)

Position	1	2	3
1	69.8	29.4	137.1
2	58.5	33.9	120.1
3	57.6	77.2	34.3
4	36.0	80.0	72.2
5	26.7	31.9	30.6
6	71.5	12.8	22.7
7	21.2	33.1	67.0
8	30.5	19.5	37.1
9	20.5	19.4	16.9
10	20.5	22.4	16.9

Measured in $CDCl_3$.

showed absorption band at 3252 cm^{-1} in the IR spectrum. The molecular formula $C_{10}H_{18}O_2$ was determined from the electron ionization (EI)-MS [*m/z* 170 (M^+)] and by high-resolution MS measurement. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **2** indicated the presence of two secondary methyls [δ 0.88 (3H, d, J=6.8 Hz, 8-H_3), 0.93 (3H, d, J=6.6 Hz, 9-H_3)], a tertiary methyl [δ 1.24 (3H, s, 10-H_3)], a cyclopropane ring [δ 0.31, 0.68 (1H each, both m, 6-H_2)], a methylene [δ 1.12 (1H, dd, J=3.8, 8.2 Hz, 5-H)], a methylene [δ 1.55 (1H, m, 2β-H), 2.02 (1H, dd, J=7.4, 12.4 Hz, 2α-H)], a methine bearing an oxygen function [δ 3.68 (1H, dd, J=7.9, 7.9 Hz, 3-H)], a methine [δ 1.20 (1H, m, 7-H)] and two quaternary carbons [δ_C 29.4 (1-C), 80.0 (4-C)]. The thujane-type monoterpene structure of **2** was clarified by ^{1}H–^{1}H COSY and HMBC experiments as shown in Fig. 1 and the relative stereostrucutre was characterized by NOESY experiment (Fig. 2). Finally, the absolute stereostructure of **2** was determined using modified Mosher's method (Fig. 3). That is, the proton signals due to the 5 and 10-positions in the 3-mono-(*S*)-MTPA ester (**2b**) were observed at higher fields as compared to those with the 3-mono-(*R*)-MTPA ester (**2a**) ($\Delta\delta$: negative), while the signals due to the 2 and 7—9 positions of **2b** were observed at lower fields as compared to those with **2a** ($\Delta\delta$: positive). On the basis of this evidence, the absolute configuration of the 3-position was elucidated to be *R* orientation and the total structure of olibanumol B (**2**) was determined as shown.

Olibanumol C (**3**), colorless oil with negative optical rotation ($[\alpha]_D^{22}$ −6.8° in MeOH), showed absorption bands at 3400 and 1646 cm^{-1} due to hydroxyl and olefin functions in the IR spectrum. The EI-MS spectrum of **3** showed a molecular ion peak at *m/z* 170 (M^+) together with fragment ion peaks at *m/z* 152 (M^+-H_2O), *m/z* 137 ($M^+-H_2O-CH_3$), and *m/z* 127 [$M^+-CH(CH_3)_2$]. The molecular formula, $C_{10}H_{18}O_2$, was determined from the molecular ion peak and by high resolution MS measurement. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra of **3** indicated the presence of an isopropyl group [δ 1.06 (6H, d, J=6.6 Hz, 9, 10-H_3), 1.50—1.58 (1H, m, 8-H)], a methylene with an oxygen function [δ 4.03 (2H, br s, 7-H_2)], and a trisubstituted olefin function [δ 5.60 (1H, br s, 2-H)]. The *p*-menthane-type monoterpene structure of **3** was determined by the ^{1}H–^{1}H COSY and HMBC experiments (Fig. 1) and the physical data were found to be identical with those of (4*S*)-*p*-menth-1-ene-4,7-diol,[30] except for the optical rotation ($[\alpha]_D^{21}$ +18° in MeOH). On the basis of this evidence, olimanumol C (**3**) was clarified to be the enantiomer of (4*R*)-*p*-menth-1-ene-4,7-diol.

Table 2. ^{13}C-NMR Data of Olibanumols H (**4**), I (**5**), and J (**6**), and **14**

Position	4	5	6	14
1	33.3	33.2	37.3	33.2
2	25.4	25.4	27.7	25.3
3	76.2	76.2	79.2	76.1
4	37.5	37.5	39.0	37.5
5	48.9	49.8	50.6	48.8
6	18.3	18.3	18.2	18.2
7	34.4	34.1	33.8	34.3
8	41.6	40.9	43.6	41.5
9	50.1	50.0	48.9	50.0
10	37.2	37.3	35.0	37.1
11	21.3	20.8	24.0	21.2
12	25.4	27.1	117.9	28.7
13	37.6	37.5	145.6	37.5
14	43.6	42.7	51.3	43.5
15	27.5	27.4	34.0	27.5
16	35.5	35.5	28.5	35.4
17	44.7	43.1	53.1	44.6
18	48.0	49.0	27.4	47.9
19	47.4	52.8	13.2	47.2
20	75.3	213.0	36.5	75.2
21	27.6	27.6	18.9	27.4
22	40.5	39.9	32.4	40.4
23	28.2	28.3	29.1	28.2
24	22.2	22.2	79.5	22.1
25	16.0	15.89	73.2	15.9
26	16.2	15.91	26.7	16.1
27	14.9	14.6	23.3	14.9
28	18.9	18.0	27.3	18.9
29	67.4		14.8	67.2
30	24.8	29.0	22.2	24.7

Measured in $CDCl_3$.

Absolute Stereostructures of Olibanumols H (4), I (5), and J (6) Olibanumol H (**4**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{30}$ +1.5°, MeOH). In the positive-ion fast atom bombardment (FAB)-MS of **4**, a quasi-molecular ion peak was observed at *m/z* 483 $(M+Na)^+$ and the molecular formula $C_{30}H_{52}O_3$ was determined by high resolution FAB-MS measurement. The proton and carbon signals in the ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 2) spectra of **4** showed signals assignable to seven methyls [δ 0.79, 0.83, 0.86, 0.94, 0.95, 1.06, 1.20 (3H ech, all s, 28, 24, 25, 23, 27, 26, 30-H_3)], a methine [δ 3.39 (1H, br s, 3-H)], a methylene [δ 3.43, 3.63 (1H each, both d, J=10.7 Hz, 29-H_2)], and quaternary carbon [δ_C 75.3 (20-C)] bearing an oxygen function together with 10 methylenes, five methines, and five quaternary carbons. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment on **4** indicated the presence of partial structures written in bold lines. In the HMBC experiment on **4**, long-range correlations were observed between the following protons and carbons: 19-H and 20-C; 23-H_3 and 3—5, 24-C; 24-H_3 and 3—5, 23-C; 25-H_3 and 1, 5, 9, 10-C; 26-H_3 and 7—9, 14-C; 27-H_3 and 8, 13—15-C; 28-H_3 and 16—18, 22-C; 29-H_2 and 20, 30-C; 30-H_3 and 20, 29-C. Thus, the connectivities of quaternary carbons (4, 8, 10, 14, 17, 20-C) in **4** were clarified and its lupane-type triterpene structure was elucidated. In order to clarify the absolute stereostructure of **4**, we carried out the chemical modification from epilupeol (**13**) to **4**. As shown in Chart 2, oxidation of **13** with osmium tetraoxide (OsO_4) yielded **4** and its diastereomer (**14**) in an approximate 3 : 1 ratio. In addition, **4** was also yielded by treatment of **13**

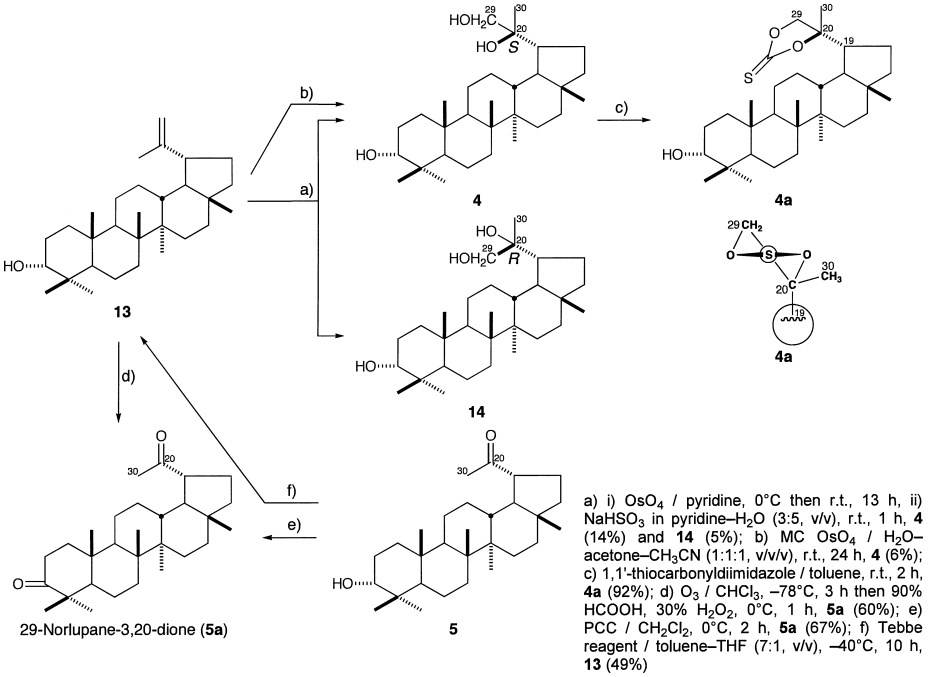

Chart 2

with microcapsule osmium tetraoxide (MC OsO_4)[31] as an oxidizing agent. Next, **4** was treated with 1,1′-thiocarbonyldiimidazole in the presence of 4-DMAP to give the 20,29-thionocarbonate (**4a**), which showed a positive Cotton effect [304 nm ($\Delta\varepsilon$ +1.40) in MeOH] associated by the n→π* transition of cyclic thionocarbonate moiety.[32—34] On the basis of these evidence, the absolute configuration at the 20-position was determined as *S* orientation and the total structure of olibanumol H (**4**) was determined to be as shown.

Olibanumol I (**5**) was also isolated as a white powder with negative optical rotation ($[\alpha]_D^{28}$ −22.7°). Its molecular formula $C_{29}H_{48}O_2$ was determined from the positive-ion FAB-MS and by high resolution FAB-MS measurements. The ^{1}H-($CDCl_3$) and ^{13}C-NMR (Table 2) spectra of **5** were similar to those of **13**, except for the signals due to around the 20-position [δ 0.77, 0.82, 0.84, 0.93, 0.98, 1.02, 2.13 (3H ech, all s, 28, 24, 25, 23, 27, 26, 29-H_3), 2.55 (1H, m, 19-H), 3.39 (1H, br s, 3-H)]. As shown in Fig. 1, the ^{1}H–^{1}H COSY experiment indicated the presence of partial structure in bold lines and long-range correlations were observed between the following protons and carbons in the HMBC experiment: 19-H and 20-C; 23-H_3 and 3—5, 24-C; 24-H_3 and 3—5, 23-C; 25-H_3 and 1,5, 9, 10-C; 26-H_3 and 7—9, 14-C; 27-H_3 and 8, 13—15-C; 28-H_3 and 16—18, 22-C; 29-H_3 and 19, 20-C. Thus, the connectivity of carbonyl carbon [δ_C 213.0 (20-C)] in **5** was clarified, so that the 29-norlupane-type triterpene structure was elucidated. Finally, the total structure including the absolute stereostructure of **5** was determined by the chemical conversion from **13**. As shown in Chart 2, ozone oxidation of **13** followed by treatment of 90% aqueous formic acid and 30% aqueous hydrogen peroxide yielded 29-norlupane-3,20-dione[35] (**5a**), which was also obtained by pyridinium chlorochromate (PCC) oxidation of **5**. Furthermore, **5** was directly derived to **13** using Tebbe reagent.[36,37] On the basis of above-mentioned evidence, the absolute stereostructure of olibanumol I (**5**) was determined to be as shown.

Olibanumol J (**6**) was also as a white powder with positive optical rotation ($[\alpha]_D^{22}$ +1.1° in MeOH). The molecular formula, $C_{30}H_{52}O_3$, of **6** was determined by a quasimolecular ion peak in the positive-ion FAB-MS and the high resolution FAB-MS measurements. The ^{1}H- ($CDCl_3$) and ^{13}C-NMR (Table 2) spectra of **6** showed signals assignable to six tertiary methyls [δ 0.75, 0.83, 0.86, 0.92, 0.97, 1.16, 1.22 (3H ech, all s, 19, 30, 29, 18, 28, 27, 26-H_3)], a secondary methyl [δ 0.86 (3H, d, J=5.9 Hz, 21-H_3)], two methines bearing an oxygen function [δ 3.27 (1H, dd-like, 3-H), 3.29 (1H, dd-like, 24-H)], and an olefin [δ 5.25 (1H, br s, 12-H)] and together with a quaternary carbon bearing an oxygen function [δ_C 73.2 (25-C)]. The dammarane-type triterpene structure of **6** was clarified by ^{1}H–^{1}H COSY and HMBC experiments as shown in Fig. 1 and the relative stereostrucutre was characterized by NOESY experiment (Fig. 2). Thus, the stereostructure of **6** was clarified except for the 24-position. Finally, the stereochemistry at a vicinal glycol moiety including the 24-position in **6** was elucidated by means of the circular dichroic (CD) spectroscopic method using europium tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate) [Eu(fod)$_3$] as a chelating reagent.[38] Namely, a solution of 24(*R*),25-dihydroxycholesterol (i) and [Eu(fod)$_3$] (1 : 1 ratio) in $CHCl_3$ or CCl_4 solution was observed a negative Cotton effect [308 nm ($\Delta\varepsilon$ −11±1)], while a solution of 24(*S*),25-dihydroxycholesterol (ii) and [Eu(fod)$_3$] (1 : 1 ratio) in $CHCl_3$ or CCl_4 solution was observed a positive Cotton effect [308 nm ($\Delta\varepsilon$ +12±1)].[38] Using this method, the absolute configuration at the 24-position in **6** was determined as *S* orientation {**6** and [Eu(fod)$_3$] (1 : 1 ratio) in CCl_4: 308 nm ($\Delta\varepsilon$ +2.44)} and the total structure of olibanumol J (**6**) was determined to be as shown.

Inhibitory Effects on Production of NO in LPS-Activated Macrophages The inorganic free radical NO has been implicated in physiologic and pathologic processes,

Table 3. Inhibitory Effects of 80% Aqueous Acetone Extract and Constituents from *B. carterii* on NO Production Induced by LPS in Mouse Peritoneal Macrophages

	Inhibition (%)					
	0 μg/ml	1 μg/ml	3 μg/ml	10 μg/ml	30 μg/ml	100 μg/ml
80% aqueous acetone ext.	0.0±2.6	8.1±2.7	11.3±1.0**	14.2±1.3**	22.3±1.9**	59.8±2.2**,*a)*

	Inhibition (%)					
	0 μM	1 μM	3 μM	10 μM	30 μM	100 μM
Olibanumol A (**1**)	0.0±4.3	5.2±2.9	−3.0±4.8	−1.1±3.9	15.9±1.0*	16.5±1.2*
Olibanumol B (**2**)	0.0±5.5	5.3±1.0	7.6±3.9	9.8±5.7	0.2±3.9	0.3±4.1
Olibanumol C (**3**)	0.0±2.3	11.0±5.5	7.7±5.3	16.7±5.9	7.4±4.9	18.8±4.1
Olibanumol H (**4**)	0.0±1.8	5.5±1.6	6.1±2.5	11.5±2.8**	37.4±1.2**	100.1±0.2**,*a)*
Olibanumol I (**5**)	0.0±2.5	9.2±1.1*	10.3±2.3**	12.8±1.2**	32.5±3.3**	97.8±0.3**,*a)*
3,6-Dihydroxy-*p*-menth-1-ene (**7**)	0.0±5.8	−11.0±2.8	−6.2±1.9	−11.0±2.3	1.2±1.2	6.1±4.6
p-Menth-1-en-4α,6β-diol (**8**)	0.0±10.2	10.1±8.3	−4.6±4.0	−4.6±1.8	15.4±7.5	8.8±4.0
(−)-*trans*-Sobrerol (**9**)	0.0±6.9	−11.9±4.3	−0.7±9.0	−0.4±2.1	−1.4±4.2	3.1±2.8
p-Menth-4-en-1,2-diol (**10**)	0.0±3.7	6.9±6.0	16.9±5.5	3.7±4.4	8.3±7.7	8.5±3.9
p-Menth-5-en-1,2-diol (**11**)	0.0±5.1	−13.5±4.6	−5.8±6.6	−16.0±4.6	3.3±5.4	9.7±3.7
Isofpuquierol (**12**)	0.0±0.9	−0.3±2.3	0.6±2.0	4.9±1.7	18.4±3.6**	86.6±0.2**,*a)*
Epilupeol (**13**)	0.0±2.1	1.9±0.6	−1.3±1.2	0.0±0.4	2.0±1.6	12.6±0.7**
L-NMMA[45]	0.0±3.1	1.0±2.4	1.4±2.8	19.9±2.8**	43.0±2.1**	70.9±1.6**
CAPE[45]	0.0±2.1	−1.1±2.3	5.9±5.2	44.4±3.2**	86.2±1.1**	99.6±0.1**,*a)*

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, * p<0.05, ** p<0.01. *a*) Cytotoxic effect was observed.

such as vasodilation, nonspecific host defense, ischemia-reperfusion injury, and chronic or acute inflammation. NO is produced by the oxidation of L-arginine by NO synthase (NOS). In the family of NOS, inducible NOS (iNOS) is specifically involved in pathologic aspects with the overproduction of NO and can be expressed in response to proinflammatory agents such as interleukin-1β, tumor necrosis factor-α, and LPS in various cell types including macrophages, endothelial cells, and smooth muscle cells. As a part of our studies to characterize the bioactive components of natural medicines, we have investigated various NO production inhibitors, *i.e.*, higher unsaturated fatty acids,[39] polyacetylenes,[40—42] coumarins,[40,42,43] flavonoids,[41,44,45] stilbenes,[46,47] lignans,[48—50] sesquiterpenes,[51—58] diterpenes,[59,60] triterpenes,[19,61—63] diarylheptanoids,[64—66] cyclic peptides,[63] alkaloids,[67,68] and phenylpropanoids.[50,69,70] As a continuation of these studies on bioactive constituents of natural medicines, the effects of the mono- and triterpene constituents (**1**—**5**, **7**—**13**) from the gum-resin of *B. carterii* on NO production from LPS-activated macrophages were examined, and the results were summarized in Table 3. Among them, olibanumols A [**1**, inhibition (%) at 30 μM 15.9±1.0], H (**4**, 37.4±1.2), and I (**5**, 32.5±3.3), and isofouquierol (**12**, 18.4±3.6) exhibited the inhibitory activity.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; ^{1}H-NMR spectra, JEOL JNM-LA500 (500 MHz) and EX-270 (270 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-LA500 (125 MHz) and EX-270 (68 MHz) spectrometers with tetramethylsilane as an internal standard; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and high resolution FAB-MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index detector; HPLC column, YMC-Pack ODS-A (YMC Co., Ltd., Kyoto, Japan) (250 mm×4.6 mm i.d.) and (250 mm×20 mm i.d.) columns were used for analytical and preparative purposes, respectively.

The following experimental conditions were used for chromatography: normal-phase silica gel column chromatography (CC), silica gel 60N (Kanto Chemical Co., Ltd., 63—210 mesh, spherical, neutral); reversed-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); normal-phase TLC, pre-coated TLC plates with silica gel 60F$_{254}$ (Merck, 0.25 mm); reversed-phase TLC, pre-coated TLC plates with silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm); reversed-phase HPTLC, pre-coated TLC plates with silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm), detection was achieved by spraying with 1% Ce(SO$_4$)$_2$–10% aqueous H$_2$SO$_4$, followed by heating.

Plant Material The gum-resin from *B. carterii* collected in Yemen and purchased at Cairo of Egypt, in February 2000, and the identification was kindly undertaken by Professor Gisho Honda, Graduate School of Pharmaceutical Sciences, Kyoto University. A voucher of the plant material is on file in our laboratory.

Extraction and Isolation The gum-resin from *B. carterii* (2.7 kg) was extracted three times with 80% aqueous acetone at room temperature for 24 h. Evaporation of the solvent under reduced pressure provided an aqueous acetone extract (2131 g, 78.9%). The aqueous acetone extract (360.0 g) was subjected to normal-phase silica gel CC [3.0 kg, *n*-hexane–EtOAc (30 : 1→20 : 1→10 : 1→1 : 1, v/v)→MeOH] to give 11 fractions [Fr. 1 (6.3 g), Fr. 2 (11.2 g), Fr. 3 (30.5 g), Fr. 4 (181.4 g), Fr. 5 (12.7 g), Fr. 6 (9.3 g), Fr. 7 (18.2 g), Fr. 8 (14.6 g), Fr. 9 (7.1 g), Fr. 10 (5.9 g), and Fr. 11 (15.6 g)]. The fraction 4 (1.0 g) was subjected to reversed-phase silica gel CC [30 g, MeOH–H$_2$O (90 : 10, v/v)→MeOH] to give epilupeol (**13**, 948.3 mg, 37.7%). The fraction 11 (15.0 g) was purified by normal-phase silica gel CC [450 g, *n*-hexane–EtOAc (1 : 1→2 : 3→1 : 2, v/v)→MeOH] to give five fractions [Fr. 11-1 (95.0 mg), Fr. 11-2 (5.1 g), Fr. 11-3 (2.0 g), Fr. 11-4 (1.6 g), and Fr. 11-5 (5.0 g)]. The fraction 11-2 (5.1 g) was subjected by reversed-phase silica gel CC [153 g, MeOH–H$_2$O (50 : 50→60 : 40→70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [MeOH–H$_2$O (90 : 10, v/v)] to furnish olibanumol A (**1**, 168.8 mg, 0.037%), 3,6-dihydroxy-*p*-menth-1-ene (**7**, 95.8 mg, 0.021%), *p*-menth-1-en-4α,6β-diol (**8**, 82.1 mg, 0.018%), *p*-menth-4-en-1,2-diol (**10**, 63.8 mg, 0.014%) and *p*-menth-5-en-1,2-diol (**11**, 196.1 mg, 0.043%). The fraction 11-3 (2.0 g) was subjected by reversed-phase silica gel CC [60 g, MeOH–H$_2$O (50 : 50→60 : 40→70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [MeOH–H$_2$O (90 : 10, v/v)] to furnish olibanumols H (**4**, 68.4 mg, 0.015%) and J (**6**, 14.1 mg, 0.0031%), and isofouquierol (**12**, 10.4 mg, 0.0023%). The fraction 11-4 (1.6 g) was subjected by reversed-phase silica gel CC [50 g, MeOH–H$_2$O (50 : 50→60 : 40→70 : 30→80 : 20→90 : 10, v/v)→MeOH] and HPLC [MeOH–H$_2$O (90 : 10, v/v)] to furnish olibanumols B (**2**, 118.6 mg, 0.026%), C (**3**,

40.6 mg, 0.0089%), and I (**5**, 3.3 mg, 0.00074%), and (−)-*trans*-sobrerol (**9**, 45.6 mg, 0.010%).

Olibanumol A (**1**): Colorless oil, $[\alpha]_D^{28}$ +7.6° (c=1.00, MeOH). High-resolution EI-MS: Calcd for $C_{10}H_{18}O_3$ (M^+) 186.1256; Found 186.1270. IR (film, cm^{-1}): 3426, 2950. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.00, 1.06 (3H each, both d, J=6.6 Hz, 9, 10-H_3), 1.35 (3H, s, 7-H_3), 1.52 (1H, m, 5β-H), 1.75 (1H, m, 8-H), 1.87 (1H, m, 4-H), 1.95 (1H, m, 5α-H), 3.06 (1H, d, J=4.0 Hz, 2-H), 3.40 (1H, dd-like, 3-H), 3.62 (1H, dd-like, 6-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 186 (M^+, 1), 168 (M^+-H_2O, 3), 87 (100).

Olibanumol B (**2**): Colorless oil, $[\alpha]_D^{24}$ −72.7° (c=1.00, MeOH). High-resolution EI-MS: Calcd for $C_{10}H_{18}O_2$ (M^+) 170.1307; Found 170.1295. IR (film, cm^{-1}): 3252, 2954, 2871. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.31, 0.68 (1H each, both m, 6-H_2), 0.88 (3H, d, J=6.8 Hz, 8-H_3), 0.93 (3H, d, J=6.6 Hz, 9-H_3), 1.12 (1H, dd, J=3.8, 8.2 Hz, 5-H), 1.20 (1H, m, 7-H), 1.24 (3H, s, 10-H_3), 1.55 (1H, m, 2β-H), 2.02 (1H, dd, J=7.4, 12.4 Hz, 2α-H), 3.68 (1H, dd, J=7.9, 7.9 Hz, 3-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 170 (M^+, 1), 152 (M^+-H_2O, 9), 134 (33), 109 (100).

Olibanumol C (**3**): Colorless oil, $[\alpha]_D^{22}$ −6.8° (c=1.00, MeOH). High-resolution EI-MS: Calcd for $C_{10}H_{18}O_2$ (M^+) 170.1307; Found 170.1302. IR (film, cm^{-1}): 3400, 2991, 1646. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.06 (6H, d, J=6.6 Hz, 9, 10-H_3), 1.50—1.58 (5H, m, 5-H_2, 6-H_2, and 8-H), 2.05, 2.30 (1H each, both m, 3-H_2), 4.03 (2H, br s, 7-H_2), 5.60 (1H, br s, 2-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z (%): 170 (M^+, 1), 152 (M^+-H_2O, 34), 137 (34), 127 (13), 71 (100).

Olibanumol H (**4**): A white powder, $[\alpha]_D^{30}$ +1.5° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{52}O_3Na$ $(M+Na)^+$ 483.3814; Found 483.3817. IR (KBr, cm^{-1}): 3475, 2942. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.79, 0.83, 0.86, 0.94, 0.95, 1.06, 1.20 (3H each, all s, 28, 24, 25, 23, 27, 26, 30-H_3), 3.39 (1H, br s, 3-H), 3.43, 3.63 (1H each, both d, J=10.7 Hz, 29-H_2). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 483 $(M+Na)^+$.

Olibanumol I (**5**): A white powder, $[\alpha]_D^{28}$ −22.7° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{29}H_{49}O_2$ $(M+H)^+$ 429.3733; Found 429.3762. IR (KBr, cm^{-1}): 3475, 2944, 1703. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.77, 0.82, 0.84, 0.93, 0.98, 1.02, 2.13 (3H each, all s, 28, 24, 25, 23, 27, 26, 29-H_3), 2.55 (1H, m, 19-H), 3.39 (1H, br s, 3-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 429 $(M+H)^+$.

Olibanumol J (**6**): A white powder, $[\alpha]_D^{22}$ +1.1° (c=1.00, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{52}O_3Na$ $(M+Na)^+$ 483.3814; Found 483.3805. IR (KBr, cm^{-1}): 3432, 2952, 1636. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.75, 0.83, 0.86, 0.92, 0.97, 1.16, 1.22 (3H ech, all s, 19, 30, 29, 18, 28, 27, 26-H_3), 0.86 (3H, d, J=5.9 Hz, 21-H_3), 1.44 (1H, m, 20-H), 3.27 (1H, dd-like, 3-H), 3.29 (1H, dd-like, 24-H), 5.25 (1H, br s, 12-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 483 $(M+Na)^+$.

Preparation of the (*R*)-MTPA Ester (1a) and (*S*)-MTPA Ester (1b) from 1 A solution of **1** (1.5 mg) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-2-methoxy-2-trifluoromethylphenylacetic acid [(*R*)-MTPA, 5.0 mg] in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl, 5.0 mg) and 4-dimethylaminopyridine (4-DMAP, 1.0 mg), and the mixture was stirred under reflux for 2 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (4 : 1, v/v)] to give **1a** (1.1 mg, 38%). Through a similar procedure, **1b** (1.0 mg, 37%) was obtained from **1** (1.4 mg) using (*S*)-MTPA (5.0 mg), EDC·HCl (5.0 mg), and 4-DMAP (1.0 mg).

Compound **1a**: Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.90, 0.95 (3H each, both d, J=6.6 Hz, 9, 10-H_3), 1.30 (3H, s, 7-H_3), 1.54 (1H, m, 4-H), 1.68 (1H, m, 8-H), 1.68, 1.72 (1H each, both m, 5-H_2), 3.00 (1H, d, J=4.0 Hz, 2-H), 3.31 (1H, dd-like, 3-H), 3.55 (3H, s, OCH_3), 4.87 (1H, br d, J=*ca.* 6 Hz, 6-H), 7.29—7.52 (5H, m, Ph-H).

Compound **1b**: Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.94, 1.00 (3H each, both d, J=6.6 Hz, 9, 10-H_3), 1.20 (3H, s, 7-H_3), 1.58 (1H, m, 4-H), 1.71, 1.75 (1H each, both m, 5-H_2), 1.75 (1H, m, 8-H), 2.99 (1H, d, J=4.0 Hz, 2-H), 3.37 (1H, dd-like, 3-H), 3.51 (3H, s, OCH_3), 4.87 (1H, dd-like, 6-H), 7.30—7.52 (5H, m, Ph-H).

Preparation of the (*R*)-MTPA Ester (2a) and (*S*)-MTPA Ester (2b) from 2 A solution of **2** (1.5 mg) in CH_2Cl_2 (1.0 ml) was treated with (*R*)-MTPA (5.0 mg) in the presence of EDC·HCl (5.0 mg) and 4-DMAP (1.0 mg), and the mixture was stirred under reflux for 2 h. The reaction mixture was poured into ice-water and extracted with EtOAc. Work-up of the EtOAc extract as described above gave a product, which was purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (4 : 1, v/v)] to give **2a** (1.1 mg, 32%). Through a similar procedure, **2b** (1.0 mg, 32%) was obtained from **2** (1.4 mg) using (*S*)-MTPA (5.0 mg), EDC·HCl (5.0 mg), and 4-DMAP (1.0 mg).

Compound **2a**: Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.90, 0.95 (3H each, both d, J=6.6 Hz, 8, 9-H_3), 1.23 (1 H, m, 5-H), 1.30 (1H, m, 7-H), 1.30 (3H, s, 10-H_3), 1.49, 2.02 (1H each, both m, 2-H_2), 3.51 (3H, s, OCH_3), 4.27 (1H, m, 3-H), 7.30—7.52 (5H, m, Ph-H).

Compound **2b**: Colorless oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.93, 0.97 (3H each, both d, J=6.6 Hz, 8, 9-H_3), 1.20 (1H, m, 5-H), 1.35 (1H, m, 7-H), 1.28 (3H, s, 10-H_3), 1.55, 2.04 (1H each, both m, 2-H_2), 3.51 (3H, s, OCH_3), 4.26 (1H, m, 3-H), 7.30—7.52 (5H, m, Ph-H).

Osmium Tetraoxide (OsO_4) Oxidation of 13 A solution of **13** (60.0 mg) in dry-pyridine (10.0 ml) was treated with osmium tetraoxide (OsO_4, 120.0 mg) at 0 °C, and then the mixture was stirred at room temperature for 13 h. Then a solution of $NaHSO_3$ (26.0 mg) in pyridine–H_2O (3 : 5, v/v, 5.0 ml) was added to the reaction mixture and stirred at room temperature for 1 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel CC [4.0 g, *n*-hexane–EtOAc (10 : 1, v/v)] to give **4** (29.5 mg, 14%) and **14** (11.0 mg, 5%).

Compound **14**: A white powder, $[\alpha]_D^{24}$ −15.0° (c=0.32, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{30}H_{52}O_3Na$ $(M+Na)^+$ 483.3814; Found 483.3820. IR (KBr, cm^{-1}): 3474, 2942. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.81, 1.03, 1.08, 1.09, 1.20 (3H each, all s, 28, 23, 27, 26, 30-H_3), 0.94 (6H, s, 24, 25-H_3), 3.43, 3.63 (1H each, both d, J=10.8 Hz, 29-H_2), 3.48 (1H, br s, 3-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 2. Positive-ion FAB-MS m/z: 483 $(M+Na)^+$.

Microcapsule Osmium Tetraoxide (MC OsO_4) Oxidation of 13 A solution of **13** (20.0 mg) in H_2O–acetone–CH_3CN (1 : 1 : 1, v/v/v, 5.0 ml) was treated with microcapsule osmium tetraoxide (MC OsO_4, 50.0 mg) and the mixture was stirred at room temperature for 24 h. The reaction mixture was filtered and evaporated under reduced pressure gave a residue. The residue was purified by normal-phase silica gel CC [4.0 g, *n*-hexane–EtOAc (10 : 1, v/v)] to give **4** (5.0 mg, 6%), which was identified by comparison of the physical data with those of natural **4**.

Thiocarbonylation of 4 To a solution of **4** (3.0 mg) in dry-toluene (1.0 ml) was added 1,1′-thiocarbonyldiimidazole (2.5 mg) and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The extract was successively washed with 5% aqueous HCl and brine, and filtrated. Removal of the solvent under reduced pressure gave a residue, which was purified by normal-phase silica gel CC [10.0 g, *n*-hexane–EtOAc (2 : 1, v/v)] to give **4a** (3.0 mg, 92%).

Compound **4a**: Colorless oil. High-resolution positive-ion FAB-MS: Calcd for $C_{31}H_{50}O_3SNa$ $(M+Na)^+$ 525.3378; Found 525.3368. CD (MeOH, nm, $\Delta\varepsilon$): 230 (−1.61), 304 (+1.40). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.82, 0.82, 0.83, 0.93, 0.94, 1.05, 1.26 (3H ech, all s, 28, 25, 24, 27, 23, 26, 30-H_3), 3.39 (1H, br s, 3-H), 4.33, 4.55 (1H each, both d, J=8.7 Hz, 29-H_2). Positive-ion FAB-MS m/z: 525 $(M+Na)^+$.

Ozone Oxidation of 13 A solution of **13** (205.0 mg) in $CHCl_3$ (10.0 ml) was oxidized with O_3 gas (3.0 g/h) at −78 °C for 3 h to give ozonide. Successively, the reaction mixture was treated with 90% aqueous formic acid (10.0 ml) and 30% aqueous hydrogen peroxide (4.0 ml), and the mixture was stirred at 0 °C for 1 h. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue. The residue was purified by normal-phase silica gel CC [6.0 g, *n*-hexane–EtOAc (10 : 1, v/v)] to give 29-norlupane-3,20-dione (**5a**, 122.0 mg, 60%), which was identified by comparison of the physical data with reported values.[35]

PCC Oxidation of 5 A solution of **5** (5.0 mg) in dry-CH_2Cl_2 (1.0 ml) was treated with pyridinium chlorochromate (PCC, 5.0 mg), and the whole mixture was stirred at room temperature for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with saturated aqueous $NaHCO_3$ and brine, then dried

over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was then purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (10 : 1, v/v)] to give **5a** (3.4 mg, 67%).

Ketone Methylation Using Tebbe Reagent of 5 A solution of **5** (10.0 mg) in dry-toluene–THF (7 : 1, v/v, 2.0 ml) was treated with Tebbe reagent [0.4 ml (*ca.* 0.5 M solution in toluene)] and dry-pyridine (25 μl) at −40 °C for 10 h. The reaction mixture was treated with Et_2O (2.0 ml) and 5 drops of 0.4 N NaOH, and then the whole was extracted with EtOAc. The EtOAc extract was successively washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by normal-phase silica gel CC [1.0 g, *n*-hexane–EtOAc (10 : 1, v/v)] to give **13** (6.2 mg, 49%).

Bioassay. Reagents Lipopolysaccharide (LPS, from *Salmonella enteritidis*), N^G-monomethyl-L-arginine (L-NMMA), and RPMI 1640 medium were purchased from Sigma; 3-(4,5-dimethyl-2-thiazolyl) 2,5-diphenyl tetrazolium bromide (MTT) was from Dojindo Laboratories; protease inhibitor cocktail (Complete Mini) was from Roche Diagnostics GmbM; fetal calf serum (FCS) was from Gibco; thioglycolate (TGC) medium was from Nissui Seiyaku; other reagents was from Wako Pure Chemical.

Effects on Production of NO in LPS-Stimulated Mouse Peritoneal Macrophages Screening test for NO production using TGC-induced mouse peritoneal macrophages was performed as described previously.[45,58] Briefly, peritoneal exudate cells (5×10^5 cells/well) were collected from the peritoneal cavities of male ddY mice and were suspended in 100 μl of RPMI 1640 supplemented with 5% fetal calf serum (FCS), penicillin (100 units/ml) and streptomycin (100 μg/ml), and pre-cultured in 96-well microplates at 37 °C in 5% CO_2 in air for 1 h. Nonadherent cells were removed by washing with PBS, and the adherent cells were cultured in 200 μl of fresh medium containing 10 μg/ml LPS and various concentrations of test compound for 20 h. NO production in each well was assessed by measuring the accumulation of nitrite (NO_2^-) in the culture medium using Griess reagent. Cytotoxicity was determined by the MTT colorimetric assay, after 20 h incubation with test compounds. Each test compound was dissolved in dimethyl sulfoxide (DMSO), and the solution was added to the medium (final DMSO concentration was 0.5%). Inhibition (%) was calculated using the following formula and IC_{50} was determined graphically ($n=4$).

$$\text{inhibition (\%)} = [(A—B)/(A—C)]\times 100$$

A—C: NO_2^- concentration (μM)

[A: LPS (+), sample (−); B: LPS (+), sample (+); C: LPS (−), sample (−)]

Statistics Values are expressed as means±S.E.M. One-way analysis of variance followed by Dunnett's test was used for statistical analysis.

Acknowledgements The authors are sincerely grateful to Professor Gisho Honda, Graduate School of Pharmaceutical Sciences, Kyoto University, for the botanical identification of olibanum. M. Yoshikawa and H. Matsuda were supported by the 21st COE Program, Academic Frontier Project, and a Grant-in Aid for Scientific Research from MEXT (the Ministry of Education, Culture, Sports, Science and Technology of Japan). T. Morikawa was supported by High-tech Research Center Project (2007—2011) and a Grant-in Aid for Scientific Research from MEXT.

References and Notes

1) Shanghai Scientific and Technologic Press, "Dictionary of Chinese Traditional Medicines," Shogakkan, Tokyo, 1985, pp. 2012—2014.
2) Safayhi H., Ammon H. P. T., *Pharm. Zeit.*, **142**, 3277—3280, 3282, 3284—3286 (1997).
3) Safayhi H., Boden S. E., Schweizer S., Ammon H. P. T., *Planta Med.*, **66**, 110—113 (2000).
4) Fan A. Y., Lao L., Zhang R. X., Zhou A. N., Wang L. B., Moudgil K. D., Lee D. Y. W., Ma Z. Z., Zhang W. Y., Berman B. M., *J. Ethnopharmacol.*, **101**, 104—109 (2005).
5) Büchele B., Zugmaier W., Estrada A., Genze F., Syrovets T., Paetz C., Schneider B., Simmet T., *Planta Med.*, **72**, 1285—1289 (2006).
6) Banno N., Akihisa T., Yasukawa K., Tokuda H., Tabata K., Nakamura Y., Nishimura R., Kimura Y., Suzuki T., *J. Ethnopharmacol.*, **107**, 249—253 (2006).
7) Akihisa T., Tabata K., Banno N., Tokuda H., Nishihara R., Nakamura Y., Kimura Y., Yasukawa K., Suzuki T., *Biol. Pharm. Bull.*, **29**, 1976—1979 (2006).
8) Pardhy R. S., Bhattacharyya S. C., *Ind. J. Chem.*, **16B**, 171—173 (1978).
9) Pardhy R. S., Bhattacharyya S. C., *Ind. J. Chem.*, **16B**, 174—175 (1978).
10) Pardhy R. S., Bhattacharyya S. C., *Ind. J. Chem.*, **16B**, 176—178 (1978).
11) Singh G. B., Atal C. K., *Agents Actions*, **18**, 407—412 (1986).
12) Sharma M. L., Bani S., Singh G. B., *Int. J. Immunopharm.*, **11**, 647—652 (1989).
13) Safayhi H., Mack T., Sabieraj J., Anazodo M. I., Subramanian L. R., Ammon H. P. T., *J. Pharmacol. Exp. Ther.*, **261**, 1143—1146 (1992).
14) Ammon H. P. T., Safayhi H., Mark T., Sabieraj J., *J. Ethnopharmacol.*, **38**, 105—112 (1993).
15) Safayhi H., Sailer E.-R., *Planta Med.*, **63**, 487—493 (1997).
16) Hamm S., Bleton J., Connan J., Tchapla A., *Phytochemistry*, **66**, 1499—1514 (2005).
17) Kimura I., Yoshikawa M., Kobayashi S., Sugihara Y., Suzuki M., Oominami H., Muraokami T., Matsuda H., Doiphode V. V., *Bioorg. Med. Chem. Lett.*, **11**, 985—989 (2001).
18) Matsuda H., Morikawa T., Ando S., Oominami H., Muraokami T., Kimura I., Yoshikawa M., *Chem. Pharm. Bull.*, **52**, 1200—1203 (2004).
19) Matsuda H., Morikawa T., Ando S., Oominami H., Murakami T., Kimura I., Yoshikawa M., *Bioorg. Med. Chem.*, **12**, 3037—3046 (2004).
20) Morikawa T., Matsuda H., Oominami H., Kageura T., Toguchida I., Yoshikawa M., "Abstract of Papers," the 43rd Symposium on the Chemistry of Natural Products, Osaka, October, 2001, pp. 485—490.
21) Talapatra S. K., Pal M. K., Mallik A. K., Talapatra B., *J. Indian Chem. Soc.*, **57**, 999—1002 (1985).
22) Garside P., Halsall T. G., Hornby G. M., *J. Chem. Soc. C*, **1969**, 716—721 (1969).
23) Schmidt H., *Chem. Ber.*, **86**, 1437—1444 (1953).
24) Garg S. N., Agarwal S. K., *Phytochemistry*, **27**, 936—937 (1988).
25) De Pascual Teresa J., Torres C., González M. S., Grande M., Bellido I. S., *Phytochemistry*, **22**, 2749—2751 (1983).
26) Butruille D., Dominguez X. A., *Tetrahedron Lett.*, **8**, 639—642 (1974).
27) di Cabdi Farah Xaasan N., Minale L., Bashir M., Hussein M., Finamore E., *Rend. Accad. Sci. Fis. Mat. Naples*, **51**, 93—96 (1984).
28) The ^{1}H- and ^{13}C-NMR spectra of **1**—**6** and **14** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments.
29) Ohtani I., Kusumi T., Kashman Y., Kakisawa H., *J. Am. Chem. Soc.*, **113**, 4092—4069 (1991).
30) Ishikawa T., Sega Y., Kitajima J., *Chem. Pharm. Bull.*, **49**, 840—844 (2001).
31) Nagayama S., Endo M., Kobayashi S., *J. Org. Chem.*, **63**, 6094—6095 (1998).
32) Haines A. H., Jenkins C. S. P., *J. Chem. Soc. C*, **1971**, 1438—1442 (1971).
33) De Pascual Teresa J., Barrero A. F., San Feliciano A., Grande M., Medarde M., *Tetrahedron Lett.*, **43**, 4141—4144 (1978).
34) Muraoka O., Morikawa T., Zhang Y., Ninomiya K., Nakamura S., Matsuda H., Yoshikawa M., *Tetrahedron*, **65**, 4142—4148 (2009).
35) Cole B. J. W., Bentley M. D., Hua Y., Bu L., *J. Wood Chem. Technol.*, **11**, 209—223 (1991).
36) Tebbe F. N., Parshall G. W., Reddy G. S., *J. Am. Chem. Soc.*, **100**, 3611—3613 (1978).
37) Pine S. H., Shen G. S., Hoang H., *Systhesis*, **1991**, 165—167 (1991).
38) Partridge J. J., Toome V., Uskokovic M. R., *J. Am. Chem. Soc.*, **98**, 3739—3741 (1976).
39) Yoshikawa M., Murakami T., Shimada H., Yoshizumi S., Saka M., Matsuda H., *Chem. Pharm. Bull.*, **46**, 1008—1014 (1998).
40) Matsuda H., Murakami T., Kageura T., Ninomiya K., Toguchida I., Nishida N., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 2191—2196 (1998).
41) Yoshikawa M., Morikawa T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull.*, **48**, 651—656 (2000).
42) Yoshikawa M., Nishida N., Ninomiya K., Ohgushi T., Kubo M., Morikawa T., Matsuda H., *Bioorg. Med. Chem.*, **14**, 456—463 (2006).
43) Matsuda H., Morikawa T., Ohgushi T., Ishiwada T., Nishida N.,

Yoshikawa M., *Chem. Pharm. Bull.*, **53**, 387—392 (2005).
44) Matsuda H., Morikawa T., Ando S., Toguchida I., Yoshikawa M., *Bioorg. Med. Chem.*, **11**, 1995—2000 (2003).
45) Matsuda H., Kiyohara S., Sugimoto S., Ando S., Nakamura S., Yoshikawa M., *Biol. Pharm. Bull.*, **32**, 147—149 (2009).
46) Matsuda H., Kageura T., Morikawa T., Toguchida I., Harima S., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **10**, 323—327 (2000).
47) Kageura T., Matsuda H., Morikawa T., Toguchida I., Harima S., Oda M., Yoshikawa M., *Bioorg. Med. Chem.*, **9**, 1887—1893 (2001).
48) Yoshikawa M., Marikawa T., Xu F., Ando S., Matsuda H., *Heterocycles*, **60**, 1787—1792 (2003).
49) Matsuda H., Kageura T., Oda M., Morikawa T., Sakamoto Y., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 716—720 (2001).
50) Morikawa T., Ando S., Matsuda H., Kataoka S., Muraoka O., Yoshikawa M., *Chem. Pharm. Bull.*, **53**, 625—630 (2005).
51) Matsuda H., Morikawa T., Toguchida I., Ninomiya K., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 1558—1566 (2001).
52) Morikawa T., Matsuda H., Toguchida I., Ueda K., Yoshikawa M., *J. Nat. Prod.*, **65**, 1468—1474 (2002).
53) Matsuda H., Ninomiya K., Morikawa T, Yoshikawa M., *Bioorg. Med. Chem.*, **8**, 339—344 (1998).
54) Matsuda H., Morikawa T., Toguchida I., Ninomiya K., Yoshikawa M., *Heterocycles*, **55**, 841—846 (2001).
55) Matsuda H., Kageura T., Toguchida I., Ueda H., Morikawa T., Yoshikawa M., *Life Sci.*, **66**, 2151—2157 (2000).
56) Muraoka O., Fujimoto M., Tanabe G., Kubo M., Minematsu T., Matsuda H., Morikawa T., Toguchida I., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **11**, 2217—2220 (2001).
57) Matsuda H., Toguchida I., Ninomiya K., Kageura T., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem.*, **11**, 709—715 (2003).
58) Morikawa T., Abdel-Halim O. B., Matsuda H., Ando S., Muraoka O., Yoshikawa M., *Tetrahedron*, **62**, 6435—6442 (2006).
59) Matsuda H., Morikawa T., Sakamoto Y., Toguchida I., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 2527—2534 (2002).
60) Matsuda H., Morikawa T., Sakamoto Y., Toguchida I., Yoshikawa M., *Heterocycles*, **56**, 45—50 (2002).
61) Matsuda H., Kageura T., Toguchida I., Murakami T., Kishi A., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **9**, 3081—3086 (1999).
62) Morikawa T., Tao J., Ando S., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 638—645 (2003).
63) Tao J., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 654—662 (2003).
64) Tao J., Morikawa T., Toguchida I., Ando S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 4005—4012 (2002).
65) Morikawa T., Tao J., Toguchida I., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **66**, 86—91 (2003).
66) Matsuda H., Ando S., Kato T., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem.*, **14**, 138—142 (2006).
67) Abdel-Halim O. B., Morikawa T., Ando S., Matsuda H., Yoshikawa M., *J. Nat. Prod.*, **67**, 1119—1124 (2004).
68) Shimoda H., Nishida N., Ninomiya K., Matsuda H., Yoshikawa M., *Heterocycles*, **44**, 2043—2050 (2001).
69) Matsuda H., Ando S., Morikawa T., Kataoka S., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **15**, 1949—1953 (2005).
70) Ando S., Matsuda H., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem.*, **13**, 3289—3294 (2005).