Medicinal Foodstuffs. XIV.[1] On the Bioactive Constituents of Moroheiya. (2): New Fatty Acids, Corchorifatty Acids A, B, C, D, E, and F, from the Leaves of *Corchorus olitorius* L. (Tiliaceae): Structures and Inhibitory Effect on NO Production in Mouse Peritoneal Macrophages

Masayuki YOSHIKAWA,* Toshiyuki MURAKAMI, Hiromi SHIMADA, Satoshi YOSHIZUMI, Masami SAKA, Johji YAMAHARA, and Hisashi MATSUDA

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607–8414, Japan.
Received January 26, 1998; accepted March 12, 1998

Following the characterization of the glycosidic constituents in a medicinal foodstuff "moroheiya," the leaves of *Corchorus olitorius* L., four higher fatty acids with a trienone function, corchorifatty acids A, B, C, and D, an undecanoic acid, corchorifatty acid E, and a trihydroxyfatty acid, corchorifatty acid F, were isolated from the less polar fraction of "moroheiya". The structures and optical purity of corchorifatty acids were determined on the basis of chemical and physicochemical evidence. Corchorifatty acids A, B, and C showed an inhibitory effect on lipopolysaccharide-induced NO production in cultured mouse peritoneal macrophages.

Key words *Corchorus olitorius*; corchorifatty acid; NO production inhibitor; medicinal foodstuff; moroheiya; fatty acid

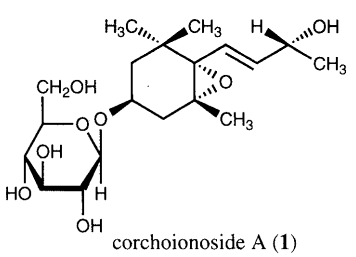
During the course of our search for bioactive constituents of medicinal foodstuffs,[1,2] we have investigated the chemical constituents of the leaves of *Corchorus olitorius* L. (Tiliaceae), which are commonly called "moroheiya" and are consumed as a health food in Japan. We have so far isolated three ionone glycosides called corchoionosides A (**1**),[3] B (**2**), and C (**3**) together with seven known glycosides from the polar fraction of the leaves and described their absolute stereostructures. Furthermore, we have reported the inhibitory activity of the glycoside constituents on the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction.[4] As a continuing study of the leaves of *C. olitorius*, we isolated new fatty acids called corchorifatty acids A (**4**), B (**5**), C (**6**), D (**7**), E (**8**), and F (**9**) together with methyl (9*S*,12*S*,13*S*)-trihydroxyoctadeca-10*E*,15*Z*-dienoate (**9a**),[5] methyl (9*S*,12*S*,13*S*)-trihydroxy-10*E*-octadecenoate (**10**),[5] azelaic acid (**11**), *trans*-3-dodecenedioic acid (**12**),[6] *S*-(+)-dehydrovomifoliol (**13**),[7] and (−)-loliolide (**14**).[8]

This paper deals with the structure elucidation of new fatty acids (**4**—**9**) and their optical purity using the NMR analyses of the 2-methoxy-2-(trifluoromethyl)phenyl (MTPA) ester. We also describe the inhibitory activities of **4**, **5**, **6**, and **9** on the lipopolysaccharide (LPS)-induced NO production in cultured mouse peritoneal macrophages.

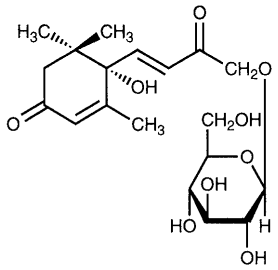
The methanolic extract from the leaves of *C. olitorius* was separated into the ethyl acetate-soluble portion, 1-butanol-soluble portion, and water-soluble portion. From the 1-butanol-soluble portion, corchoionosides (**1**—**3**) and known glycosides were isolated as described in our previous paper.[4] The ethyl acetate-soluble portion was subjected to normal-phase silica-gel column chromatography to provide six fractions (fraction 1—6). Fraction 2 was separated by normal-phase and reversed-phase silica-gel column chromatography and finally HPLC to give two known ionones **13** (0.0004% from the leaves) and **14** (0.0072%). From fraction 3, two known fatty acid methyl esters **9a** (0.0007%) and **10** (0.0003%) were isolated by a separation procedure similar to that described above. Fraction 4 was also subjected to reversed-phase silica-gel column and HPLC to afford **4** (0.0007%), **5** (0.0017%), **6** (0.0005%), **7** (0.0004%), **8** (0.0005%), and **9** (0.0056%) together with two known fatty acids **11** (0.0015%) and **12** (0.0011%). This is a first time that the two fatty acid methyl esters (**9a**, **10**), a fatty acid (**12**), and two ionones (**13**, **14**) have been isolated from *C. olitorius*. A fatty acid (**11**) has also been newly isolated from the plant source.

Corchorifatty Acids A (4) and B (5) Corchorifatty acid A (**4**) was isolated as a white powder of positive optical rotation ($[\alpha]_D^{26}$ +13.0°). The electron impact MS (EI-MS) of **4** showed a molecular ion peak at *m/z* 308 (M^+) and the molecular composition was defined as $C_{18}H_{28}O_4$ from high-resolution MS measurement. In the UV spectrum of **4**, an absorption maximum was observed at 310 nm (log ε 4.6), suggestive of a trienone structure. The IR spectrum of **4** showed absorption bands at 3431, 1736, 1701, 1655, and 1605 cm^{-1} ascribable to hydroxyl, carboxyl, ketone, and olefin functions. Methylation of **4** with ethereal diazomethane in methanol furnished the monomethyl ester (**4a**) whose proton and carbon signals in the ^{1}H-NMR and ^{13}C-NMR spectra were found to be superimposable on those of **4**, except for the signals due to the carbomethoxyl group. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) signals of **4**, which were assigned following various NMR experiments,[9] showed the presence of a propionyl moiety [**4**: δ 1.12 (t, *J*=7.3 Hz, 18-H_3), 2.58 (q, *J*=7.3 Hz, 17-H_2)], a 10*E*,12*E*,14*E*-trien-16-one moiety [**4**: δ 5.92 (dd, *J*=6.1, 15.0 Hz, 10-H), 6.18 (d, *J*=15.5 Hz, 15-H), 6.31 (dd, *J*=11.0, 15.0 Hz, 13-H), 6.33 (dd, *J*=11.0, 15.0 Hz, 11-H), 6.59 (dd, *J*=11.0, 15.0 Hz, 12-H), 7.19 (dd, *J*=11.0, 15.5 Hz, 14-H)], an allylic methine bearing a hydroxyl group [**4**: δ 4.21 (dt, *J*=6.1, 6.1 Hz, 9-H)], and seven methylenes [**4**: δ 1.26—1.39 (m, 4, 5, 6, 7-H_2), 1.56 (dt-like, 8-H_2), 1.63 (tt-like, 3-H_2), 2.34 (t, *J*=7.3 Hz, 2-H_2)]. In the EI-MS of **4**, fragment ion peaks at *m/z* 290 ($M^+ - H_2O$), *m/z* 233 [$M^+ - H_2O$ − propionyl (C_3H_5O)], and *m/z* 165 (**i**) were observed together with a molecular ion peak. On the basis of these findings, the structure of corchorifatty acid A was

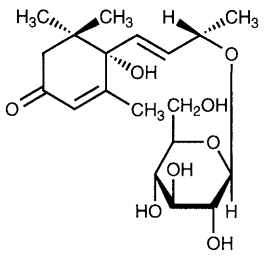
* To whom correspondence should be addressed.



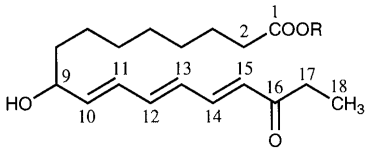
corchoionoside A (**1**)



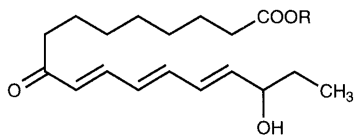
corchoionoside B (**2**)



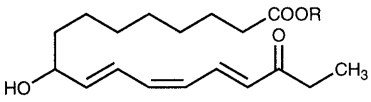
corchoionoside C (**3**)



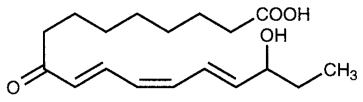
corchorifatty acid A (**4**) : R=H
4a : R=CH_3



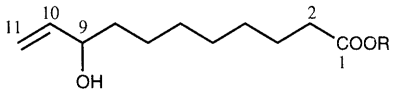
corchorifatty acid B (**5**) : R=H
5a : R=CH_3



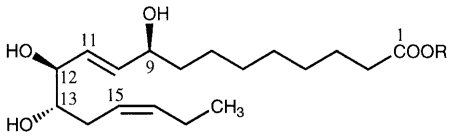
corchorifatty acid C (**6**) : R=H
6a : R=CH_3



corchorifatty acid D (**7**)

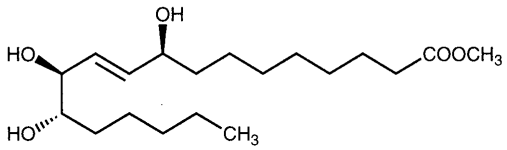


corchorifatty acid E (**8**) : R=H
8a : R=CH_3

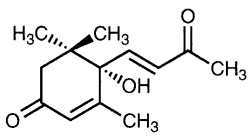


corchorifatty acid F (**9**) : R=H
9a : R=CH_3

Chart 1

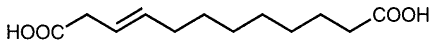


methyl-(9*S*,12*S*,13*S*)-trihydroxy-10*E*-octadecadienoate (**10**)

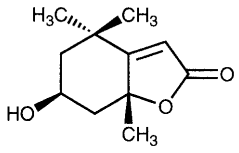


S-(+)-dehydrovomifoliol (**13**)

azelaic acid (**11**)



trans-3-dodecenedioic acid (**12**)

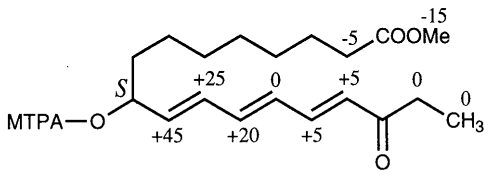


(-)-loliolide (**14**)

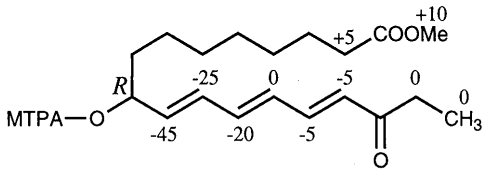
Chart 2

determined as 9-hydroxy-16-oxo-10*E*,12*E*,14*E*-octadecatrienoic acid (**4**).

To clarify the absolute configuration of the 9-position, **4a** was subjected to a modification of Mosher's method.[10] MTPA esters of **4a** were prepared by esterification with (*R*)- and (*S*)-2-methoxy-2-trifluorophenylacetic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-methoxyaminopyridine (DMAP). Both ^{1}H-NMR spectra of the esters showed two pairing signals in a ratio of *ca.* 2:3. As shown in Fig. 1, the major signals due to protons attached to C-10, 11, 12, 14, and 15 in the ^{1}H-NMR spectrum of the (*S*)-MTPA ester of **4a** were observed at lower field as compared to those of the (*R*)-MTPA ester ($\Delta\delta$: positive), while the signals due to protons on C-2 and

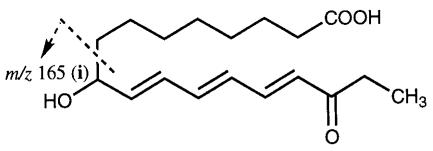


Major Signals in the (*S*)- and (*R*)-MTPA Esters of **4a**

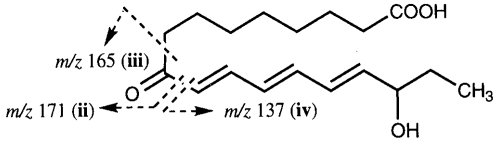


Minor Signals in the (*S*)- and (*R*)-MTPA Esters of **4a**

Fig. 1. $\Delta\delta$ ($\delta_S - \delta_R$) Values in Hz



4



5

Fig. 2. Fragment Ion Peaks in the EI-MS of **4** and **5**

the carbomethoxyl group of the (*S*)-MTPA ester were observed at higher fields than those of the (*R*)-MTPA ($\Delta\delta$: negative). The minor signals due to protons attached to C-10, 11, 12, 14, and 15 in the (*S*)-MTPA ester were observed at higher field than those of the (*R*)-MTPA, while the signals due to C-2 and the carbomethoxyl group were observed at lower field. Consequently, corchorifatty acid A (**4**) was found to be an enantiomer mixture of *ca.* a 2 : 3 ratio at the 9-position, and the absolute configurations of the major and minor components were confirmed to be *S* and *R* orientation, respectively.

Corchorifatty acid B (**5**) was also obtained as a white powder of positive optical rotation ($[\alpha]_D^{28}$ +17.3°). The UV and IR spectra of **5** showed absorption bands ascribable to hydroxyl, carboxyl, and trienone functions [UV: 309 nm (log ε 4.3); IR: 3430, 1719, 1710, 1655, 1605 cm^{-1}]. The molecular formula $C_{18}H_{28}O_4$, which was the same as that of **4**, was determined from the EI-MS [*m/z* 308 (M^+)] and by high-resolution MS measurement. Furthermore, fragment ion peaks at *m/z* 290 ($M^+ - H_2O$), *m/z* 233 (base peak, $M^+ - H_2O - C_3H_5O$), *m/z* 171 (**ii**), *m/z* 165 (**iii**), and *m/z* 137 (**iv**) were observed (Fig. 2). The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[9] of **5** showed a signal due to a 10*E*,12*E*,14*E*-trien-9-one moiety [δ 5.93 (dd, J=6.1, 15.1 Hz, 15-H), 6.17 (d, J=15.5 Hz, 10-H), 6.31 (dd, J=11.1, 15.4 Hz, 12-H), 6.32 (dd, J=10.8, 15.1 Hz, 14-H), 6.60 (dd, J=10.8, 15.4 Hz, 13-H), 7.18 (dd, J=11.1, 15.5 Hz, 11-H)], an allylic methine bearing a hydroxyl group [δ 4.15 (dt, J=6.1, 6.7 Hz, 16-H)], eight methylenes [δ 1.24—1.36 (m, 4,5,6-H_2), 1.52—1.64 (m, 3,7,17-H_2), 2.31 (t-like, 2-H_2), 2.52 (t, J=7.2 Hz, 8-H_2)], and a primary methyl group [δ 0.94 (t, J=7.3 Hz, 18-H_3)]. This evidence led us to formulate the structure of corchorifatty acid B as 16-hydroxy-9-oxo-10*E*,12*E*,14*E*-octadecatrienoic acid (**5**). Finally, methylation of **5** with diazomethane yielded the monomethyl ester (**5a**), which was identical with methyl (16*S*)-hydroxy-9-oxo-(10*E*,12*E*,14*E*)-octadecatrienoate from the marine chlorophyte *Acrosipbonia coalita*[11] by comparison of the physical data. The optical purity of **5** was also deduced by the ^{1}H-NMR analysis of the (*R*)- and (*S*)-MTPA esters as described for **4** and both ^{1}H-NMR spectra of **5** showed two pairing signals in a ratio of *ca.* 3 : 4.[12]

Corchorifatty Acids C (6) and D (7) Corchorifatty acids C (**6**) and D (**7**) were isolated as a white powder of positive optical rotation (**6**: $[\alpha]_D^{23}$ +12.9°; **7**: $[\alpha]_D^{26}$ +19.7°). The UV and IR spectra of **6** and **7** were similar to one another and showed absorption bands due to hydroxyl, carboxyl, and trienone functions [**6**, UV: 311 nm (log ε 4.4), IR: 3432, 1717, 1653, 1597 cm^{-1}; **7**, UV: 311 nm (log ε 4.3), IR: 3431, 1726, 1655, 1597 cm^{-1}]. The EI-MS of **6** and **7** showed the same molecular ion peak at *m/z* 308 (M^+) and the molecular formula of $C_{18}H_{28}O_4$ was determined by high-resolution MS measurement. Methylation of **6** with diazomethane furnished the monomethyl ester (**6a**). The ^{1}H-NMR ($CDCl_3$) spectra[9] of **6** and **6a** (shown in Experimental) showed the presence of a propionyl moiety [δ 1.13 (t, J=7.3 Hz, 18-H_3), 2.60 (q, J=7.3 Hz, 17-H_2)], a 10*E*,12*Z*,14*E*-trien-16-one moiety [δ 5.92 (dd, J=6.3, 14.9 Hz, 10-H), 6.10 (dd, J=11.3, 11.6 Hz, 13-H), 6.21 (d, J=15.0 Hz, 15-H), 6.36 (dd, J=11.3, 11.3 Hz, 12-H), 6.83 (dd, J=11.3, 14.9 Hz, 11-H), 7.66 (dd, J=11.6, 15.0 Hz, 14-H)], an allylic methine bearing a hydroxyl group [δ 4.26 (dt, J=6.3, 6.4 Hz, 9-H)], and seven methylenes [δ 1.25—1.38 (m, 4,5,6,7-H_2), 1.55 (dt, J=6.4, 7.0 Hz, 8-H_2), 1.61 (tt, J=7.0, 7.3 Hz, 3-H_2), 2.35 (t, J=7.3 Hz, 2-H_2)]. The carbon signals observed in the ^{13}C-NMR (Table 1) spectrum of **6** were found to be superimposable on those of **4**, except for the signals (C-11—C-14) due to the 12*Z*-olefin function. The fragmentation pattern of the EI-MS for **6** closely resembled that of **4** and the fragment ion peaks at *m/z* 290 ($M^+ - H_2O$), *m/z* 261 ($M^+ - H_2O - C_2H_5$), *m/z* 233 ($M^+ - H_2O - C_3H_5O$), and *m/z* 165 (**i**) were observed. In addition, photo-irradiation[13] of **6** yielded a mixture of **6** and **4** (*ca.* 1 : 1) and photo-irradiation of **4** also gave the same mixture. On the basis of this evidence, the structure of corchorifatty acid C was characterized as 9-hydroxy-16-oxo-10*E*,12*Z*,14*E*-octadecatrienoic acid (**6**).

The ^{1}H-NMR ($CDCl_3$) spectrum of **7** showed signals due to a 10*E*,12*Z*,14*E*-trien-9-one moiety [δ 5.93 (dd, J=6.1, 15.3 Hz, 15-H), 6.10 (dd, J=11.0, 11.3 Hz, 12-H), 6.19 (d, J=15.3 Hz, 10-H), 6.36 (dd, J=11.0, 11.3 Hz, 13-H), 6.85 (dd, J=11.3, 15.3 Hz, 14-H), 7.64 (dd, J=11.3,

Table 1. ^{13}C-NMR Chemical Shift Values for Corchorifatty Acids A (**4**), B (**5**), C (**6**), D (**7**), E (**8**), and F (**9**) and the Monomethyl Derivatives (**4a**, **5a**, **6a**, **8a**)

	4[a]	**4a**[a]	**5**[a]	**5a**[a]	**6**[a]	**6a**[a]	**7**[a]	**8**[a]	**8a**[a]	**9**[b]
C-1	178.6	174.3	178.7	174.3	178.8	174.3	178.5	179.4	174.3	177.8
2	33.8	34.1	33.9	34.0	33.9	34.1	33.8	34.1	34.1	35.1
3	24.6	24.9	24.8*	24.3*	24.6	24.9	24.6*	24.7	24.9	26.1
4	28.9*	29.0*	28.8**	28.9**	28.9*	29.1*	28.8**	28.9*	29.1*	30.2*
5	29.1*	29.1*	28.9**	29.1**	29.0*	29.2*	28.9**	29.1*	29.2*	30.4*
6	29.3*	29.3*	29.2**	29.1**	29.2*	29.3*	29.2**	29.3*	29.3*	30.6*
7	25.2	25.2	24.3*	24.9*	25.2	25.3	24.3*	25.2	25.2	26.5
8	37.2	37.2	40.7	40.7	37.1	37.2	40.9	36.9	37.0	38.3
9	72.3	72.3	200.9	200.7	72.4	72.4	201.0	73.3	73.2	73.0
10	141.0	141.0	129.3	129.4	141.4	141.4	130.0	141.2	141.3	126.6
11	129.4	129.3	142.3	142.0	124.7	124.7	136.6	114.7	114.6	131.0
12	140.5	140.5	130.5	130.5	137.3	137.1	127.1			75.8
13	130.6	130.6	140.7	140.6	127.1	127.1	137.3			75.9
14	141.9	141.9	129.5	129.5	136.4	136.2	124.9			31.6
15	129.2	129.2	140.9	140.7	129.5	129.7	141.1			126.4
16	201.1	201.1	73.5	73.6	201.4	201.1	73.6			134.4
17	33.9	33.9	30.1	30.1	34.4	34.3	30.1			21.7
18	8.3	8.3	9.6	9.6	8.2	8.2	9.6			14.6
OMe		51.5		51.5		51.5			51.4	

*,** Assignment may be interchangeable in the same column. *a*) $CDCl_3$. *b*) CD_3OD (500 MHz).

Table 2. Inhibition (%) of Corchorifatty Acids A (**4**), B (**5**), C (**6**), and F (**9**), Azelaic Acid (**11**), and *trans*-3-Dodecenedioic Acid (**12**) on NO Production in Mouse Peritoneal Macrophages

	Concentration (μM)				
	1	3	10	30	100
Corchorifatty acid A (**4**)	10.0±4.7	14.2±3.8	14.0±2.5	14.7±6.8	56.2±10.3**
Corchorifatty acid B (**5**)	3.7±1.4	13.3±4.2*	14.5±2.5**	35.8±3.3**	57.7±2.2**
Corchorifatty acid C (**6**)	1.2±2.3	4.2±3.2	8.6±4.7	30.7±6.6**	60.7±2.4**
Corchorifatty acid F (**9**)	2.4±2.2	1.3±1.8	1.7±2.5	7.9±4.9	16.4±8.8
Azelaic acid (**11**)	3.1±2.9	3.8±0.6	12.0±2.3	14.3±6.6	17.4±3.0*
trans-3-Dodecenedioic acid (**12**)	−1.3±5.6	0.2±3.1	−2.5±2.7	1.7±5.0	13.3±5.6

*, $p<0.05$; **, $p<0.01$, $n=4$.

15.3 Hz, 11-H)], and an allylic methine bearing a hydroxyl group [δ 4.22 (dt, J=5.8, 6.1 Hz, 16-H)] together with a primary methyl and eight methylenes. The fragmentation pattern of the EI-MS of **7** was very similar to that of **5**. Photo-irradiation of **7** yielded **5** to give a mixture of **5** and **7**, while **5** also converted **7** to provide a mixture of **5** and **7** (both *ca.* 1 : 1). Consequently, the structure of corchorifatty acid D was determined as 16-hydroxy-9-oxo-10*E*, 12*Z*,14*E*-octadecatrienoic acid (**7**).

Corchorifatty Acids E (8) and F (9) Corchorifatty acid E (**8**) was isolated as colorless oil of positive optical rotation ($[\alpha]_D^{24}$ +14.2°). The IR spectrum of **8** showed absorption bands at 3410, 1709, 1647, and 991 cm^{-1} ascribable to hydroxyl, carboxyl, and olefin functions. The positive-ion FAB-MS of **8** showed a quasimolecular ion peak at m/z 201 $(M+H)^+$ and the molecular formula $C_{11}H_{21}O_3$ was determined by high-resolution MS measurement. The ^{1}H-NMR ($CDCl_3$) and ^{13}C-NMR (Table 1) spectra[9] of **8** showed a vinyl group [δ 5.10 (d, J= 10.6 Hz), 5.21 (d, J=17.5 Hz) (11-H_2), 5.86 (ddd, J=6.3, 10.6, 17.5 Hz, 10-H)], an allylic methine bearing a hydroxyl group [δ 4.10 (dt, J=6.3, 6.3 Hz, 9-H)], and seven methylenes [δ 1.26—1.42 (m, 4,5,6,7-H_2), 1.51 (m, 8-H_2), 1.62 (m, 3-H_2), 2.33 (t, J=7.3 Hz, 2-H_2)]. Diazomethane methylation of **8** yielded the monomethyl ester (**8a**). On the basis of those findings and examination of the spectral data of **8a**, the structure of corchorifatty acid E was determined as 9-hydroxy-10-undecenoic acid (**8**). Finally, the ^{1}H-NMR spectra of the (*R*)- and (*S*)-MTPA esters from **8a** showed two pairing signals in a ratio of *ca.* 3 : 4 and corchorifatty acid E was found to be a mixture of enantiomers.[12]

Corchorifatty acid F (**9**) was obtained as a white powder of negative optical rotation ($[\alpha]_D^{24}$ −10.5°) and its IR spectrum showed absorption bands due to hydroxyl, carboxyl, and olefin functions. The negative-ion and positive-ion FAB-MS of **9** showed quasimolecular ion peaks at m/z 327 $(M-H)^-$ and m/z 351 $(M+Na)^+$, respectively, and high-resolution MS analysis of the quasimolecular ion peak $(M-H)^-$ revealed the molecular formula of **9** to be $C_{18}H_{32}O_5$. The signals in the ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[9] of **9** were similar to those of **9a** in the literature,[5] except for the signals due to the carboxyl group. Methylation of **9** with diazomethane furnished **9a** and consequently, the structure of corchorifatty acid F was determined as shown.

Inhibitory Effect on LPS-Induced NO Production During the course of our screening to find new bioactive

constituents in medicinal foodstuffs, we examined the effect of the principal fatty acids (**4**, **5**, **6**, **9**, **11**, and **12**) from "moroheiya" on the LPS-induced NO production as an index of activation of macrophages. As shown in Table 2, corchorifatty acids A (**4**), B (**5**), and C (**6**) containing a trienone function in their structure were found to inhibit the LPS-induced NO production in cultured mouse peritoneal macrophages. Furthermore, these compounds exhibited no cytotoxicity to the macrophage at the concentration showing the inhibitory activity. Since overproduction of NO is well known to be a cause of inflammation, immunological responses, and endotoxin-induced shocks,[14] these fatty acids (**4**, **5**, **6**) may be effective against inflammation and endotoxin shocks.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were described previously.[1]

Extraction and Isolation The air-dried leaves of *Corchorus olitorius* L. (5 kg, cultivated in Vietnam and purchased from Honso Pharmaceutical Co., Ltd., Nagoya) were minced and extracted three times with methanol under reflux. Removal of the solvent from the methanol extract solution under reduced pressure gave the MeOH extract (926 g) and 463 g of the MeOH extract partitioned into the AcOEt–H_2O (1 : 1) mixture. The aqueous layer was further extracted with 1-butanol. Removal of the solvent under reduced pressure from the AcOEt and 1-butanol-soluble-portion yielded 121 g and 117 g of residue, respectively. The AcOEt-soluble-portion (121 g) was subjected to normal-phase silica-gel column chromatography (2.0 kg, $CHCl_3 \rightarrow CHCl_3$–MeOH$\rightarrow CHCl_3$–MeOH–$H_2O$) to give six fractions [Fr. 1 (18.6 g), Fr. 2 (49.1 g), Fr. 3 (2.9 g), Fr. 4 (7.1 g), Fr. 5 (4.6 g), Fr. 6 (26.8 g)]. Fraction 2 (49.1 g) was further separated by normal- and reversed-phase silica-gel column chromatography and HPLC (YMC-ODS-A, MeOH–H_2O) to furnish *S*-(+)-dehydrovomifoliol (**13**, 9.5 mg) and (−)-loliolide (**14**, 179.5 mg). Fraction 3 (2.8 g) was separated by the above methods to give methyl (9*S*,12*S*,13*S*)-trihydroxyoctadeca-10*E*,15*Z*-dienoate (**9a**, 17.3 mg) and methyl (9*S*,12*S*,13*S*)-trihydroxy-10*E*-octadecenoate (**10**, 8.2 mg). Fraction 4 (7.1 g) was subjected to reversed-phase silica-gel column chromatography and HPLC (MeOH–H_2O) to furnish corchorifatty acids A (**4**, 18.3 mg), B (**5**, 43.0 mg), C (**6**, 11.9 mg), D (**7**, 10.5 mg), E (**8**, 12.8 mg), and F (**9**, 140.5 mg), azelaic acid (**11**, 39.4 mg), and *trans*-3-dodecenedioic acid (**12**, 28.5 mg). The known compounds (**9a**, **10**, **13**, **14**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-NMR, ^{13}C-NMR, MS) with reported values. Azelaic acid (**11**) was identified by TLC, IR, ^{1}H-NMR, ^{13}C-NMR, and MS spectral comparisons with commercial authentic sample (Nacalai Tesque Inc.). Since the physical data to identify *trans*-3-dodecenedioic acid (**12**) was not recorded in the literature,[6] the structure of **12** was characterized by detailed analysis of the following physical data: ^{1}H-NMR (270 MHz, C_6D_6) δ: 1.03—1.34 (8H, m, 6,7,8,9-H_2), 1.48 (2H, m, 10-H_2), 1.86 (2H, td, J=6.9, 7.3 Hz, 5-H_2), 2.11 (2H, t, J=7.3 Hz, 11-H_2), 2.96 (2H, dd-like, 2-H_2), 5.47 (1H, td, J=7.3, 18.2 Hz, 4-H), 5.62 (1H, td, J=7.3, 18.2 Hz, 3-H). ^{13}C-NMR (68 MHz, C_6D_6) δ_C: 24.9 (10-C), 27.5 (5-C), 29.1, 29.2, 29.3, 29.4 (6,7,8,9-C), 33.0 (2-C), 34.2 (11-C), 120.9 (3-C), 133.8 (4-C), 178.6 (1-C), 180.6 (12-C).

Corchorifatty Acid A (**4**): A white powder, $[\alpha]_D^{26}$ +13.0° (c=0.4, acetone). High-resolution EI-MS: Calcd for $C_{18}H_{28}O_4$ (M^+): 308.1988. Found: 308.1983. UV λ_{max}^{MeOH} nm (log ε): 310 (4.6). IR (KBr) cm^{-1}: 3431, 2926, 2853, 1736, 1701, 1655, 1605, 1001. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.12 (3H, t, J=7.3 Hz, 18-H_3), 1.26—1.39 (8H, m, 4,5,6,7-H_2), 1.56 (2H, dt-like, 8-H_2), 1.63 (2H, tt-like, 3-H_2), 2.34 (2H, t, J=7.3 Hz, 2-H_2), 2.58 (2H, q, J=7.3 Hz, 17-H_2), 4.21 (1H, dt, J=6.1, 6.1 Hz, 9-H), 5.92 (1H, dd, J=6.1, 15.0 Hz, 10-H), 6.18 (1H, d, J=15.5 Hz, 15-H), 6.31 (1H, dd, J=11.0, 15.0 Hz, 13-H), 6.33 (1H, dd, J=11.0, 15.0 Hz, 11-H), 6.59 (1H, dd, J=11.0, 15.0 Hz, 12-H), 7.19 (1H, dd, J=11.0, 15.5 Hz, 14-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 308 (M^+), 290 (M^+-H_2O), 261 ($M^+-H_2O-C_2H_5$), 233 ($M^+-H_2O-C_3H_5O$).

Corchorifatty Acid B (**5**): A white powder, $[\alpha]_D^{28}$ +17.3° (c=0.2, acetone). High-resolution EI-MS: Calcd for $C_{18}H_{28}O_4$ (M^+): 308.1988. Found: 308.1993. UV λ_{max}^{MeOH} nm (log ε): 309 (4.3). IR (KBr) cm^{-1}: 3430, 2932, 2857, 1719, 1655, 1605, 1007. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.94 (3H, t, J=7.3 Hz, 18-H_3), 1.24—1.36 (6H, m, 4,5,6-H_2), 1.52—1.64 (6H, m, 3,7,17-H_2), 2.31 (2H, t-like, 2-H_2), 2.54 (2H, t, J=7.3 Hz, 8-H_2), 4.15 (1H, dt, J=6.1, 6.7 Hz, 16-H), 5.93 (1H, dd, J=6.1, 15.1 Hz, 15-H), 6.17 (1H, d, J=15.5 Hz, 10-H), 6.31 (1H, dd, J=11.1, 15.4 Hz, 12-H), 6.32 (1H, dd, J=10.8, 15.1 Hz, 14-H), 6.60 (1H, dd, J=10.8, 15.4 Hz, 13-H), 7.18 (1H, dd, J=11.1, 15.5 Hz, 11-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 308 (M^+), 290 (M^+-H_2O), 233 (100), 223, 171, 165, 137, 79.

Corchorifatty Acid C (**6**): A white powder, $[\alpha]_D^{24}$ +12.9° (c=0.24, acetone). High-resolution EI-MS: Calcd for $C_{18}H_{28}O_4$ (M^+): 308.1988. Found: 308.1978. UV λ_{max}^{MeOH} nm (log ε): 311 (4.4). IR (KBr) cm^{-1}: 3432, 2930, 2855, 1717, 1653, 1597, 1009. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.13 (3H, t, J=7.3 Hz, 18-H_3), 1.25—1.38 (8H, m, 4,5,6,7-H_2), 1.55 (2H, dt, J=6.4, 7.0 Hz, 8-H_2), 1.61 (2H, tt, J=7.0, 7.3 Hz, 3-H_2), 2.35 (2H, t, J=7.3 Hz, 2-H_2), 2.60 (2H, q, J=7.3 Hz, 17-H_2), 4.26 (1H, dt, J=6.3, 6.4 Hz, 9-H), 5.92 (1H, dd, J=6.3, 14.9 Hz, 10-H), 6.10 (1H, dd, J=11.3, 11.6 Hz, 13-H), 6.21 (1H, d, J=15.0 Hz, 15-H), 6.36 (1H, dd, J=11.3, 11.3 Hz, 12-H), 6.83 (1H, dd, J=11.3, 14.9 Hz, 11-H), 7.66 (1H, dd, J=11.6, 15.0 Hz, 14-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 308 (M^+), 290 (M^+-H_2O), 261, 233 ($M^+-H_2O-C_3H_5O$).

Corchorifatty Acid D (**7**): A white powder, $[\alpha]_D^{26}$ +19.7° (c=0.04, acetone). High-resolution EI-MS: Calcd for $C_{18}H_{28}O_4$ (M^+): 308.1988. Found: 308.1990. UV λ_{max}^{MeOH} nm (log ε): 311 (4.3). IR (KBr) cm^{-1}: 3431, 2932, 2857, 1726, 1655, 1597, 1115. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.96 (3H, t, J=7.5 Hz, 18-H_3), 1.28—1.39 (6H, m, 4,5,6-H_2), 1.57—1.66 (6H, m, 3,7,17-H_2), 2.34 (2H, t-like, 2-H_2), 2.57 (2H, t, J=7.1 Hz, 8-H_2), 4.22 (1H, dt, J=5.8, 6.1 Hz, 16-H), 5.93 (1H, dd, J=6.1, 15.3 Hz, 15-H), 6.10 (1H, dd, J=11.0, 11.3 Hz, 12-H), 6.19 (1H, d, J=15.3 Hz, 10-H), 6.36 (1H, dd, J=11.3, 11.3 Hz, 13-H), 6.85 (1H, dd, J=11.3, 15.3 Hz, 14-H), 7.64 (1H, dd, J=11.0, 11.3 Hz, 11-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 308 (M^+), 290 (M^+-H_2O), 261, 233 ($M^+-H_2O-C_3H_5O$), 223, 171, 137, 41 (100).

Corchorifatty Acid E (**8**): Colorless oil, $[\alpha]_D^{24}$ +14.2° (c=0.7, $CHCl_3$). High-resolution Positive-ion FAB-MS: Calcd for $C_{11}H_{21}O_3$ $(M+H)^+$: 201.1491. Found: 201.1481. IR (film) cm^{-1}: 3410, 2930, 2856, 1709, 1647, 991. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.20—1.42 (8H, m, 4,5,6,7-H_2), 1.51 (2H, m, 8-H_2), 1.62 (2H, m, 3-H_2), 2.33 (2H, t, J=7.3 Hz, 2-H_2), 4.10 (1H, dt, J=6.3, 6.3 Hz, 9-H), 5.10 (1H, d, J=10.6 Hz), 5.21 (1H, d, J=17.5 Hz) (11-H_2), 5.86 (1H, ddd, J=6.3, 10.6, 17.5 Hz, 10-H). ^{13}C-NMR (68 MHz, $CDCl_3$) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 201 $(M+H)^+$.

Corchorifatty Acid F (**9**): A white powder, $[\alpha]_D^{24}$ −10.5° (c=0.7, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{18}H_{32}O_5Na$ $(M+Na)^+$: 351.2147. Found: 351.2159. IR (KBr) cm^{-1}: 3368, 2926, 2849, 1705, 1072. ^{1}H-NMR (500 MHz, CD_3OD) δ: 0.96 (3H, t, J=7.6 Hz, 18-H_3), 1.24—1.42 (8H, m, 4,5,6,7-H_2), 1.54 (2H, m, 8-H_2), 1.61 (2H, m, 3-H_2), 2.06 (2H, dq-like, 17-H_2), 2.13, 2.34 (1H each, both m, 14-H_2), 2.27 (2H, t, J=7.6 Hz, 2-H_2), 3.45 (1H, ddd, J=4.3, 5.5, 8.3 Hz, 13-H), 3.95 (1H, dd, J=5.2, 5.5 Hz, 12-H), 4.05 (1H, dt-like, 9-H), 5.45 (2H, m, 15, 16-H), 5.71 (2H, m, 10, 11-H). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: given in Table 1. Positive-ion FAB-MS m/z: 351 $(M+Na)^+$, 329 $(M+H)^+$. Negative-ion FAB-MS m/z: 327 $(M-H)^-$.

Diazomethane Methylation of Corchorifatty Acids A (4), C (6), and E (8) A solution of corchorifatty acids (3.0 mg each of **4**, **6**, and **8**) in MeOH (0.5 ml) was treated with ethereal diazomethane (*ca.* 1.0 ml) until the yellow color persisted. The solution was stirred for 10 min at room temperature, then the solvent was removed under reduced pressure and the residue was purified by normal-phase silica-gel column chromatography (1.0 g, *n*-hexane-acetone) to furnish methyl corchorifatty acids (**4a**, **6a**, **8a**, each 3.0 mg).

4a: A white powder. High-resolution EI-MS: Calcd for $C_{19}H_{30}O_4$ (M^+): 322.2144. Found: 322.2170. UV λ_{max}^{MeOH} nm (log ε): 310 (4.6). IR (KBr) cm^{-1}: 3453, 2932, 2855, 1734, 1718, 1601, 1581, 1030. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.12 (3H, t, J=7.3 Hz, 18-H_3), 1.28—1.33 (8H, m, 4,5,6,7-H_2), 1.56 (2H, dt-like, 8-H_2), 1.61 (2H, tt-like, 3-H_2), 2.30 (2H, t, J=7.6 Hz, 2-H_2), 2.59 (2H, q, J=7.3 Hz, 17-H_2), 3.66 (3H, s, OMe), 4.21 (1H, dt-like, 9-H), 5.93 (1H, dd, J=6.3, 15.1 Hz, 10-H), 6.19 (1H, d, J=15.4 Hz, 15-H), 6.32 (1H, dd, J=11.1, 14.8 Hz, 13-H), 6.34 (1H, dd, J=10.8, 15.1 Hz, 11-H), 6.59 (1H, dd, J=10.8, 14.8 Hz, 12-H), 7.19 (1H, dd, J=11.1, 15.4 Hz, 14-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 322 (M^+), 304 (M^+-H_2O), 233, 185, 165, 137 (100).

6a: A white powder. High-resolution EI-MS: Calcd for $C_{19}H_{30}O_4$ (M^+): 322.2144. Found: 322.2125. UV λ_{max}^{MeOH} nm (log ε): 310 (4.4). IR (KBr) cm^{-1}: 3453, 2930, 2855, 1743, 1655, 1598, 1010. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.13 (3H, t, J=7.3 Hz, 18-H_3), 1.25—1.36 (8H, m, 4,5,6,7-H_2), 1.55 (2H, dt-like, 8-H_2), 1.61 (2H, tt-like, 3-H_2), 2.31 (2H, t, J=7.6 Hz, 2-H_2), 2.61 (2H, q, J=7.3 Hz, 17-H), 3.67 (3H, s, OMe), 4.25 (1H, dt, J=6.4, 6.4 Hz, 9-H), 5.92 (1H, dd, J=6.4, 15.3 Hz, 10-H), 6.12 (1H, dd, J=11.0, 11.9 Hz, 13-H), 6.20 (1H, d, J=15.4 Hz, 15-H), 6.35 (1H, dd, J=11.0, 11.9 Hz, 12-H), 6.82 (1H, dd, J=11.9, 15.3 Hz, 11-H), 7.65 (1H, dd, J=11.9, 15.4 Hz, 14-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. EI-MS m/z: 322 (M^+), 304 ($M^+ - H_2O$), 261, 233, 185, 165, 137 (100).

8a: Colorless oil. High-resolution FAB-MS: Calcd for $C_{12}H_{23}O_3$ $(M+H)^+$: 215.1647. Found: 215.1663. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 1.20—1.40 (8H, m, 4,5,6,7-H_2), 1.51 (2H, m, 8-H_2), 1.62 (2H, m, 3-H_2), 2.30 (2H, t, J=7.4 Hz, 2-H_2), 3.66 (3H, s, OMe), 4.09 (1H, dt, J=6.3, 6.3 Hz, 9-H), 5.10 (1H, dd, J=1.3, 10.6 Hz), 5.21 (1H, dd, J=1.3, 17.2 Hz) (11-H_2), 5.87 (1H, ddd, J=6.3, 10.6, 17.2 Hz, 10-H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: given in Table 1. FAB-MS m/z: 237 $(M+Na)^+$, 215 $(M+H)^+$.

Isomerization of Corchorifatty Acids A (4), B (5), C (6), and D (7) Under Irradiation with Fluorescent Lamps A $CHCl_3$ solution (10 ml) of corchorifatty acids (5 mg each of **4**, **5**, **6**, and **7**) in a Pyrex tube was irradiated (distance 5 cm) externally by five fluorescent lamps for 12 h, and the ratio of **4**:**6** (*ca.* 1.0:1.0) from **4** and **6**, or **5**:**7** (*ca.* 1.0:1.0) from **5** and **7** in the solution was characterized by HPLC [YMC-Pack (250 × 4.6 mm, i.d.), MeOH–H_2O (60:40, v/v), detection: UV 254 nm].

Preparation of the (*S*)-MTPA Esters from 4a, 5a, and 8a A solution of **4a** (1.5 mg) in CH_2Cl_2 (0.5 ml) was treated with (*S*)-MTPA (5.9 mg), DCC (5.2 mg), and 4-DMAP (1.9 mg) and the whole mixture was stirred at room temperature (25 °C) for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, and then dried over $MgSO_4$ and filtered. Evaporation of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica-gel column chromatography (1.0 g, *n*-hexane–AcOEt) to give the (*S*)-MTPA ester of **4a** (1.7 mg), colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the peaks due to major constituents) 1.12 (3H, t, J=7.3 Hz, 18-H_3), 2.28 (2H, t, J=7.3 Hz, 2-H_2), 2.59 (2H, q, J=7.3 Hz, 17-H_2), 3.53 (3H, s, OMe), 5.83 (1H, d, J=7.5, 15.3 Hz, 10-H), 6.21 (1H, d, J=15.3 Hz, 15-H), 6.26 (1H, dd, J=11.0, 15.3 Hz, 13-H), 6.38 (1H, dd, J=10.9, 15.3 Hz, 11-H), 6.54 (1H, dd, J=10.9, 15.3 Hz, 12-H), 7.17 (1H, dd, J=11.0, 15.3 Hz, 14-H); (the peaks due to minor constituents) 1.12 (3H, t, J=7.3 Hz, 18-H_3), 2.29 (2H, t, J=7.3 Hz, 2-H_2), 2.59 (2H, q, J=7.3 Hz, 17-H_2), 3.56 (3H, s, OMe), 5.74 (1H, d, J=7.3, 15.0 Hz, 10-H), 6.20 (1H, d, J=15.3 Hz, 15-H), 6.25 (1H, dd, J=11.0, 15.2 Hz, 13-H), 6.33 (1H, dd, J=10.7, 15.0 Hz, 11-H), 6.50 (1H, dd, J=10.7, 15.2 Hz, 12-H), 7.16 (1H, dd, J=11.0, 15.3 Hz, 14-H).

The (*S*)-MTPA esters were also prepared from **5a** and **8a** (both 1.5 mg) by the procedure described above; the (*S*)-MTPA ester from **5a**, colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the signals of major constituents) 0.85 (3H, t, J=7.3 Hz, 18-H_3), 1.77 (1H, m, 17-H_2), 2.30 (2H, t, J=7.5 Hz, 2-H_2), 2.54 (2H, t, J=7.3 Hz, 8-H_2), 3.53 (3H, s, OMe), 5.47 (1H, m, 16-H), 5.84 (1H, dd, J=7.6, 15.5 Hz, 15-H), 6.20 (1H, d, J=15.0 Hz, 10-H), 6.26 (1H, dd, J=11.0, 15.0 Hz, 12-H), 6.39 (1H, dd, J=11.0, 15.5 Hz, 14-H), 6.56 (1H, dd, J=11.0, 15.0 Hz, 13-H), 7.16 (1H, dd, J=11.0, 15.0 Hz, 11-H); (the signals of minor constituents) 0.95 (3H, t, J=7.3 Hz, 18-H_3), 1.72 (1H, m, 17-H_2), 2.30 (2H, t, J=7.5 Hz, 2-H_2), 2.54 (2H, t, J=7.3 Hz, 8-H_2), 3.57 (3H, s, OMe), 5.47 (1H, m, 16-H), 5.74 (1H, dd, J=7.3, 15.2 Hz, 15-H), 6.19 (1H, d, J=15.0 Hz, 10-H), 6.22 (1H, dd, J=11.0, 14.6 Hz, 12-H), 6.33 (1H, dd, J=11.0, 15.2 Hz, 14-H), 6.51 (1H, dd, J=11.0, 14.6 Hz, 13-H), 7.15 (1H, dd, J=11.0, 15.0 Hz, 11-H).

The (*S*)-MTPA ester from **8a**, colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the signals of major constituents) 5.24 (1H, dd, J=1.2, 10.4 Hz), 5.35 (1H, dd, J=1.2, 17.1 Hz, 11-H_2), 5.45 (1H, dt-like, 9-H), 5.82 (1H, ddd, J=7.0, 10.4, 17.1 Hz, 10-H); (the signals of minor constituents) 5.19 (1H, dd, J=1.2, 10.7 Hz), 5.25 (1H, dd, J=1.2, 17.4 Hz, 11-H_2), 5.43 (1H, dt-like, 9-H), 5.72 (1H, ddd, J=7.3, 10.7, 17.4 Hz, 10-H).

Preparation of the (*R*)-MTPA Esters from 4a, 5a, and 8a A solution of **4a** (1.5 mg) in CH_2Cl_2 (0.5 ml) was treated with (*R*)-MTPA (5.9 mg), DCC (5.2 mg), and 4-DMAP (1.9 mg) and the whole mixture was stirred at room temperature (25 °C) for 1 h. It was worked up as described for the above $CHCl_3$ extract, which was purified by silica-gel column chromatography (1.0 g, *n*-hexane–AcOEt) to give the (*R*)-MTPA ester of **4a** (1.7 mg), colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the peaks due to major constituents) 1.12 (3H, t, J=7.3 Hz, 18-H_3), 2.29 (2H, t, J=7.3 Hz, 2-H_2), 2.59 (2H, q, J=7.3 Hz, 17-H_2), 3.56 (3H, s, OMe), 5.74 (1H, d, J=7.3, 15.3 Hz, 10-H), 6.20 (1H, d, J=15.6 Hz, 15-H), 6.26 (1H, dd, J=11.0, 15.0 Hz, 13-H), 6.33 (1H, dd, J=10.9, 15.3 Hz, 11-H), 6.50 (1H, dd, J=10.9, 15.0 Hz, 12-H), 7.16 (1H, dd, J=11.0, 15.6 Hz, 14-H); (the peaks due to minor constituents) 1.12 (3H, t, J=7.3 Hz, 18-H_3), 2.28 (2H, t, J=7.3 Hz, 2-H_2), 2.59 (2H, q, J=7.3 Hz, 17-H_2), 3.53 (3H, s, OMe), 5.83 (1H, d, J=7.3, 15.3 Hz, 10-H), 6.21 (1H, d, J=15.6 Hz, 15-H), 6.25 (1H, dd, J=11.0, 15.5 Hz, 13-H), 6.38 (1H, dd, J=10.7, 15.3 Hz, 11-H), 6.54 (1H, dd, J=10.7, 15.5 Hz, 12-H), 7.17 (1H, dd, J=11.0, 15.6 Hz, 14-H).

The (*R*)-MTPA esters were also prepared from **5a** and **8a** (both 1.5 mg) by the above procedure; the (*R*)-MTPA ester from **5a**, colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the signals of major constituents) 0.85 (3H, t, J=7.3 Hz, 18-H_3), 1.77 (1H, m, 17-H_2), 2.29 (2H, t, J=7.5 Hz, 2-H_2), 2.54 (2H, t, J=7.6 Hz, 8-H_2), 3.53 (3H, s, OMe), 5.47 (1H, m, 16-H), 5.84 (1H, dd, J=7.5, 15.1 Hz, 15-H), 6.20 (1H, d, J=14.9 Hz, 10-H), 6.26 (1H, dd, J=11.6, 15.0 Hz, 12-H), 6.39 (1H, dd, J=11.0, 15.1, 14-H), 6.56 (1H, dd, J=11.0, 15.0 Hz, 13-H), 7.16 (1H, dd, J=11.6, 14.9 Hz, 11-H); (the signals of minor constituents) 0.95 (3H, t, J=7.3 Hz, 18-H_3), 1.72 (1H, m, 17-H_2), 2.29 (2H, t, J=7.5 Hz, 2-H_2), 2.54 (2H, t, J=7.6 Hz, 8-H_2), 3.57 (3H, s, OMe), 5.47 (1H, m, 16-H), 5.74 (1H, dd, J=7.0, 15.3 Hz, 15-H), 6.19 (1H, d, J=15.3 Hz, 10-H), 6.22 (1H, dd, J=11.3, 14.6 Hz, 12-H), 6.33 (1H, dd, J=11.0, 15.3 Hz, 14-H), 6.51 (1H, dd, J=11.0, 14.6 Hz, 13-H), 7.15 (1H, dd, J=11.3, 15.3 Hz, 11-H).

The (*R*)-MTPA ester from **8a**, colorless oil, ^{1}H-NMR ($CDCl_3$) δ: (the signals of major constituents) 5.25 (1H, dd, J=1.2, 10.4 Hz), 5.35 (1H, dd, J=1.2, 17.4 Hz, 11-H_2), 5.45 (1H, dt-like, 9-H), 5.82 (1H, ddd, J=7.3, 10.4, 17.4 Hz, 10-H); (the signals of minor constituents) 5.19 (1H, dd, J=1.1, 10.4 Hz), 5.25 (1H, dd, J=1.2, 17.4 Hz, 11-H_2), 5.43 (1H, dt-like, 9-H), 5.72 (1H, ddd, J=7.3, 10.4, 17.4 Hz, 10-H).

Diazomethane Methylation of Corchorifatty Acids B (5) and F (9) A solution of **5** (3 mg) and **9** (6.5 mg) in MeOH (0.7 ml) was treated with ethereal diazomethane (*ca.* 1 ml) until the yellow color persisted. The solution was stirred for 10 min at room temperature, then the solvent was removed under reduced pressure and the residue was purified by normal-phase silica-gel column chromatography (1 g, *n*-hexane–acetone) to furnish **5a** (3 mg) and **9a** (6.5 mg). **5a** and **9a** were identified by comparison of their physical data (UV, IR, ^{1}H-NMR, and ^{13}C-NMR) with reported values.[5,11]

Bioassay Methods

NO Production from Macrophages Stimulated by LPS Cells were collected from the peritoneal cavities of mice by washing with 6—7 ml of PBS. The cells were washed with PBS and resuspended in RPMI 1640 medium containing 10% fetal calf serum (FCS). The total cell number was counted with a hemocytometer, and the peritoneal cells were inoculated into a 96-well tissue culture plate (5×10^5 cells/100 μl in a well). After incubation for 1 h at 37 °C in 5% CO_2 atmosphere, nonadherent cells were removed by washing with PBS. The cells were then incubated with RPMI 1640 medium (200 μl/well) containing 10% FCS, LPS (from *Salmonella enteritidis*, Sigma) and a test sample. After 20 h incubation of the cells, 100 μl of the medium was removed for measurement of NO. The nitrite was measured spectrophotometrically for the assessment of NO production using Griess reagent with $NaNO_2$ as a standard.

Cytotoxicity The cytotoxicity of a test compound to the cell was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. After 20 h incubation of the cells in the 200 μl of the medium containing 10% FCS and a test compound, 100 μl of the medium was removed. Ten μl of MTT (5 mg/ml in PBS) solution was added to the wells and

incubated for 4 h. The medium was then removed, and 100 μl of isopropanol containing 0.04 N HCl was added to dissolve the formazan. The optical density (O.D.) of the formazan solution was measured by microplate reader at 570 nm (reference: 655 nm).

Statistical Analysis Each value was expressed as the mean $\pm$ S.E. and the statistical significance was assessed by one-way analysis of variance following Dunnett's test.

References and Notes

1) Part XIII: Komatsu H., Murakami T., Matsuda H., Yoshikawa M., *Heterocycles*, **48**, 703—710 (1998).
2) *a*) Yoshikawa M., Murakami T., Komatsu H., Murakami N., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 81—87 (1997); *b*) Yoshikawa M., Shimada H., Komatsu H., Sakurama T., Nishida N., Yamahara J., Shimoda H., Matsuda H., Tani T., *ibid.*, **45**, 877—882 (1997); *c*) Yoshikawa M., Shimada H., Morikawa T., Yoshizumi S., Matsumura N., Murakami T., Matsuda H., Hori K., Yamahara J., *ibid.*, **45**, 1300—1305 (1997); *d*) Yoshikawa M., Murakami T., Kadoya M., Li Y., Murakami N., Yamahara J., Matsuda H., *ibid.*, **45**, 1671—1676 (1997); *e*) Yoshikawa M., Murakami T., Matsuda H., *ibid.*, **45**, 2034—2038 (1997); *f*) Yoshikawa M., Murakami T., Komatsu H., Yamahara J., Matsuda H., *Heterocycles*, **47**, 397—405 (1997).
3) We previously reported[4] the absolute configuration of the 9-position in corchoionoside A as *S* by our careless application of a Mosher's method. We devised here the 9*S* configuration of corchoionoside A to the 9*R* configuration (**1**).
4) Yoshikawa M., Shimada H., Saka M., Yoshizumi S., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 464—469 (1997).
5) *a*) Kato T., Yamaguchi Y., Abe N., Uyehara T., Namai T., Kodama M., Shiobara Y., *Tetrahedron Lett.*, **26**, 2357—2360 (1987); *b*) Kato T., Yamaguchi Y., Ohmura S., Uyehara T., Namai T., Kodama M, Shiobara Y., *Chem. Lett.*, **1986**, 577—580; *c*) Kato T., Yamaguchi Y., Hirukawa T., Hoshino N., *Agric. Biol. Chem.*, **55**, 1349—1357 (1991).
6) Jin S. J., Tserng K. Y., *Biochemistry*, **29**, 8540—8547 (1990).
7) Takasugi M., Anetai M., Katsui N., Masamune T., *Chem. Lett.*, **1973**, 245—248.
8) *a*) Hodges R., Porte A. L., *Tetrahedron*, **20**, 1463—1467 (1964); *b*) Wada T., *Chem. Pharm. Bull.*, **13**, 43—49 (1965).
9) The ^{1}H- and ^{13}C-NMR spectra of corchorifatty acids (**4**—**9**) were assigned with the aid of homo- and hetero-correlation spectroscopy (^{1}H–^{1}H, ^{1}H–^{13}C COSY), distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple bond correlation (HMBC) experiments.
10) Ohtani I., Kusumi T., Kashman H., Kakisawa H., *J. Am. Chem. Soc.*, **13**, 4092—4096 (1991).
11) Bernary M. W., Whatley G. G., Gerwick W. H., *J. Nat. Prod.*, **56**, 245—259 (1993).
12) The absolute configuration of the major or minor enantiomer in **5** and **8** could not be confirmed by the NMR analysis of their (*R*)- and (*S*)-MTPA esters.
13) Kitagawa I., Kobayashi M., Yasuzawa T., Son B. W., Yoshihara M., Kyogoku Y., *Tetrahedron*, **41**, 995—1005 (1985).
14) Kilbourn R. G., Griffith O. W., *J. Natl. Cancer Inst.*, **84**, 827—831 (1992).