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Benzoic Acid Derivatives, Acetophenones, and Anti-inflammatory Constituents from *Melicope semecarpifolia*

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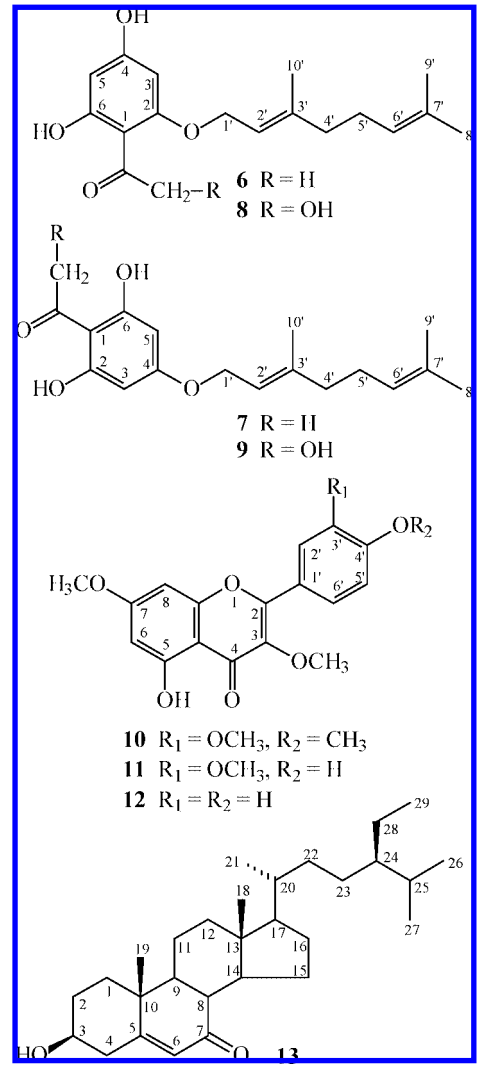
Two new benzoic acid derivatives, (*E*)-3-acetyl-6-(3,7-dimethylocta-2,6-dienyloxy)-2,4-dihydroxybenzoic acid (**1**) and (*E*)-3-acetyl-4-(3,7-dimethylocta-2,6-dienyloxy)-2,6-dihydroxybenzoic acid (**2**), and three new acetophenones, (*E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-7-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)ethanone (**3**), (*E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-7-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2*H*-chromen-8-yl)ethanone (**4**), and (*R,E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-3,7-dihydroxy-2,2-dimethylchroman-8-yl)ethanone (**5**), have been isolated from the fruits of *Melicope semecarpifolia*, together with eight known compounds. The structures were determined through in-depth NMR and mass spectrometric analyses. Among the isolated compounds, 2-(1′-geranyloxy)-4,6,*β*-trihydroxyacetophenone (**8**), 4-(1′-geranyloxy)-2,6,*β*-trihydroxyacetophenone (**9**), 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**10**), 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**11**), and 5,4′-dihydroxy-3,7-dimethoxyflavone (**12**) exhibited potent inhibition (IC_{50} < 4 μg/mL) on superoxide anion generation and elastase release by human neutrophils in response to fMet-Leu-Phe/cytochalasin B.

Melicope semecarpifolia (Merr.) T. G. Hartley (Rutaceae) [*Euodia merrillii* Kanehira & Sasaki ex Kanehira; *Melicope confusa* (Merr.) Liu] is a small- to medium-sized evergreen tree found at low altitude in forests of Taiwan and the Philippines.[1] The roots of this plant have been used as a carminative in folk medicine.[2] Previous chemical studies of this plant (fruits,[3,4] leaves,[5–7] root bark,[8] root wood,[9] flowers,[10] and stems[10]) have yielded acetophenones, furoquinolines, quinolones, benzenoids, coumarins, and flavones.[3–10] Antiplatelet aggregation[6,8,9] and cytotoxic[7,9] activities have been reported for some of these compounds. In our studies of Formosan plants for *in vitro* anti-inflammatory activity, *M. semecarpifolia* was found to be an active species. In our search for compounds with anti-inflammatory activities, two new benzoic acids (**1** and **2**), three new acetophenones (**3**–**5**), and eight known compounds (**6**–**13**) have been isolated from the fruits of *M. semecarpifolia.* This paper describes the structural elucidation of **1**–**5** and the anti-inflammatory activities of these compounds.



Results and Discussion

Extensive fractionation of the EtOAc-soluble portion of a MeOH extract of fruits of *M. semecarpifolia* using silica gel column chromatography (CC) and preparative TLC afforded compounds **1**–**13**.

Compound **1** was isolated as a colorless oil. The HRFABMS gave an $[M]^+$ ion at *m/z* 348.1564 (calcd for $C_{19}H_{24}O_6$, 348.1573), consistent with a molecular formula of $C_{19}H_{24}O_6$. The UV absorption at 295 nm was similar to that of 4-(1′-geranyloxy)-2,6-dihydroxy-3-isopentenylacetophenone[3] and suggested the presence of a 2,4,6-trioxygenated acetophenone nucleus.[3] IR absorptions for OH (3269 cm^{-1}) and carbonyl functions (1620 cm^{-1}) were observed. The ^1H NMR spectrum of **1** was similar to that of 4-(1′-geranyloxy)-2,6-dihydroxyacetophenone (**7**),[3] except for an additional carboxy group [δ 3.78 (1H, s, D_2O exchangeable)] of **1**, replacing H-3 [δ 5.95 (1H, s)] of **7**. This was supported by HMBC correlations observed between COOH (δ 3.78) and both C-1 (δ 106.4) and COOH (δ 163.5). The ^1H NMR spectrum of **1** showed the presence of a 3,7-dimethylocta-2,6-dienyloxy group [δ 1.59,

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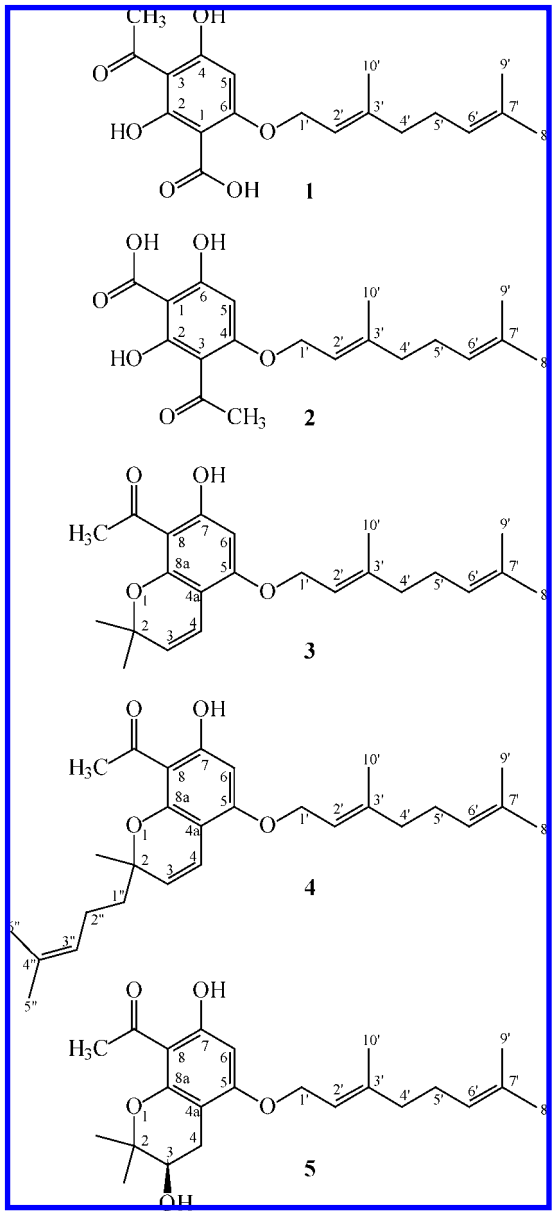

1.66, 1.71 (each 3H, each d, $J = 0.8$, 0.8, and 1.2 Hz, H-9′, H-8′, and H-10′), δ 2.10 (4H, m, H-4′ and H-5′), δ 4.54 (2H, d, $J = 6.4$ Hz, H-1′), δ 5.06 (1H, br. t, $J = 6.8$ Hz, H-6′), and δ 5.47 (1H, br t, $J = 6.4$ Hz, H-2′)], two OH groups [δ 9.36, 16.13 (each 1H, each s, D_2O exchangeable, OH-4 and OH-2), an acetyl group [δ 2.63 (3H, s, Ac-3)], a carboxy group [δ 3.78 (1H, s, COOH-1)], and an unsubstituted aromatic proton [δ 6.02 (1H, s, H-5)]. The locations of the substituents were determined by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between H-1′ (δ 4.54) and H-5 (δ 6.02). (b) HMBC correlations were observed between OH-4 (δ 9.36) and C-3 (δ 105.5), C-4 (δ 163.5), and C-5 (δ 93.6). (c) HMBC correlations were observed between C-3 (δ 105.5) and both Ac-3 (δ 2.63) and OH-2 (δ 16.13). (d) HMBC correlations were observed between H-5 (δ 6.02) and C-1 (δ 106.4), C-3 (δ 105.5), and C-6 (δ 163.2). The ^{13}C NMR resonances were assigned by DEPT, HSQC, and HMBC techniques. Thus, the structure of **1** was elucidated as (*E*)-3-acetyl-6-(3,7-dimethylocta-2,6-dienyloxy)-2,4-dihydroxybenzoic acid.

Compound **2** was isolated as a colorless oil. The ESIMS afforded the sodiated ion $[M + Na]^+$ at *m/z* 371, implying a molecular formula of $C_{19}H_{24}O_6$, which was confirmed by the HRESIMS (*m/z* 371.1475 $[M + Na]^+$, calcd for $C_{19}H_{24}O_6Na$, 371.1471). The IR spectrum suggested the presence of OH (3269 cm^{-1}) and conjugated carbonyl groups (1620 cm^{-1}). The ^1H NMR spectrum of **2** showed the presence of a 3,7-dimethylocta-2,6-dienyloxy group, two OH groups, an acetyl group, and an unsubstituted aromatic proton very similar to signals described previously for 2-(1′-geranyloxy)-4,6-dihydroxyacetophenone (**6**),[4] except for the presence of an additional carboxy group [δ 3.70 (1H, s, D_2O exchangeable)] of **2**, replacing H-5 [δ 6.00 (1H, d, $J = 2.4$ Hz)] of **6**. This was supported by HMBC correlations observed between COOH (δ 3.70) and both C-1 (δ 104.8) and COOH (δ 160.8). In the NOESY spectrum of **2**, H-5 (δ 6.13) showed correlations with H-1′ (δ 4.72). HMBC correlations were observed between OH-6 (δ 8.75) and C-1 (δ 104.8), C-5 (δ 93.3), and C-6 (δ 162.5) and between C-1 (δ 104.8) and COOH (δ 3.70), OH-2 (δ 13.71), and H-5 (δ 6.13). Thus, the acetyl group was at C-3. Assignments of the carbon resonances were confirmed by DEPT, HSQC, and HMBC techniques. On the basis of the above data, the structure of **2** was elucidated as (*E*)-3-acetyl-4-(3,7-dimethylocta-2,6-dienyloxy)-2,6-dihydroxybenzoic acid.

Compound **3** was isolated as a colorless oil with molecular formula $C_{23}H_{30}O_4$ as determined by positive-ion HRESIMS, showing an $[M + Na]^+$ ion at *m/z* 393.2044 (calcd for $C_{23}H_{30}O_4Na$, 393.2042). The presence of OH and carbonyl groups in **3** was revealed by the bands at 3446 and 1613 cm^{-1}, respectively, in the IR spectrum. The ^1H NMR spectrum of **3** showed resonances for a 3,7-dimethylocta-2,6-dienyloxy group, an acetyl group, an OH group, an unsubstituted aromatic proton, two methyl groups, and two mutually coupled protons of a dimethylchromene derivative. The locations of the above groups of **3** were determined by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between H-1′ (δ 4.56) and H-6 (δ 6.01) and between Me-2 (δ 1.49) and Ac-8 (δ 2.67). (b) HMBC correlations were observed between OH-7 (δ 13.82) and C-6 (δ 93.4), C-7 (δ 166.5), and C-8 (δ 106.1). (c) HMBC correlations were observed between C-8 (δ 106.1) and both H-6 (δ 6.01) and Ac-8 (δ 2.67). (d) HMBC correlations were observed between H-6 (δ 6.01) and C-5 (δ 160.6), C-7 (δ 166.5), and C-4a (δ 103.2). (e) HMBC correlations were observed between C-5 (δ 160.6) and both H-4 (δ 6.58) and H-1′ (δ 4.56). According to the above data, the structure of **3** was elucidated as (*E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-7-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)ethanone. This was confirmed by $^1H-^1H$ COSY and NOESY experiments. This is the first report of the occurrence of **3** in a natural source, although it has been synthesized by Chou et al.[3]

Compound **4** had the molecular formula $C_{28}H_{38}O_4$, as indicated by the sodiated HRESIMS ion peak at *m/z* = 461.2662 $[M + Na]^+$ (calcd for $C_{28}H_{38}O_4Na$, 461.2668). IR absorptions for OH (3446 cm^{-1}) and conjugated carbonyl functions (1613 cm^{-1}) were observed. The ^1H NMR spectrum of **4** showed resonances for a 3,7-dimethylocta-2,6-dienyloxy group, an acetyl group, an OH group, an unsubstituted aromatic proton, a methyl group, and two mutually coupled protons of a dimethylchromene derivative. The spectrum was very similar to the spectrum of **3**,[3] except for the resonances of a 4-methylpent-3-enyl group at C-2 in the spectrum of **4**, replacing that of Me-2 of **3**.[3] This was supported by HMBC correlations between H-1″ (δ 2.10) and both C-2 (δ 80.9) and C-3 (δ 123.2). On the basis of the above data, the structure of **4** was elucidated as (*E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-7-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2*H*-chromen-8-yl)ethanone.

Compound **5** was a colorless oil having the molecular formula $C_{23}H_{32}O_5$, as deduced from the sodiated ion at *m/z* 411.2150 $[M + Na]^+$ in the HRESI mass spectrum. The presence of a conjugated carbonyl group was revealed by a band at 1619 cm^{-1} in the IR spectrum, which was confirmed by the resonance at δ 203.3 in the ^{13}C NMR spectrum. Comparison of the ^1H NMR data of **5** with that of **3**[3] suggested that their structures were closely related except that the 2,2-dimethyl-3-hydroxychromane moiety of **5** replaced a 2,2-dimethylchromene moiety of **3**.[3] This was supported by HMBC

Table 1. Inhibitory Effects of **1**–**13** on Superoxide Anion Generation and Elastase Release by Human Neutrophils in Response to fMet-Leu-Phe/Cytochalasin B[a]

compound	IC_{50} (μg/mL[b] or Inh %)	
	superoxide anion	elastase
1	6.62 ± 1.01***	5.79 ± 1.55***
2	8.02 ± 0.89***	(46.11 ± 8.23)**
3	(21.37 ± 1.29)***	(27.35 ± 3.83)**
4	(23.24 ± 1.44)**	(26.62 ± 3.55)*
5	(30.61 ± 5.79)***	(28.73 ± 8.32)**
6[c]	elicit superoxide anion generation and elastase release	
7[c]	elicit superoxide anion generation and elastase release	
8	0.73 ± 0.29***	0.60 ± 0.07***
9	3.75 ± 1.13***	0.91 ± 0.34***
10	1.43 ± 0.42***	1.69 ± 0.11***
11	1.00 ± 0.31***	0.99 ± 0.15***
12	1.20 ± 0.18***	0.70 ± 0.03***
13	(22.56 ± 6.45)**	(7.48 ± 6.53)
diphenyleneiodonium	0.52 ± 0.23***	
phenylmethylsulfonyl fluoride		35.68 ± 5.89***

[a] Percentage of inhibition (Inh %) at 10 μg/mL concentration. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive control. Results are presented as average ± SEM ($n = 4$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with the control. [b] Concentration necessary for 50% inhibition (IC_{50}). [c] Compounds **6** and **7** (10 μg/mL) alone elicited superoxide anion generation and elastase release by human neutrophils in the absence of fMet-Leu-Phe/cytochalasin B.

correlations observed between H-4 (δ 2.60 and 2.84) and both C-2 (78.4) and C-5 (δ 163.4) and by NOESY correlations between H-3 (δ 3.79) and both H-4 (δ 2.60) and Me-2 (δ 1.38). Compound **5** was dextrorotatory, $[\alpha]^{25}_D = +46.2$, similar to that of lomatin ($[\alpha]^{23}_D = +52$),[11] and the absolute configuration of C-3 in **5** must be *R*.[11] According to the above data, the structure of **5** was elucidated as (*R,E*)-1-(5-(3,7-dimethylocta-2,6-dienyloxy)-3,7-dihydroxy-2,2-dimethylchroman-8-yl)ethanone.

Compounds **6**–**13** were readily identified, by comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values, as 2-(1′-geranyloxy)-4,6-dihydroxyacetophenone (**6**),[3] 4-(1′-geranyloxy)-2,6-dihydroxyacetophenone (**7**),[3] 2-(1′-geranyloxy)-4,6,β-trihydroxyacetophenone (**8**),[4] 4-(1′-geranyloxy)-2,6,β-trihydroxyacetophenone (**9**),[3] 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**10**),[12] 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**11**),[13] 5,4′-dihydroxy-3,7-dimethoxyflavone (**12**),[14] and 3β-hydroxystigmast-5-en-7-one (**13**).[15]

The distribution of acetophenones in the Rutaceae seems to be restricted. Adsersen et al.[16] (2007) reviewed the distribution of acetophenones in the Rutaceae and indicated that acetophenones with geranyloxy substituents have until now been reported only from *Melicope*. The restricted occurrence of *O*-geranylated acetophenones in Rutaceae indicates that they are valuable as chemotaxonomic markers for the genus *Melicope*, an assumption supported by the isolation of compounds of this type from *M. semecarpifolia*.

The anti-inflammatory effects of compounds isolated from the fruits of *M. semecarpifolia* were evaluated by suppressing fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced superoxide anion ($O_2^{\bullet-}$) generation and elastase release by human neutrophils. The anti-inflammatory activity data are summarized in Table 1. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls for $O_2^{\bullet-}$ generation and elastase release, respectively. From the results of our anti-inflammatory tests, the following conclusions can be drawn: (a) 2-(1′-Geranyloxy)-4,6,β-trihydroxyacetophenone (**8**), 4-(1′-geranyloxy)-2,6,β-trihydroxyacetophenone (**9**), 5-hydroxy-3,7,3′,4′-tetramethoxyflavone (**10**), 5,4′-dihydroxy-3,7,3′-trimethoxyflavone (**11**), and 5,4′-dihydroxy-3,7-dimethoxyflavone (**12**) exhibited potent inhibitory activities ($IC_{50} < 4$ μg/mL) on human neutrophil $O_2^{\bullet-}$ generation and elastase release. (b) Compounds **8** and **9**, with a 1-hydroxyacetyl group, exhibited more effective inhibition than their corresponding analogues **6** and **7**, with an acetyl group, against $O_2^{\bullet-}$ generation and elastase release. (c) Compounds **6** and **7** alone elicited superoxide anion generation and elastase release by human neutrophils in the absence of fMLP/CB at 10 μg/mL. (d) 2-(1′-Geranyloxy)-4,6,β-trihydroxyacetophenone (**8**) was the most effective among the isolated compounds, with IC_{50} values of 0.73 ± 0.29 and 0.60 ± 0.07 μg/mL, respectively, against fMLP-induced superoxide anion generation and elastase release.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanaco micromelting point apparatus and were uncorrected. Optical rotations were measured using a Jasco DIP-370 polarimeter in $CHCl_3$. UV spectra were obtained on a Jasco UV-240 spectrophotometer. IR spectra (KBr or neat) were recorded on a Perkin-Elmer system 2000 FT-IR spectrometer. NMR spectra, including COSY, NOESY, HMBC, and HSQC experiments, were recorded on a Varian Unity 400 or a Varian Inova 500 spectrometer operating at 400 and 500 MHz (^{1}H) and 100 and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) using TMS as an internal standard. EI, ESI, and HRESI mass spectra were recorded on a Bruker APEX II mass spectrometer. FAB and HRFAB mass spectra were recorded on a JEOL JMX-HX 110 mass spectrometer. Silica gel (70–230, 230–400 mesh) (Merck) was used for CC. Silica gel 60 F-254 (Merck) were used for TLC and preparative TLC.

Plant Material. The fruit of *M. semecarpifolia* was collected from Lai-I, Pingtung County, Taiwan, in May 2006 and identified by Dr. I. S. Chen. A voucher specimen (Chen 5685) was deposited in the herbarium of the Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation. The dried fruit of *M. semecarpifolia* (9.55 kg) was extracted with cold MeOH, and the extract concentrated under reduced pressure. The MeOH extract (1.5 kg), when partitioned between H_2O−EtOAc (1:1), afforded an EtOAc-soluble fraction (fraction A, 950 g). Part (100 g) of fraction A was purified by CC (4.2 kg SiO_2, 70–230 mesh; CH_2Cl_2−MeOH gradient) to give 11 fractions: A1 (5 L, CH_2Cl_2), A2 (3 L, CH_2Cl_2−MeOH, 100:1), A3 (5 L, CH_2Cl_2−MeOH, 90:1), A4 (4 L, CH_2Cl_2−MeOH, 80:1), A5 (3 L, CH_2Cl_2−MeOH, 60:1), A6 (4 L, CH_2Cl_2−MeOH, 50:1), A7 (4 L, CH_2Cl_2−MeOH, 30:1), A8 (5 L, CH_2Cl_2−MeOH, 10:1), A9 (5 L, CH_2Cl_2−MeOH, 5:1), A10 (4 L, CH_2Cl_2−MeOH, 1:1), and A11 (3 L, MeOH). Fraction A1 (4.78 g) was subjected to CC (160 g SiO_2, 230–400 mesh; *n*-hexane−EtOAc, 30:1) to give nine fractions (each 1.5 L, A1-1−A1-9). Fraction A1-2 (198 mg) was purified by PTLC (*n*-hexane−acetone, 20:1) to afford **3** (9.4 mg). Fraction A1-3 (185 mg) was purified by PTLC (*n*-hexane−CH_2Cl_2, 1:2) to obtain **4** (10.8 mg). Fraction A1-4 (162 mg) was purified further by PTLC (*n*-hexane−EtOAc, 10:1) to yield **1** (5.7 mg). Fraction A1-5 (144 mg) was purified by PTLC ($CHCl_3$−EtOAc, 40:1) to give **2** (3.4 mg). Fraction A1-6 (216 mg) was purified by PTLC (CH_2Cl_2−EtOAc, 30:1) to obtain **10** (24.4 mg). Fraction A1-7 (223 mg) was purified by PTLC (CH_2Cl_2−acetone, 20:1) to provide **11** (22.7 mg). Fraction A3

(5.31 g) was purified by CC (175 g SiO_2, 230–400 mesh; *n*-hexane−EtOAc, 30:1) to afford 11 fractions (each 1.5 L, A3-1−A3-11). Fraction A3-1 (283 mg) was purified by PTLC (CH_2Cl_2−EtOAc, 30:1) to obtain **6** (17.9 mg). Fraction A3-3 (207 mg) was purified by PTLC ($CHCl_3$−EtOAc, 5:1) to yield **8** (17.2 mg). Fraction A3-7 (187 mg) was purified by PTLC (*n*-hexane−acetone, 3:1) to give **7** (9.4 mg). Fraction A3-9 (196 mg) was purified by PTLC (CH_2Cl_2−acetone, 10:1) to obtain **9** (7.9 mg). Fraction A5 (3.7 g) was subjected to CC (135 g SiO_2, 230–400 mesh; *n*-hexane−acetone, 3:2) to afford 10 fractions (each 1.5 L, A5-1−A5-10). Fraction A5-2 (198 mg) was purified by PTLC (*n*-hexane−acetone, 3:2) to obtain **13** (27.7 mg). Fraction A5-3 (222 mg) was purified PTLC (*n*-hexane−acetone, 3:2) to yield **12** (3.5 mg).

Biological Assay. The anti-inflammatory effects of the compounds isolated from *M. semecarpifolia* were evaluated by inhibiting the generation of $O_2^{\bullet-}$ and the release of elastase in fMLP-activated human neutrophils in a concentration-dependent manner.

Preparation of Human Neutrophils. Human neutrophils from venous blood of healthy, adult volunteers (20–28 years old) were isolated using a standard method of dextran sedimentation prior to centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of erythrocytes.[17] Purified neutrophils containing >98% viable cells, as determined by the trypan blue exclusion method, were resuspended in a calcium (Ca^{2+})-free HBSS buffer at pH 7.4 and were maintained at 4 °C prior to use.

Measurement of $O_2^{\bullet-}$ Generation. The assay for measurement of $O_2^{\bullet-}$ generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[18] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were activated by 100 nM fMLP for 10 min, and 1 μg/mL cytochalasin B was incubated for 3 min before activation by peptide. Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were continuously monitored in a double-beam, six-cell positioner spectrophotometer with constant stirring (Hitachi U-3010, Tokyo, Japan). Calculations were based on differences in the reactions with and without SOD (100 U/mL) divided by the extinction coefficient for the reduction of ferricytochrome *c* (ϵ = 21.1/mM/10 mm).

Measurement of Elastase Release. Degranulation of azurophilic granules was determined by measuring elastase release as described previously.[19] Experiments were performed using MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. Briefly, after supplementation with MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (100 μM), neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with drugs for 5 min. Cells were stimulated with fMLP (100 nM)/cytochalasin B (0.5 μg/mL), and changes in absorbance at 405 nm were continuously being monitored in order to assay elastase release. The results were expressed as the percent of the initial rate of elastase release in the fMLP/cytochalasin B-activated, drug-free control system.

Statistical Analysis. Results are expressed as the mean ± SEM, and comparisons were made using Student's *t*-test. A probability of 0.05 or less was considered significant.

(*E*)-3-Acetyl-6-(3,7-dimethylocta-2,6-dienyloxy)-2,4-dihydroxybenzoic acid (1): colorless oil; UV (MeOH) λ_{max} (log ϵ) 295 (4.17) nm; IR (neat) ν_{max} 3269 (OH), 1620 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.59 (3H, d, J = 0.8 Hz, H-9′), 1.66 (3H, d, J = 0.8 Hz, H-8′), 1.71 (3H, d, J = 1.2 Hz, H-10′), 2.10 (4H, m, H-4′ and H-5′), 2.63 (3H, s, Ac-3), 3.78 (1H, s, D_2O exchangeable, COOH-1), 4.54 (2H, d, J = 6.4 Hz, H-1′), 5.06 (1H, br t, J = 6.8 Hz, H-6′), 5.47 (1H, br t, J = 6.4 Hz, H-2′), 6.02 (1H, s, H-5), 9.36 (1H, s, D_2O exchangeable, OH-4), 16.13 (1H, s, D_2O exchangeable, OH-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.8 (C-10′), 17.9 (C-9′), 25.9 (C-8′), 26.4 (C-5′), 32.9 (*Me*CO), 39.6 (C-4′), 65.9 (C-1′), 93.6 (C-5), 105.5 (C-3), 106.4 (C-1), 118.6 (C-2′), 123.8 (C-6′), 132.2 (C-7′), 142.4 (C-3′), 161.8 (C-2), 163.2 (C-6), 163.5 (C-4), 163.5 (COOH), 204.0 (Me*C*O); FABMS *m/z* (rel int) 348 ([M]$^+$, 11); HRFABMS *m/z* 348.1564 [M]$^+$ (calcd for $C_{19}H_{24}O_6$, 348.1573).

(*E*)-3-Acetyl-4-(3,7-dimethylocta-2,6-dienyloxy)-2,6-dihydroxybenzoic acid (2): colorless oil; UV (MeOH) λ_{max} (log ϵ) 285 (4.19) nm; IR (neat) ν_{max} 3269 (OH), 1620 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.61 (3H, s, H-9′), 1.67 (3H, s, H-8′), 1.81 (3H, d, J = 1.2 Hz, H-10′), 2.15 (4H, m, H-4′ and H-5′), 2.67 (3H, s, Ac-3), 3.70 (1H, s, D_2O exchangeable, COOH-1), 4.72 (2H, d, J = 6.8 Hz, H-1′), 5.11 (1H, br t, J = 6.8 Hz, H-6′), 5.59 (1H, br t, J = 6.8 Hz, H-2′), 6.13 (1H, s, H-5), 8.75 (1H, s, D_2O exchangeable, OH-6), 13.71 (1H, s, D_2O exchangeable, OH-2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.7 (C-10′), 17.8 (C-9′), 25.9 (C-8′), 26.4 (C-5′), 33.4 (*Me*CO), 39.8 (C-4′), 66.5 (C-1′), 93.3 (C-5), 104.8 (C-1), 107.0 (C-3), 116.8 (C-2′), 123.6 (C-6′), 131.9 (C-7′), 141.8 (C-3′), 160.8 (COOH), 161.9 (C-2), 162.5 (C-6), 162.8 (C-4), 204.5 (Me*C*O); ESIMS *m/z* (rel int) 371 ([M + Na]$^+$, 45); HRESIMS *m/z* 371.1475 [M + Na]$^+$ (calcd for $C_{19}H_{24}O_6Na$, 371.1471).

(*E*)-1-(5-(3,7-Dimethylocta-2,6-dienyloxy)-7-hydroxy-2,2-dimethyl-2*H*-chromen-8-yl)ethanone (3): colorless oil; UV (MeOH) λ_{max} (log ϵ) 284 (4.25) nm; IR (neat) ν_{max} 3446 (OH), 1613 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.49 (6H, s, Me-2 × 2), 1.61 (3H, s, H-9′), 1.68 (3H, s, H-8′), 1.72 (3H, s, H-10′), 2.10 (4H, m, H-4′ and H-5′), 2.67 (3H, s, Ac-8), 4.56 (2H, d, J = 6.4 Hz, H-1′), 5.09 (1H, br t, J = 6.8 Hz, H-6′), 5.41 (1H, d, J = 10.0 Hz, H-3), 5.45 (1H, br t, J = 6.4 Hz, H-2′), 6.01 (1H, s, H-6), 6.58 (1H, d, J = 10.0 Hz, H-4), 13.82 (1H, s, D_2O exchangeable, OH-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.0 (C-10′), 18.0 (C-9′), 25.9 (C-8′), 26.5 (C-5′), 28.1 (Me-2 × 2), 33.4 (*Me*CO), 39.7 (C-4′), 65.7 (C-1′), 78.1 (C-2), 93.4 (C-6), 103.2 (C-4a), 106.1 (C-8), 117.0 (C-4), 118.9 (C-2′), 123.9 (C-6′), 124.6 (C-3), 132.2 (C-7′), 142.0 (C-3′), 156.5 (C-8a), 160.6 (C-5), 166.5 (C-7), 203.5 (Me*C*O); EIMS *m/z* (rel int) 370 ([M]$^+$, 7), 234 (14), 219 (100), 203 (18), 147 (22), 121 (13), 105 (19), 91 (15), 81 (32); HRESIMS *m/z* 393.2044 [M + Na]$^+$ (calcd for $C_{23}H_{30}O_4$, 393.2042).

(*E*)-1-(5-(3,7-Dimethylocta-2,6-dienyloxy)-7-hydroxy-2-methyl-2-(4-methylpent-3-enyl)-2*H*-chromen-8-yl)ethanone (4): colorless oil; UV (MeOH) λ_{max} (log ϵ) 278 (4.22) nm; IR (neat) ν_{max} 3446 (OH), 1613 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.44 (3H, s, Me-2), 1.57 (3H, s, H-6″), 1.61 (3H, s, H-9′), 1.67 (3H, s, H-5″), 1.68 (3H, s, H-8′), 1.72 (3H, s, H-10′), 2.10 (8H, m, H-4′, H-5′, H-1″, and H-2″), 2.66 (3H, s, Ac-8), 4.56 (2H, d, J = 6.8 Hz, H-1′), 5.10 (2H, br t, J = 6.8 Hz, H-6′ and H-3″), 5.36 (1H, d, J = 10.2 Hz, H-3), 5.45 (1H, br t, J = 6.8 Hz, H-2′), 5.99 (1H, s, H-6), 6.63 (1H, d, J = 10.2 Hz, H-4), 13.83 (1H, s, D_2O exchangeable, OH-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.9 (C-10′), 17.8 (C-6″), 17.9 (C-9′), 23.3 (C-2″), 25.9 (C-8′), 25.9 (C-5″), 26.5 (C-5′), 26.8 (Me-2), 33.4 (*Me*CO), 39.7 (C-4′), 41.7 (C-1″), 65.7 (C-1′), 80.9 (C-2), 93.2 (C-6), 102.9 (C-4a), 105.9 (C-8), 117.6 (C-4), 118.9 (C-2′), 123.2 (C-3), 123.9 (C-6′), 124.0 (C-3″), 132.1 (C-7′), 132.3 (C-4″), 141.9 (C-3′), 156.7 (C-8a), 160.6 (C-5), 166.5 (C-7), 203.4 (Me*C*O); ESIMS *m/z* (rel int) 461 ([M + Na]$^+$, 100); HRESIMS *m/z* 461.2662 [M + Na]$^+$ (calcd for $C_{28}H_{38}O_4Na$, 461.2668).

(*R,E*)-1-(5-(3,7-Dimethylocta-2,6-dienyloxy)-3,7-dihydroxy-2,2-dimethylchroman-8-yl)ethanone (5): colorless oil; $[\alpha]^{25}_D$ +46.2 (*c* 0.12, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 288 (4.18) nm; IR (neat) ν_{max} 3424 (OH), 1619 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.38 (3H, s, Me-2), 1.41 (3H, s, Me-2), 1.61 (3H, s, H-9′), 1.69 (3H, s, H-8′), 1.72 (3H, s, H-10′), 2.10 (4H, m, H-4′ and H-5′), 2.60 (1H, dd, J = 17.0, 5.6 Hz, H_α−4), 2.64 (3H, s, Ac-8), 2.84 (1H, dd, J = 17.0, 5.6 Hz, H_β−4), 3.79 (1H, t, J = 5.6 Hz, H-3), 4.55 (2H, d, J = 6.4 Hz, H-1′), 5.10 (1H, br t, J = 6.8 Hz, H-6′), 5.45 (1H, br t, J = 6.4 Hz, H-2′), 6.05 (1H, s, H-6), 13.86 (1H, s, D_2O exchangeable, OH-7); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.0 (C-10′), 17.9 (C-9′), 22.2 (Me-2), 25.0 (Me-2), 25.9 (C-8′), 26.2 (C-4), 26.5 (C-5′), 33.5 (*Me*CO), 39.7 (C-4′), 65.7 (C-1′), 68.9 (C-3), 78.4 (C-2), 93.4 (C-6), 99.0 (C-4a), 106.0 (C-8), 118.9 (C-2′), 123.9 (C-6′), 132.1 (C-7′), 141.9 (C-3′), 155.4 (C-8a), 163.4 (C-5), 166.0 (C-7), 203.3 (Me*C*O); ESIMS *m/z* (rel int) 411 ([M + Na]$^+$, 100); HRESIMS *m/z* 411.2150 [M + Na]$^+$ (calcd for $C_{23}H_{32}O_5Na$, 411.2147).

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