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Amides and Benzenoids from *Zanthoxylum ailanthoides* with Inhibitory Activity on Superoxide Generation and Elastase Release by Neutrophils

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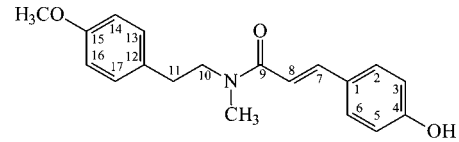
Five new compounds, ailanthamide (**1**), *N*-(4-methoxyphenethyl)-*N*-methylbenzamide (**2**), (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde (**4**), and (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (**5**), and 17 known compounds have been isolated from the stem bark of *Zanthoxylum ailanthoides*. The structures were determined through spectroscopic and MS analyses. Compounds **1**, **3**, xanthyletin, decarine, (+)-episesamin, (−)-hinokinin, and evofolin-B exhibited inhibition ($IC_{50} \leq 5.34$ μg/mL) of superoxide anion generation by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). Compounds **1**, xanthyletin, decarine, and (+)-episesamin also inhibited fMLP/CB-induced elastase release with IC_{50} values ≤ 5.53 μg/mL.

Zanthoxylum ailanthoides Sieb. & Zucc. (Rutaceae) is a medium- to large-sized tree found at low altitude in forests of China, Korea, Japan, the Philippines, and Taiwan.[1] Its leaves are used as a folk medicine to treat the common cold in Taiwan.[2] Benzo[*c*]phenanthridines, quinolines, coumarins, flavonoids, lignans, and terpenoids have been identified as constituents of this plant.[3−12] Antiplatelet aggregation[11] and anti-HIV[12] activities have been reported for some of these compounds. In our studies on constituents of Formosan plants for *in vitro* inhibitory activity on neutrophil pro-inflammatory responses, *Z. ailanthoides* was found to be an active species. Three new amides, ailanthamide (**1**), *N*-(4-methoxyphenethyl)-*N*-methylbenzamide (**2**), and (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), two new benzenoids, 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde (**4**) and (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (**5**), and 17 known compounds have been isolated and identified from the stem bark of *Z. ailanthoides*. This paper describes the structural elucidation of **1**−**5** and the inhibitory activities of all isolates on superoxide generation and elastase release by neutrophils.



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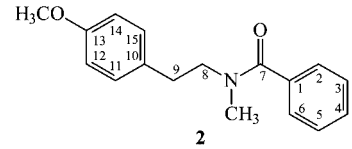

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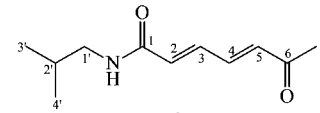

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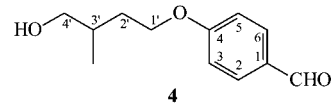

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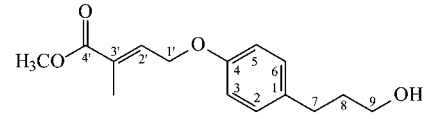

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Results and Discussion

Chromatographic purification of the EtOAc-soluble fraction of a MeOH extract of stem bark of *Z. ailanthoides* on a silica gel column (CC) and preparative thin-layer chromatography (TLC) afforded five new (**1**−**5**) and 17 known compounds.

Ailanthamide (**1**) was isolated as a colorless oil. The HRESIMS gave an $[M + Na]^+$ ion at *m*/*z* 334.1421 (calcd for $C_{19}H_{21}NO_3Na$, 334.1419), consistent with a molecular formula of $C_{19}H_{21}NO_3$. The UV absorptions at 225, 294, and 313 nm were similar to those of *N*-*p*-coumaroyltyramine,[13] and the bathochromic shift in alkaline solution suggested the presence of an *N*-*p*-coumaroyltyramine nucleus.[13] IR absorptions for OH (3345 cm^{-1}) and carbonyl (1650 cm^{-1}) functions were observed. The ^1H NMR data of **1** were similar to those of beecheyamide,[14] except that the 4-hydroxyphenyl group at C-7 of **1** replaced the 3,4-dimethoxyphenyl group at C-7 of beecheyamide.[14] This was supported by the following NOESY and HMBC correlations: (a) NOESY correlations were observed between H-2 and both H-3 and H-7; (b) HMBC correlations were observed between H-2 and C-4 and C-7; (c) HMBC correlations were observed between H-3 and C-1 and C-5. At room temperature, two isomers (**1a** and **1b**), with integrated intensities of 2 (**1a**):3 (**1b**), were detected in the ^1H NMR spectrum of **1**, but at a temperature above 70 °C, only one component could be detected in the ^1H NMR spectrum of **1**, as in the case of beecheyamide.[14] Consequently, the temperature-dependent ^1H NMR spectra can be explained in terms of slow interconversion of the *syn* and *anti* amide isomers due to *syn*−*anti* isomerism.[15] The *syn* and *anti* isomers of **1** were determined by the NOESY experiments (Figure 1). The major component (**1b**) exhibited a NOESY correlation between H-8 (δ 6.38) and H-10 (δ 3.65), while the same experiment for H-8 (δ 6.69) in the minor component (**1a**) correlated with N-Me (δ 3.03). Thus, the amide configurations in the major (**1b**) and minor (**1a**) components are *syn* and *anti* form, respectively. On the basis of the above data, the structure of **1** was elucidated as 3-(4-hydroxyphenyl)-*N*-[2-(4-methoxyphenyl)ethyl]-*N*-methylacryla-

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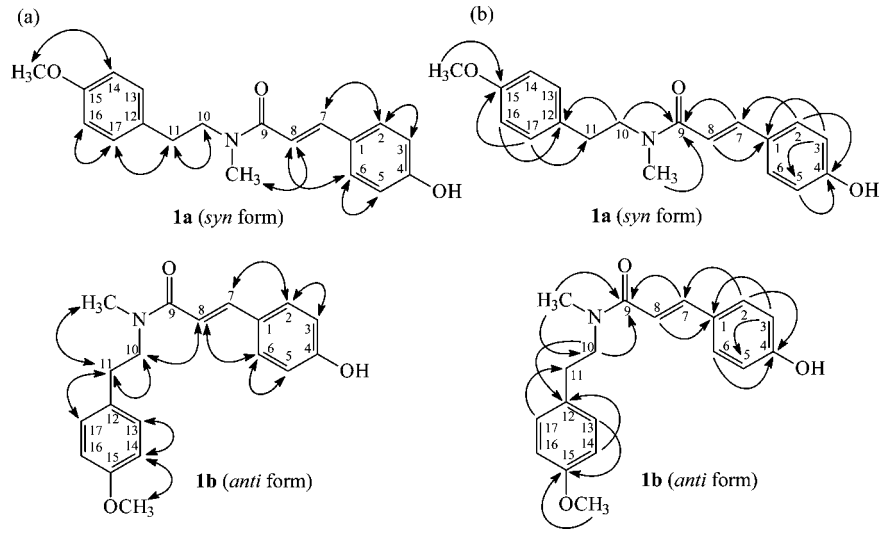

Figure 1. NOESY (a) and HMBC (b) correlations of **1**.

mide. This was confirmed by $^1H-^1H$ COSY and NOESY experiments (Figure 1). Assignments of the carbon resonances were confirmed by DEPT, HSQC, and HMBC techniques.

Compound **2** was isolated as a yellowish, amorphous powder. The ESIMS afforded the sodiated ion $[M + Na]^+$ at *m/z* 292, implying a molecular formula of $C_{17}H_{19}NO_2$, which was confirmed by the HRESIMS (*m/z* 292.1314 $[M + Na]^+$, calcd for $C_{17}H_{19}NO_2Na$, 292.1313). UV absorptions at 224, 274, and 283 nm were similar to those of dihydroalatamide[16] and suggested the presence of a benzoylamide moiety. The presence of a carbonyl group was revealed by the band at 1628 cm^{-1} in the IR spectrum of **2** and was confirmed by a signal at δ 172.2 in the ^{13}C NMR spectrum. The ^1H NMR spectrum of **2** showed the presence of a benzoyl group, an *N*-methyl group, a 4-methoxyphenyl group, and four mutually coupled methylene protons very similar to signals described previously for dihydroalatamide,[16] except for the presence of an additional *N*-methyl group [δ 3.14 (3H, s)] of **2**, replacing an NH group of dihydroalatamide.[16] This was supported by HMBC correlations observed between N-Me (δ 3.14) and both C-7 (δ 172.2) and C-8 (δ 53.1) and by NOESY correlations between N-Me (δ 3.14) and both H-6 (δ 7.33) and H-9 (δ 2.74). The ^1H NMR spectrum of **2** also exhibited the *syn* and *anti* amide isomers due to *syn*−*anti* isomerism[15] as in the case of **1** and beecheyamide.[14] The *syn* amide component (**2a**) exhibited a NOESY correlation between N-Me (δ 3.14) and H-6 (δ 7.33), while the same experiment for N-Me (δ 2.82) in the *anti* amide component (**2b**) correlated with H-9 (δ 2.95). Assignments of the carbon resonances were confirmed by DEPT, HSQC, and HMBC techniques (Figure 2). On the basis of the above data, the structure of **2** was elucidated as *N*-(4-methoxyphenethyl)-*N*-methylbenzamide, which was confirmed by the $^1H-^1H$ COSY and NOESY experiments (Figure 2).

Compound **3** was isolated as a colorless oil with molecular formula $C_{11}H_{17}NO_2$ as determined by positive-ion HRESIMS, showing an $[M + Na]^+$ ion at *m/z* 218.1159 (calcd for $C_{11}H_{17}NO_2Na$, 218.1157). The presence of NH and carbonyl groups in **3** was revealed by the bands at 3292, 1666, and 1627 cm^{-1}, respectively, in the IR spectrum. The ^1H NMR spectrum of **3** showed resonances for an *N*-isobutyl group, an NH group, an acetyl group, and four mutually coupled protons of a 6-oxohepta-2,4-dienamide moiety. The locations of the above groups of **3** were determined by the following NOESY and HMBC correlations. NOESY correlations were observed between H-2′ (δ 1.83) and both H-1′ (δ 3.20) and H-3′ (δ 0.95); between NH (δ 5.64) and both H-1′ (δ 3.20) and H-2 (δ 6.22); between H-2 (δ 6.22) and both NH (δ 5.64) and H-4 (δ 7.15); and between H-5 (δ 6.42) and both H-3 (δ 7.30) and H-7 (δ 2.32). HMBC correlations were observed

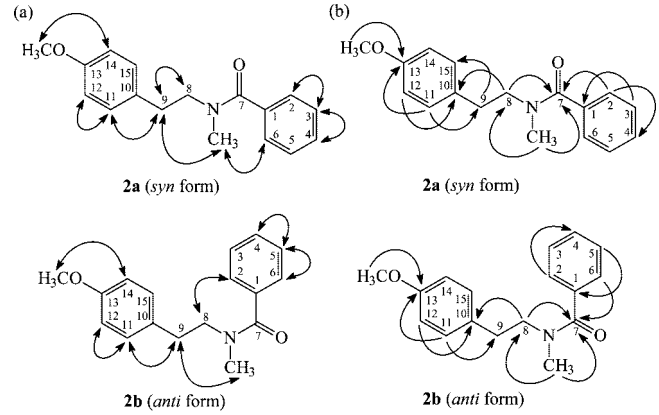

Figure 2. NOESY (a) and HMBC (b) correlations of **2**.

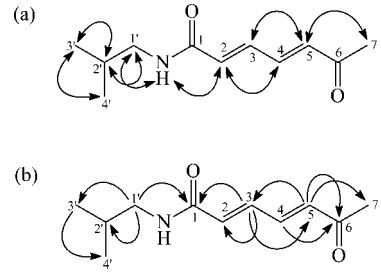

Figure 3. NOESY (a) and HMBC (b) correlations of **3**.

between H-1′ (δ 3.20) and both C-1 (δ 165.3) and C-3′ (δ 20.1); between H-3 (δ 7.30) and C-1 (δ 165.3), C-2 (δ 131.7), and C-5 (δ 135.1); and between H-5 (δ 6.42) and C-3 (δ 137.9), C-6 (δ 198.3), and C-7 (δ 28.1). In addition, the 2*E*,4*E*-configuration of **3** was established by the coupling constants between H-2/H-3 (J = 15.0 Hz) and H-4/H-5 (J = 15.5 Hz) and by the NOESY correlations between H-2/H-4 and H-3/H-5. Thus, the structure of **3** was elucidated as (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide. This structure was confirmed by the $^1H-^1H$ COSY, NOESY, DEPT, HSQC, and HMBC techniques (Figure 3).

Compound **4** had the molecular formula $C_{12}H_{16}O_3$, as indicated by the sodiated HRESIMS ion peak at *m/z* = 231.0996 $[M + Na]^+$ (calcd for $C_{12}H_{16}O_3Na$, 231.0997). IR absorptions for OH (3425 cm^{-1}) and conjugated carbonyl functions (1680 cm^{-1}) were observed. The ^1H NMR spectrum of **4** showed resonances for a 4-hydroxy-3-methylbutoxy group [δ 1.02 (3H, d, J = 6.5 Hz, Me-3′), δ 1.69, 1.98 (each 1H, each m, H-2′), δ 1.94 (1H, m, H-3′), δ

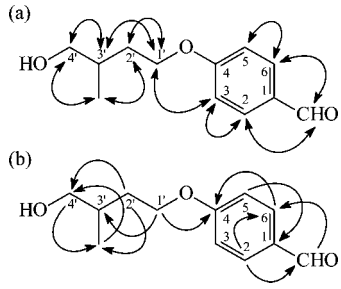

Figure 4. NOESY (a) and HMBC (b) correlations of **4**.

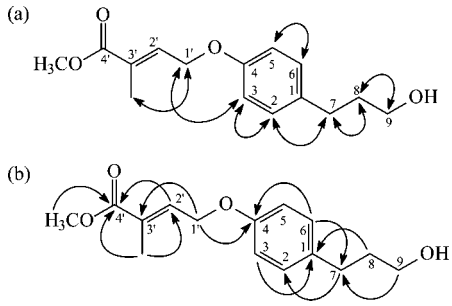

Figure 5. NOESY (a) and HMBC (b) correlations of **5**.

3.57 (2H, d, $J = 5.5$ Hz, H-4′), and δ 4.14 (2H, m, H-1′)], a formyl group [δ 9.89 (1H, s, CHO-1)], and an AA′BB′ spin system [δ 7.00 (2H, d, $J = 9.0$ Hz, H-3 and H-5) and δ 7.84 (2H, d, $J = 9.0$ Hz, H-2 and H-6)]. On the basis of NOESY correlations between H-2 (δ 7.84) and both CHO-1 (δ 9.89) and H-3 (δ 7.00), and between H-3 (δ 7.00) and both H-2 (δ 7.84) and H-1′ (δ 4.14), the 4-hydroxy-3-methylbutoxy group was assigned to C-4. The structure of **4** was thus elucidated as 4-(4′-hydroxy-3′-methylbutoxy)benzaldehyde. This was confirmed by ^{1}H−^{1}H COSY and NOESY experiments (Figure 4). The assignment of ^{13}C NMR resonances was confirmed by DEPT, HSQC, and HMBC techniques.

Compound **5** was isolated as colorless needles having the molecular formula $C_{15}H_{20}O_4$ as deduced from the sodiated ion at *m/z* 287.1254 $[M + Na]^+$ in the HRESI mass spectrum. The presence of OH and carbonyl groups in **5** was revealed by bands at 3394 and 1715 cm^{-1}, respectively, in the IR spectrum. Comparison of the ^{1}H NMR data of **5** with that of cuspidiol[17] suggested that their structures were closely related except that a 3′-methoxycarbonyl group [δ 3.76 (3H, s)] of **5** replaced the 3′-hydroxymethyl group of cuspidiol.[17] This was supported by HMBC correlations observed between OMe-4′ (δ 3.76) and C-4′ (δ 167.8) and between H-2′ (δ 6.93) and C-4′ (δ 167.8). NOESY correlations of **5** were observed between H-1′ (δ 4.70) and Me-3′ (δ 1.93). Thus, the *E*-configuration of **5** was established. According to the above data, the structure of **5** was elucidated as (*E*)-methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate, which was further supported by the ^{1}H−^{1}H COSY, NOESY, DEPT, HSQC, and HMBC experiments (Figure 5). This is the first isolation of **5** from a natural source, although it has been synthesized by Ishii et al.[18]

The known isolates were readily identified by a comparison of physical and spectroscopic data (UV, IR, ^{1}H NMR, $[\alpha]_D$, and MS) with corresponding authentic samples or literature values. The known compounds were identified as xanthyletin,[7] bergapten,[19] aesculetin dimethyl ether,[20] 6,7,8-trimethoxycoumarin,[21] decarine,[22] norchelerythrine,[22] γ-fagarine,[23] (+)-episesamin,[24] (−)-hinokinin,[25] methyl (*E*)-*p*-hydroxycinnamate,[26] *trans*-*p*-coumarylaldehyde,[27] methyl 4-hydroxybenzoate,[28] ailanthoidiol,[29] evofolin-B,[30] syringaldehyde,[31] 4-hydroxybenzaldehyde,[32] and β-amyrin.[33]

Human neutrophils are known to play significant roles in host defense against microorganisms and in pathogenesis of various diseases such as rheumatoid arthritis, chronic obstructive pulmonary disease (COPD), ischemia-reperfusion injury, and asthma.[34−38] In response to different stimuli, activated neutrophils secrete a series of cytotoxins, such as the superoxide anion radical ($O_2^{\bullet-}$), a precursor to other reactive oxygen species (ROS), granule proteases, bioactive lipids, and neutrophil elastase, a major contributor to destruction of tissue in chronic inflammatory disease.[35,39−41] Suppression of the extensive or inappropriate activation of neutrophils by drugs has been proposed as a way to ameliorate inflammatory diseases. The effects on neutrophil pro-inflammatory responses of compounds isolated from the stem bark of *Z. ailanthoides* 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 inhibitory activity data on neutrophil pro-inflammatory responses 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 biological tests, the following conclusions can be drawn: (a) Ailanthamide (**1**), (2*E*,4*E*)-*N*-isobutyl-6-oxohepta-2,4-dienamide (**3**), xanthyletin, decarine, (+)-episesamin, (−)-hinokinin, and evofolin-B exhibited potent inhibitory activities ($IC_{50} \leq 5.34$ μg/mL) on human neutrophil $O_2^{\bullet-}$ generation. Among them, (−)-hinokinin was the most effective, with an IC_{50} value of 0.60 ± 0.12 μg/mL against fMLP-induced superoxide anion generation. (b) Compounds **1**, xanthyletin, decarine, and episesamin inhibited fMLP/CB-induced elastase release with IC_{50} values ≤ 5.53 μg/mL. Among them, (+)-episesamin exhibited the most effective inhibition, with an IC_{50} value of 1.38 ± 0.17 μg/mL against fMLP-induced elastase release. (c) Ailanthamide (**1**), with an *N*-(*E*)-3-(4-hydroxyphenyl)acryloyl group, exhibited more effective inhibition than its analogue, *N*-(4-methoxyphenethyl)-*N*-methylbenzamide (**2**), with an *N*-benzoyl substituent, against fMLP-induced $O_2^{\bullet-}$ generation and elastase release. (d) Among the coumarin analogues, xanthyletin, with a 2,2-dimethyl-3,4-dehydroxypyran moiety at C-6,7, exhibited more effective inhibition than analogues bergapten, aesculetin dimethyl ether, and 6,7,8-trimethoxycoumarin against fMLP-induced $O_2^{\bullet-}$ generation and elastase release. (e) Decarine, with an 8-hydroxy group, showed strong inhibition against fMLP-induced superoxide production, but its analogue, norchelerythrine, with a 8-methoxy group, was inactive.

Table 1. Inhibitory Effects of Compounds Isolated from Stem Bark of *Z. ailanthoides* on Superoxide Radical Anion Generation and Elastase Release by Human Neutrophils in Response to fMet-Leu-Phe/Cytochalasin B[a]

	IC_{50} (μg/mL)[b] or (Inh %)[c]	
compound	superoxide anion	elastase
1	3.71 ± 0.06***	4.23 ± 1.01**
2	8.49 ± 0.31***	7.48 ± 0.58***
3	4.20 ± 0.72**	6.20 ± 0.72***
xanthyletin	4.10 ± 0.33***	5.53 ± 0.23***
aesculetin dimethyl ether	8.25 ± 1.52***	(43.1 ± 5.2)***
decarine	1.29 ± 0.19***	1.94 ± 0.30***
γ-fagarine	7.17 ± 0.44***	(19.1 ± 3.9)**
(+)-episesamin	4.30 ± 0.59***	1.38 ± 0.17***
(−)-hinokinin	0.60 ± 0.12***	(24.7 ± 6.2)*
evofolin-B	5.34 ± 0.41***	6.15 ± 1.10***
diphenyleneiodonium	0.54 ± 0.21***	
phenylmethylsulfonyl fluoride		35.24 ± 5.62***

[a] Diphenyleneiodonium and phenylmethylsulfonyl fluoride were used as positive controls. 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] Percentage of inhibition (Inh %) at 10 μg/mL.

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) was used for TLC and preparative TLC.

Plant Material. The stem bark of *Z. ailanthoides* was collected from Taitung District Agricultural Research and Extension Station, Taitung County, Taiwan, in June 2007 and identified by one of the authors (J.F.C.). A voucher specimen (Chen 5645) was deposited in the herbarium of the Faculty of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Separation. The dried stem bark of *Z. ailanthoides* (7.1 kg) was pulverized and extracted three times with MeOH (20 L each) for 3 days. The MeOH extracts were concentrated under reduced pressure at 35 °C, and the residue (730 g) was partitioned between EtOAc and H_2O (1:1). The EtOAc layer was concentrated to give a residue (fraction A, 125 g). The water layer was further extracted with *n*-BuOH, and the *n*-BuOH-soluble part (fraction B, 230 g) and the water-solubles (fraction C, 365 g) were separated. Fraction A (125 g) was chromatographed on silica gel (70−230 mesh, 4.9 kg), eluting with CH_2Cl_2, gradually increasing the polarity with MeOH to give 13 fractions: A1 (8 L, CH_2Cl_2), A2 (5.5 L, CH_2Cl_2/MeOH, 98:1), A3 (5 L, CH_2Cl_2/MeOH, 95:1), A4 (6 L, CH_2Cl_2/MeOH, 90:1), A5 (6 L, CH_2Cl_2/MeOH, 80:1), A6 (4 L, CH_2Cl_2/MeOH, 70:1), A7 (7 L, CH_2Cl_2/MeOH, 50:1), A8 (7 L, CH_2Cl_2/MeOH, 30:1), A9 (7 L, CH_2Cl_2/MeOH, 10:1), A10 (6 L, CH_2Cl_2/MeOH, 5:1), A11 (6 L, CH_2Cl_2/MeOH, 3:1), A12 (5.5 L, CH_2Cl_2/MeOH, 1:1), A13 (5 L, MeOH). Fraction A1 (10.5 g) was chromatographed further on silica gel (70−230 mesh, 410 g) eluting with *n*-hexane/acetone (8:1) to give 14 fractions (each 1.0 L, A1-1−A1-14). Fraction A1-5 (850 mg) was washed with MeOH and filtered to yield β-amyrin (236 mg) after recrystallization (MeOH). Fraction A1-6 (185 mg) was separated by preparative TLC (PTLC) (silica gel, *n*-hexane/EtOAc, 2:1) to afford norchelerythrine (3.7 mg). Fraction A1-8 (230 mg) was separated by PTLC (silica gel, *n*-hexane/EtOAc, 2:1) to yield xanthyletin (5.7 mg), bergapten (6.4 mg), and (+)-episesamin (16.8 mg). Fraction A1-11 (200 mg) was subjected to PTLC (silica gel, *n*-hexane/acetone, 2:1) to obtain (−)-hinokinin (21.5 mg). Fraction A2 (9.8 g) was chromatographed on silica gel (70−230 mesh, 350 g) eluting with CH_2Cl_2/acetone (20:1) to give 12 fractions (each 1.0 L, A2-1−A2-12). Fraction A2-5 (780 mg) was purified by MPLC (33 g silica gel, 230−400 mesh, CH_2Cl_2/MeOH, 20:1, 200 mL fractions) to obtain 11 subfractions: A2-5-1−A2-5-11. Fraction A2-5-4 (82 mg) was purified further by PTLC (silica gel, CH_2Cl_2/acetone, 30:1) to give 6,7,8-trimethoxycoumarin (12 mg). Fraction A2-5-5 (90 mg) was purified further by PTLC (silica gel, *n*-hexane/EtOAc, 2:1) to afford syringaldehyde (3.7 mg). Fraction A2-5-6 (98 mg) was washed with *n*-hexane and filtered to yield aesculetin dimethyl ether (5.3 mg) after recrystallization (CH_2Cl_2/MeOH). Fraction A3 (9.4 g) was chromatographed on silica gel (70−230 mesh, 385 g) eluting with CH_2Cl_2/MeOH (40:1) to afford 12 fractions (each 1.3 L, A3-1−A3-12). Fraction A3-5 (1.1 g) was separated by MPLC (38 g silica gel, 230−400 mesh, *n*-hexane/EtOAc, 2:1, 250 mL fractions) to obtain nine subfractions: A3-5-1−A3-5-9. Fraction A3-5-3 (165 mg) was purified by PTLC (silica gel, CH_2Cl_2/acetone, 20:1) to yield methyl 4-hydroxybenzoate (5.8 mg). Fraction A3-5-5 (155 mg) was fractionated by PTLC (silica gel, CH_2Cl_2/acetone, 20:1) to obtain methyl (*E*)-*p*-hydroxycinnamate (3.5 mg). Fraction A4 (9.5 g) was chromatographed on silica gel (70−230 mesh, 390 g) eluting with *n*-hexane/EtOAc (1:1) to afford 12 fractions (each 500 mL, A4-1−A4-12). Fraction A4-3 (840 mg) was purified by MPLC (37 g silica gel, 230−400 mesh, CH_2Cl_2/MeOH, 40:1, 500 mL fractions) to obtain 11 subfractions: A4-3-1−A4-3-11. Fraction A4-3-4 (120 mg) was subjected to PTLC (silica gel, $CHCl_3$/MeOH, 30:1) to obtain decarine (3.5 mg). Fraction A4-3-5 (110 mg) was purified by PTLC (silica gel, *n*-hexane/EtOAc, 1:1) to yield **2** (2.5 mg). Fraction A4-3-6 (95 mg) was purified further by PTLC (silica gel, *n*-hexane/EtOAc, 1:1) to obtain γ-fagarine (6.3 mg). Fraction A4-3-7 (115 mg) was separated by PTLC (silica gel, $CHCl_3$/acetone, 10:1) to afford **5** (2.3 mg). Fraction A4-3-8 (98 mg) was purified further by PTLC (silica gel, $CHCl_3$/acetone, 10:1) to give **4** (2.3 mg). Fraction A4-4 (160 mg) was subjected to PTLC (silica gel, $CHCl_3$) to yield *trans*-*p*-coumarylaldehyde (3.6 mg) and 4-hydroxybenzaldehyde (3.2 mg). Fraction A4-5 (190 mg) was subjected to PTLC (silica gel, *n*-hexane/acetone, 3:2) to yield **3** (2.4 mg). Fraction A5 (4.6 g) was chromatographed on silica gel (70−230 mesh, 205 g) eluting with *n*-hexane/acetone (5:1) to afford 15 fractions (each 600 mL, A5-1−A5-15). Fraction A5-9 (175 mg) was fractionated further by PTLC (silica gel, $CHCl_3$/MeOH, 15:1) to give evofolin-B (3.2 mg). Fraction A7 (7.6 g) was chromatographed on silica gel (70−230 mesh, 315 g) eluting with $CHCl_3$/EtOAc (5:1) to afford 13 fractions (each 900 mL, A7-1−A7-13). Fraction A7-6 (195 mg) was separated by PTLC (silica gel, *n*-hexane/acetone, 3:2) to give **1** (2.8 mg). Fraction A7-8 (180 mg) was separated by PTLC (silica gel, *n*-hexane/acetone, 1:1) to give ailanthoidiol (5.7 mg).

Biological Assay. The effects of the isolated compounds on neutrophil pro-inflammatory responses 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.[42] Purified neutrophils containing >98% viable cells, as determined by the trypan blue exclusion method,[43] were resuspended in a Ca^{2+}-free HBSS buffer at pH 7.4 and were maintained at 4 °C prior to use.

Measurement of $O_2^{\bullet-}$ Generation. Measurement of $O_2^{\bullet-}$ generation was based on the SOD-inhibitable reduction of ferricytochrome *c*.[44,45] In brief, after supplementation with 0.5 mg/mL ferricytochrome *c* and 1 mM Ca^{2+}, neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with different concentrations of compounds or DMSO (as control) for 5 min. Cells were activated with 100 nM fMLP for 10 min. When fMLP was used as a stimulant, CB (1 μg/mL) was incubated for 3 min before activation by peptide (fMLP/CB). 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.[45] 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 compounds for 5 min. Cells were stimulated with fMLP (100 nM)/cytochalasin B (0.5 μg/mL), and changes in absorbance at 405 nm were monitored continuously in order to assay elastase release. The results were expressed as the percent 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. The software SigmaPlot was used for the statistical analysis.

Ailanthamide (1): colorless oil; UV (MeOH) λ_{max} (log ϵ) 225 (4.09), 294 (4.13), 313 (4.15) nm; IR (neat) ν_{max} 3345 (OH), 1650 (C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) of **1a** (*syn* form) δ 2.85 (2H, t, J = 7.4 Hz, H-11), 3.03 (3H, s, N-Me), 3.63 (2H, t, J = 7.4 Hz, H-10), 3.79 (3H, s, OMe-15), 5.32 (1H, br s, D_2O exchangeable, OH-4), 6.69 (1H, d, J = 15.6 Hz, H-8), 6.82 (2H, d, J = 8.8 Hz, H-3 and H-5), 6.84 (2H, d, J = 8.4 Hz, H-14 and H-16), 7.17 (2H, d, J = 8.4 Hz, H-13 and H-17), 7.43 (2H, d, J = 8.8 Hz, H-2 and H-6), 7.63 (1H, d, J = 15.6 Hz, H-7); **1b** (*anti* form) δ 2.85 (2H, t, J = 7.4 Hz, H-11), 3.03 (3H, s, N-Me), 3.65 (2H, t, J = 7.4 Hz, H-10), 3.72 (3H, s, OMe-15), 5.32 (1H, br s, D_2O exchangeable, OH-4), 6.38 (1H, d, J = 15.6 Hz, H-8), 6.75 (2H, d, J = 8.8 Hz, H-3 and H-5), 6.84 (2H, d, J = 8.4 Hz, H-14 and H-16), 7.09 (2H, d, J = 8.4 Hz, H-13 and H-17), 7.31 (2H, d, J = 8.8 Hz, H-2 and H-6), 7.46 (1H, d, J = 15.6 Hz, H-7); ^{13}C NMR ($CDCl_3$, 100 MHz) of **1a** (*syn* form) δ 33.9 (C-11), 36.2 (N-Me), 50.7 (C-10), 55.1 (MeO-15), 113.9 (C-14), 113.9 (C-16), 115.7 (C-3), 115.7 (C-5), 118.2 (C-8), 127.6 (C-1), 129.7 (C-13), 129.7 (C-17), 130.1 (C-2), 130.1 (C-6), 131.2 (C-12), 141.8 (C-7), 157.8 (C-4), 158.0 (C-15), 166.7 (C-9); **1b** (*anti* form) δ 34.3 (C-11), 34.3 (N-Me),

52.0 (C-10), 55.1 (MeO-15), 113.9 (C-14), 113.9 (C-16), 115.5 (C-3), 115.5 (C-5), 118.1 (C-8), 127.3 (C-1), 129.7 (C-13), 129.7 (C-17), 130.0 (C-2), 130.0 (C-6), 130.2 (C-12), 141.6 (C-7), 157.7 (C-4), 158.4 (C-15), 166.3 (C-9); ESIMS *m/z* 334 $[M + Na]^+$; HRESIMS *m/z* 334.1421 $[M + Na]^+$ (calcd for $C_{19}H_{21}NO_3Na$, 334.1419).

***N*-(4-Methoxyphenethyl)-*N*-methylbenzamide (2):** yellowish, amorphous powder; UV (MeOH) λ_{max} (log ϵ) 224 (4.29), 274 (3.43), 283 (3.32) nm; IR (neat) ν_{max} 1628 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) of **2a** (*syn* form) δ 2.74 (2H, t, $J = 7.6$ Hz, H-9), 3.14 (3H, s, N-Me), 3.43 (2H, t, $J = 7.6$ Hz, H-8), 3.78 (3H, s, OMe-13), 6.87 (2H, d, $J = 8.8$ Hz, H-12 and H-14), 7.22 (2H, d, $J = 8.8$ Hz, H-11 and H-15), 7.33 (2H, m, H-2 and H-6), 7.35 (2H, m, H-3 and H-5), 7.36 (1H, m, H-4); **2b** (*anti* form) δ 2.82 (3H, s, N-Me), 2.95 (2H, t, $J = 7.2$ Hz, H-9), 3.74 (2H, t, $J = 7.2$ Hz, H-8), 3.80 (3H, s, OMe-13), 6.78 (2H, d, $J = 8.4$ Hz, H-12 and H-14), 6.86 (2H, d, $J = 8.4$ Hz, H-11 and H-15), 7.14 (2H, d, $J = 7.6$ Hz, H-2 and H-6), 7.34 (2H, m, H-3 and H-5), 7.36 (1H, m, H-4); ^{13}C NMR ($CDCl_3$, 100 MHz) of **2a** δ 33.1 (N-Me), 33.9 (C-9), 53.1 (C-8), 55.3 (OMe-13), 113.9 (C-12), 113.9 (C-14), 126.9 (C-2), 126.9 (C-6), 128.3 (C-3), 128.3 (C-5), 129.4 (C-4), 129.8 (C-11), 129.8 (C-15), 131.5 (C-10), 136.9 (C-1), 158.1 (C-13), 172.2 (C-7); **2b** δ 32.5 (C-9), 38.3 (N-Me), 49.5 (C-8), 55.3 (OMe-13), 114.0 (C-12), 114.0 (C-14), 126.4 (C-2), 126.4 (C-6), 128.3 (C-3), 128.3 (C-5), 129.2 (C-4), 129.7 (C-11), 129.7 (C-15), 131.2 (C-10), 136.6 (C-1), 158.0 (C-13), 171.3 (C-7); ESIMS *m/z* 292 $[M + Na]^+$; HRESIMS *m/z* 292.1314 $[M + Na]^+$ (calcd for $C_{17}H_{19}NO_2Na$, 292.1313).

(2*E*,4*E*)-*N*-Isobutyl-6-oxohepta-2,4-dienamide (3): colorless oil; UV (MeOH) λ_{max} (log ϵ) 272 (4.30) nm; IR (neat) ν_{max} 3292 (NH), 1666 (C═O), 1627 (C═O); ^1H NMR ($CDCl_3$, 500 MHz) δ 0.95 (6H, d, $J = 6.5$ Hz, H-3′ and H-4′), 1.83 (1H, m, H-2′), 2.32 (3H, s, H-7), 3.20 (2H, t, $J = 6.5$ Hz, H-1′), 5.64 (1H, br s, D_2O exchangeable, NH), 6.22 (1H, d, $J = 15.0$ Hz, H-2), 6.42 (1H, d, $J = 15.5$ Hz, H-5), 7.15 (1H, dd, $J = 15.5$, 11.5 Hz, H-4), 7.30 (1H, d, $J = 15.0$, 11.5 Hz, H-3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.1 (C-3′), 20.1 (C-4′), 28.1 (C-7), 28.6 (C-2′), 47.1 (C-1′), 131.7 (C-2), 135.1 (C-5), 137.9 (C-3), 139.4 (C-4), 165.3 (C-1), 198.3 (C-6); ESIMS *m/z* 218 $[M + Na]^+$; HRESIMS *m/z* 218.1159 $[M + Na]^+$ (calcd for $C_{11}H_{17}NO_2Na$, calcd 218.1157).

4-(4′-Hydroxy-3′-methylbutoxy)benzaldehyde (4): colorless, amorphous powder; $[\alpha]^{25}_D$ −34 (*c* 0.11, $CHCl_3$). UV (MeOH) λ_{max} (log ϵ) 283 (4.13) nm; IR (neat) ν_{max} 3425 (OH), 1680 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.02 (3H, d, $J = 6.5$ Hz, Me-3′), 1.69 (1H, m, H-2′), 1.94 (1H, m, H-3′), 1.98 (1H, m, H-2′), 3.57 (2H, d, $J = 5.5$ Hz, H-4′), 4.14 (2H, m, H-1′), 7.00 (2H, d, $J = 9.0$ Hz, H-3 and H-5), 7.84 (2H, d, $J = 9.0$ Hz, H-2 and H-6), 9.89 (1H, s, CHO-1); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 16.7 (Me-3′), 32.5 (C-2′), 33.0 (C-3′), 66.5 (C-1′), 67.9 (C-4′), 114.7 (C-3), 114.7 (C-5), 129.9 (C-1), 132.0 (C-2), 132.0 (C-6), 163.9 (C-4), 190.8 (CHO); ESIMS *m/z* 231 $[M + Na]^+$; HRESIMS *m/z* 231.0996 $[M + Na]^+$ (calcd for $C_{12}H_{16}O_3Na$, 231.0997).

(*E*)-Methyl 4-[4-(3-hydroxypropyl)phenoxy]-2-methylbut-2-enoate (5): colorless needles ($CHCl_3$/MeOH); mp 47−49 °C; UV (MeOH) λ_{max} (log ϵ) 223 (4.35), 285 (3.99), 308 (4.02) nm; IR (neat) ν_{max} 3394 (OH), 1715 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.87 (2H, tt, $J = 7.6$, 6.4 Hz, H-8), 1.93 (3H, s, Me-3′), 2.66 (2H, t, $J = 7.6$ Hz, H-7), 3.67 (2H, t, $J = 6.4$ Hz, H-9), 3.76 (3H, s, OMe-4′), 4.70 (2H, d, $J = 5.6$ Hz, H-1′), 6.82 (2H, d, $J = 8.8$ Hz, H-3 and H-5), 6.93 (1H, br t, $J = 5.6$ Hz, H-2′), 7.12 (2H, d, $J = 8.8$ Hz, H-2 and H-6); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.0 (Me-3′), 31.2 (C-7), 34.4 (C-8), 51.5 (OMe), 62.3 (C-9), 64.9 (C-1′), 114.6 (C-3), 114.6 (C-5), 129.4 (C-2), 129.4 (C-6), 129.6 (C-3′), 134.5 (C-1), 137.0 (C-2′), 156.6 (C-4), 167.8 (C-4′); ESIMS *m/z* 287 $[M + Na]^+$; HRESIMS *m/z* 287.1254 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

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