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New Anti-Inflammatory Aporphine and Lignan Derivatives from the Root Wood of *Hernandia nymphaeifolia*

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Received: 15 August 2018; Accepted: 1 September 2018; Published: 7 September 2018

Abstract: A new aporphine, 3-hydroxyhernandonine (**1**) and a new lignin, 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), have been isolated from the root wood of *Hernanadia nymphaeifolia*, together with thirteen known compounds (**3**–**15**). The structures of these compounds were determined through mass spectrometry (MS) and spectroscopic analyses. The known isolate, 2-*O*-methyl-7-oxolaetine (**3**), was first isolated from natural sources. Among the isolated compounds, 3-hydroxyhernandonine (**1**), 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), hernandonine (**4**), oxohernangerine (**5**), and oxohernagine (**6**) displayed inhibition (IC_{50} values $\leq$5.72 μg/mL) of superoxide anion production by human neutrophils in response to formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin B (fMLP/CB). In addition, 3-hydroxyhernandonine (**1**), 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), oxohernangerine (**5**), and oxohernagine (**6**) suppressed fMLP/CB-induced elastase release with IC_{50} values $\leq$5.40 μg/mL.

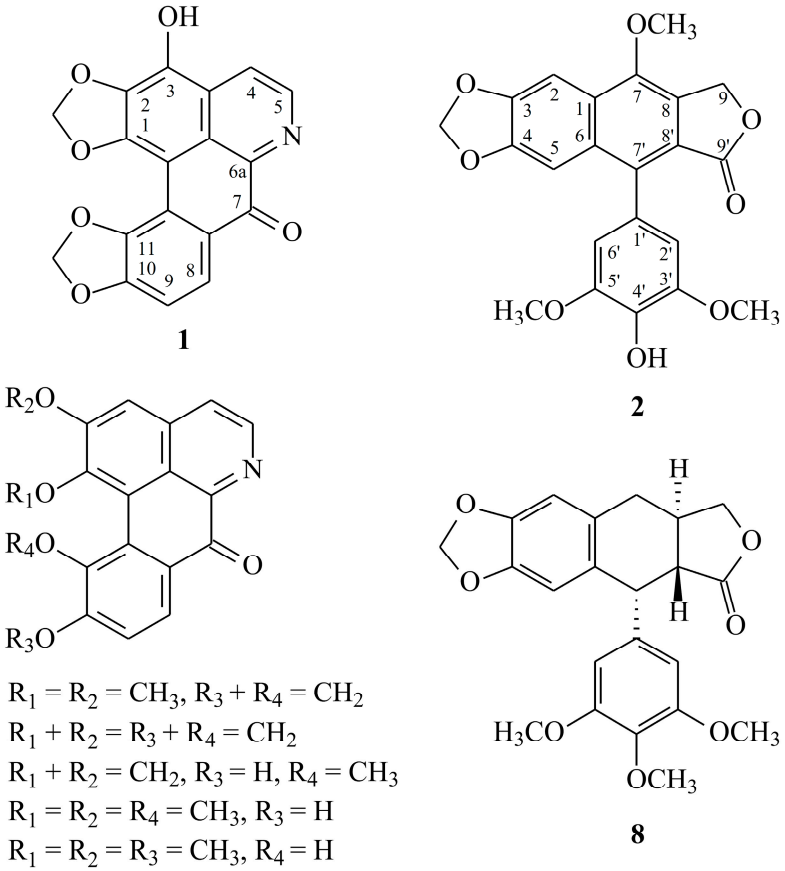
Keywords: *Hernanadia nymphaeifolia*; Hernandiaceae; root wood; structure elucidation; aporphine; lignan; anti-inflammatory activity

1. Introduction

Hernanadia nymphaeifolia (Presl) Kubitzki (Hernandiaceae) is an evergreen tree that is distributed in the tropical island shores of the Indian and western Pacific Oceans [1]. Its seed is used as a cathartic [2]. Various aporphines [3–7], isoquinolones [4,5], lignans [4,7,8], benzylisoquinoline [5], steroids [7], and their derivatives were isolated from this species in past studies. Many of these isolates display cytotoxic [4,5,8], vasorelaxing [6], antioxidant [6], and antiplatelet aggregation [7] activities.

The extensive or inappropriate activation of neutrophils leads to many inflammatory disorders such as chronic obstructive pulmonary disease (COPD), ischemia-reperfusion injury, asthma, rheumatoid arthritis, and metabolic diseases [9,10]. In response to various stimuli, activated neutrophils secrete a series of cytotoxins, such as granule proteases, bioactive lipids, and superoxide anion ($O_2^{\bullet-}$), a precursor of other reactive oxygen species (ROS) [10–12]. The inhibition of the abnormal activation of neutrophils by medicines has been recommended as a way to improve inflammatory diseases. In our researches on the anti-inflammatory constituents of Formosan plants, numerous species have been screened for anti-inflammatory activity, and *H. nymphaeifolia* has been found to be an active species. A new aporphine, 3-hydroxyhernandonine (**1**), a new lignin, 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), and thirteen known compounds (**3**–**15**) have been isolated and determined from the root wood of *Hernanadia nymphaeifolia*, and their structures are described in Figure 1.

This article describes the structural elucidation of new compounds **1** and **2** and the inhibitory effects of all isolates on elastase release and superoxide generation by human neutrophils.



3 $R_1 = R_2 = CH_3$, $R_3 + R_4 = CH_2$
4 $R_1 + R_2 = R_3 + R_4 = CH_2$
5 $R_1 + R_2 = CH_2$, $R_3 = H$, $R_4 = CH_3$
6 $R_1 = R_2 = R_4 = CH_3$, $R_3 = H$
7 $R_1 = R_2 = R_3 = CH_3$, $R_4 = H$

Figure 1. *Cont.*

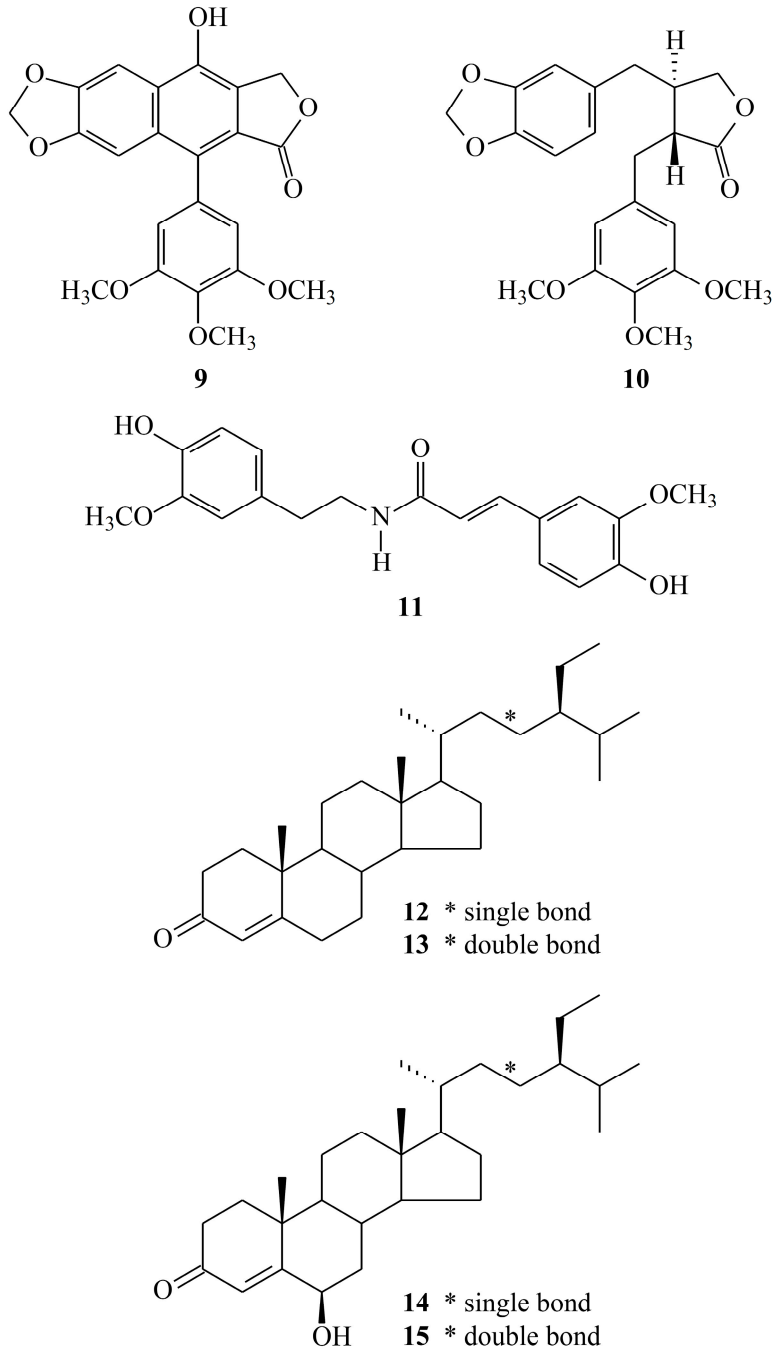


Figure 1. The chemical structures of compounds **1–15** isolated from *H. nymphaeifolia*.

2. Results and Discussion

2.1. Isolation and Structural Elucidation

Chromatographic purification of the CH_2Cl_2-soluble fraction of a MeOH extract of root wood of *H. nymphaeifolia* through a silica gel column, medium pressure liquid chromatography (MPLC), and preparative thin-layer chromatography (TLC) yielded two new (**1** and **2**) and thirteen known compounds (**3–15**) (Figure 1).

The aporphine, 3-hydroxyhernandonine (**1**), was obtained as yellow needles. The electrospray ionization mass spectrometry (ESI-MS) (Figure S1) afford the quasi-molecular ion $[M + Na]^+$ at m/z 358, implying a molecular formula of $C_{18}H_9NO_6Na$, which was confirmed by the high-resolution

(HR)-ESI-MS (*m*/*z* 358.0325 [M + Na]$^+$, calcd 358.0328) (− 0.84 ppm) (Figure S2) and by the ^{13}C-, ^{1}H-, and distortionless enhancement by polarization transfer (DEPT) NMR data. IR absorptions for OH (3439 cm^{-1}) and conjugated carbonyl (1646 cm^{-1}) functions were observed. The ^{1}H-NMR spectrum (Figure S3) of **1** showed the presence of a hydroxy group at δ_H 6.52 (1H, s, D_2O exchangeable, OH-3), two methylenedioxy groups at δ_H 6.20 (2H, s, OCH_2O-10,11) and 6.28 (2H, s, OCH_2O-1,2), and two pairs of AB-doublets at δ_H 7.08 (1H, d, *J* = 8.5 Hz, H-9), 8.10 (1H, d, *J* = 5.0 Hz, H-4), 8.28 (1H, d, *J* = 8.5 Hz, H-8), and 8.88 (1H, d, *J* = 5.0 Hz, H-5). The ^{1}H- and ^{13}C-NMR (Figure S4) data of **1** were similar to those of hernandonine [13,14], except that the 3-hydroxy group [δ_H 6.52 (1H, s, D_2O exchangeable)] of **1** replaced H-3 of hernandonine [13,14]. This was supported by HMBC correlations between OH-3 (δ_H 6.52) and C-2 (δ_C 139.2), as well as between C-3 (δ_C 148.4), and C-3a (δ_C 123.9). The full assignment of ^{1}H- and ^{13}C-NMR resonances was supported by DEPT, ^{1}H–^{1}H COSY (Figure S5), NOESY (Figure 2 and Figure S6), HMBC (Figure 2 and Figure S7), and HSQC (Figure S8) spectral analyses. Based on the above data, the structure of **1** was revealed as 3-hydroxyhernandonine.

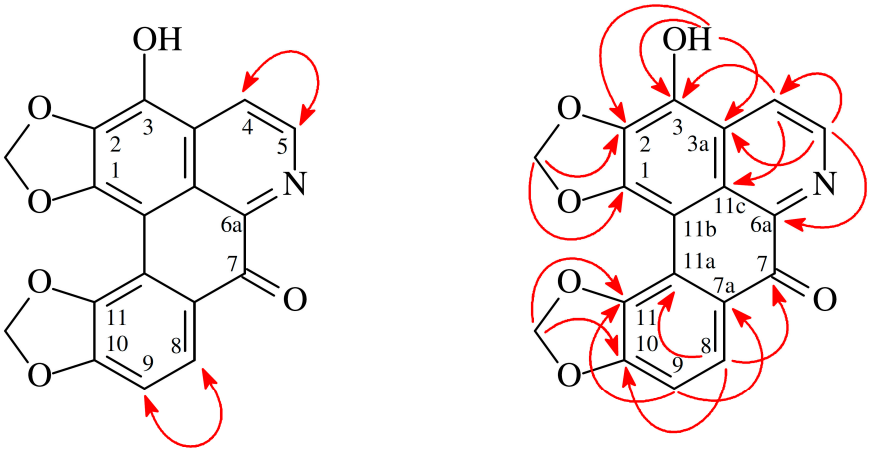


Figure 2. Key NOESY (↔) and HMBC (⇢) correlations of **1**.

4′-*O*-Demethyl-7-*O*-methyldehydropodophyllotoxin (**2**) was isolated as colorless needles. The ESI-MS (Figure S9) display the sodium adduct ion [M + Na]$^+$ at *m*/*z* 433, hinting a molecular formula of $C_{22}H_{18}O_8$, which was supported by the HR-ESI-MS (*m*/*z* 433.0898 [M + Na]$^+$, calcd 433.0899) (– 0.23 ppm) (Figure S10). The IR spectrum showed the presence of OH (3452 cm^{-1}) and γ-lactone carbonyl (1764 cm^{-1}) groups. The ^{1}H-NMR spectrum (Figure S11) of **2** showed the presence of three methoxy groups at δ_H 3.88 (6H, s, OMe-3′ and OMe-5′) and 4.09 (3H, s, OMe-7), a hydroxyl group at δ_H 5.65 (1H, br s, D_2O exchangeable, OH-4′), a methylenedioxy group at δ_H 6.09 (2H, s, OCH_2O), a γ-lactone methylene proton at 5.52 (2H, s, H-9), and four aromatic protons at δ_H 6.57 (2H, s, H-2′ and H-6′), 7.07 (1H, s, H-5), and 7.57 (1H, s, H-2). The ^{1}H- and ^{13}C-NMR (Figure S12) data of **2** were similar to those of 4′-*O*-demethyldehydropodophyllotoxin [15], except that the 7-methoxy groups [δ_H 4.09 (3H, s); δ_C 59.9 (OMe-7)] of **2** replaced the 7-OH group of 4′-*O*-demethyldehydropodophyllotoxin [15]. This was supported by NOESY correlations between OMe-7 (δ_H 4.09) and H-2 (δ_H 7.57) and by HMBC correlations between OMe-7 (δ_H 4.09) and C-7 (δ_C 148.5). According to the above evidence, the structure of **2** was elucidated as 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin. This was further affirmed by the ^{1}H–^{1}H-COSY (Figure S13), NOESY (Figure 3 and Figure S14), DEPT, HMBC (Figure 3 and Figure S15), and HSQC (Figure S16) experiments.

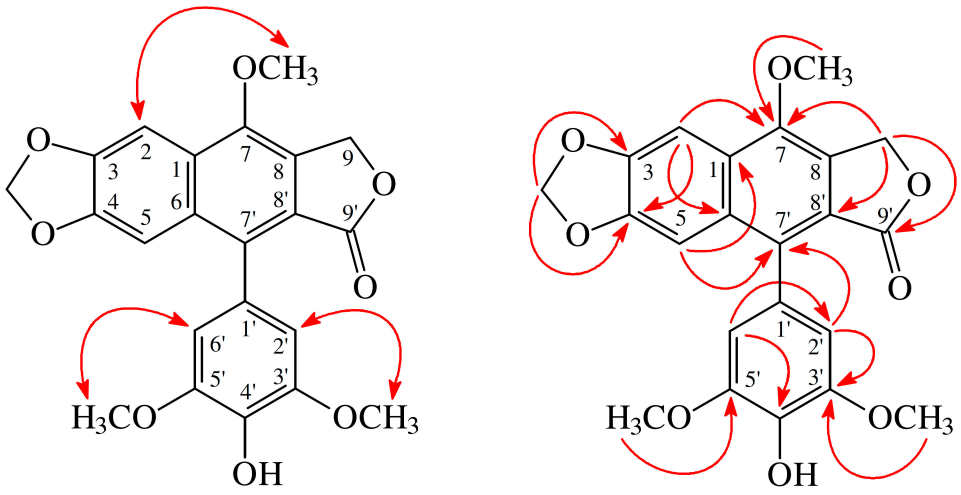


Figure 3. Key NOESY (↔) and HMBC (↷) correlations of **2**.

2.2. Structure Identification of the Known Isolates

The known isolated compounds were readily confirmed by a comparison of spectroscopic and physical data (IR, UV, ^{1}H-NMR, MS, and $[\alpha]_D$) with the literature values or corresponding authentic samples, and this included five aporphines, 2-*O*-methyl-7-oxolaetine (**3**) [16], hernandonine (**4**) [13,14], oxohernangerine (**5**) [17], oxohernagine (**6**) [17], and 7-oxonorisocorydine (**7**) [18], three lignans, (–)-deoxypodophyllotoxin (**8**) [19,20], dehydropodophyllotoxin (**9**) [20,21], (–)-yatein (**10**) [20], an amide, *N-trans*-feruloylmethoxytyramine (**11**) [22], four steroids, a mixture of β-sitostenone (**12**) [23] and stigmasta-4,22-dien-3-one (**13**) [23], and mixture of 6 β-hydroxystigmast-4-en-3-one (**14**) [24,25] and 6 β-hydroxystigmasta-4,22-dien-3-one (**15**) [24,25].

2.3. Biological Studies

Granule proteases (e.g., cathepsin G, elastase, and proteinase-3) and reactive oxygen species (ROS) (e.g., hydrogen peroxide and superoxide anion ($O_2^{\bullet-}$)) generated by human neutrophils are involved in the pathogenesis of various NMR data [10–12,26]. The activities during neutrophil proinflammatory responses to isolates from the root wood of *H. nymphaeifolia* were assessed by inhibiting fMet-Leu-Phe/cytochalasin B (fMLP/CB)-induced $O_2^{\bullet-}$ production and elastase release by human neutrophils. The inhibitory activity data on neutrophil proinflammatory responses are shown in Table 1. Diphenyleneiodonium and phenylmethylsulfonyl fluoride were employed as positive controls for $O_2^{\bullet-}$ generation and elastase release, respectively [26]. From the results of our biological assays, the following conclusions can be summarized: (a) 3-hydroxyhernandonine (**1**), 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), hernandonine (**4**), oxohernangerine (**5**), and oxohernagine (**6**) exhibited potent inhibition ($IC_{50} \leq 5.72$ μg/mL) of superoxide anion ($O_2^{\bullet-}$) generation by human neutrophils in response to fMLP/CB; (b) 3-hydroxyhernandonine (**1**), 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), oxohernangerine (**5**), and oxohernagine (**6**) exhibited potent inhibition ($IC_{50} \leq 5.40$ μg/mL) of fMLP-induced elastase release; (c) the aporphine alkaloid, 3-hydroxyhernandonine (**1**) (with a 3-hydroxy group), exhibited more effective inhibition than its analogue, hernandonine (**4**) (without any substitutant at C-3), against fMLP-induced $O_2^{\bullet-}$ generation and elastase release; (d) oxohernagine (**6**) (with 10-hydroxy and 11-methoxy groups) exhibited more effective inhibition of fMLP-induced $O_2^{\bullet-}$ generation and elastase release than its analogue, 7-oxonorisocorydine (**7**) (with 11-hydroxy and 10-methoxy groups; (e) the lignan compound, 4′-*O*-demethyl-7-*O*-methyldehydroodophyllotoxin (**2**) (with 7-methoxy and 4′-hydroxy groups) exhibited more effective inhibition of fMLP-induced $O_2^{\bullet-}$ generation and elastase release than its analogue, dehydropodophyllotoxin (**9**) (with 7-hydroxy and 4′-methoxy groups); (f) oxohernangerine (**5**) was the most effective among these compounds, with an IC_{50} value of 2.65 ± 0.97 μg/mL, against

fMLP-induced superoxide anion generation; (g) 3-hydroxyhernandonine (**1**) was the most effective among the isolates, with an IC_{50} value of 3.93 ± 0.48 μg/mL against fMLP-induced elastase release.

Table 1. Inhibitory effects of compounds **1**–**15** from the root wood of *H. nymphaeifolia* on superoxide radical anion generation and elastase release by human neutrophils in response to fMet-Leu-Phe/cytochalasin B [a].

Compounds	Superoxide anion	Elastase
	IC_{50} [μg/mL] [b] or (Inh %) [c]	
3-Hydroxyhernandonine (**1**)	4.09 ± 0.44 ***	3.93 ± 0.48 ***
4′-*O*-Demethyl-7-*O*-methyldehydro-podophyllotoxin (**2**)	5.72 ± 0.42 ***	5.40 ± 0.40 ***
2-*O*-Methyl-7-oxolaetine (**3**)	7.37 ± 0.46 ***	6.82 ± 0.09 ***
Hernandonine (**4**)	4.41 ± 0.76 ***	(45.76 ± 6.92) ***
Oxohernangerine (**5**)	2.65 ± 0.97 ***	4.82 ± 0.39 ***
Oxohernagine (**6**)	2.86 ± 0.85 ***	4.87 ± 0.27 ***
7-Oxonorisocorydine (**7**)	6.62 ± 0.28 ***	6.58 ± 0.08 ***
(–)-Deoxypodophyllotoxin (**8**)	(38.95 ± 4.83) **	(33.76 ± 3.82)
Dehydropodophyllotoxin (**9**)	(43.91 ± 3.86) ***	9.53 ± 0.84 ***
(–)-Yatein (**10**)	(42.36 ± 3.41) *	(36.74 ± 3.05) **
N-*trans*-Feruloylmethoxytyramine (**11**)	6.26 ± 0.65 ***	7.03 ± 0.21 ***
Mixture of β-sitostenone (**12**) and stigmasta-4,22-dien-3-one (**13**)	(24.71 ± 2.67)	(29.15 ± 2.89)
Mixture of 6β-hydroxystigmast-4-en-3-one (**14**) and 6β-hydroxystigmasta-4,22-dien-3-one (**15**)	(16.74 ± 2.66) **	7.91 ± 1.20 **
Diphenyleneiodonium [d]	0.55 ± 0.22 ***	–
Phenylmethylsulfonyl fluoride [d]	–	34.5 ± 5.3 ***

[a] Results are displayed as averages ± SEM (n = 4). [b] Concentration necessary for 50% inhibition (IC_{50}). If IC_{50} value of tested compound was <10 μg/mL, it was presented as IC_{50} [μg/mL]. [c] Percentage of inhibition (Inh %) at 10 μg/mL. If IC_{50} value of tested compound was ≥10 μg/mL, it was displayed as (Inh %) at 10 μg/mL. [d] Diphenyleneiodonium and phenylmethylsulfonyl were employed as positive controls for superoxide anion ($O_2^{\bullet-}$) production and elastase release, respectively. * $p < 0.05$ compared with the control. ** $p < 0.01$ compared with the control. *** $p < 0.001$ compared with the control.

3. Experimental Section

3.1. General Procedures

Melting points were determined on a Yanaco micromelting point apparatus and were uncorrected. Ultraviolet (UV) spectra were measured on a Jasco UV-240 spectrophotometer. Optical rotations were acquired using a Jasco DIP-370 polarimeter (Japan Spectroscopic Corporation, Tokyo, Japan) in $CHCl_3$. Infrared (IR) spectra (KBr or neat) were recorded on a Perkin Elmer 2000 FT-IR spectrometer (Perkin Elmer Corporation, Norwalk, CT, USA). Nuclear magnetic resonance (NMR) spectra, including correlation spectroscopy (COSY), nuclear overhauser effect spectrometry (NOESY), heteronuclear multiple-bond correlation (HMBC) experiments, and heteronuclear single-quantum coherence (HSQC), were obtained using a Varian Inova 500 spectrometer (Varian Inc., Palo Alto, CA, USA) operating at 500 MHz (^{1}H) and 125 MHz (^{13}C), respectively, with chemical shifts given in ppm (δ) and applying tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) and high-resolution electrospray ionization (HRESI)-mass spectra were recorded on a VG Platform Electrospray ESI/MS mass spectrometer (Fison, Villeurbanne, France) or a Bruker APEX II (Bruker, Bremen, Germany). Silica gel (70–230, 230–400 mesh, Merck) was used for column chromatography (CC). Silica gel 60 F-254 (Merck, Darmstadt, Germany) was employed for thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC).

3.2. Plant Material

The root wood of *Hernanadia nymphaeifolia* (Presl) Kubitzki (Hernandiaceae) was collected from Mudan Township, Pingtung County, Taiwan, in August 2008 and identified by Prof. I.-S. Chen. A voucher specimen (Chen 5521) was deposited in the Herbarium of School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan.

3.3. Extraction and Isolation

The dried root wood (5.1 kg) of *H. nymphaeifolia* was sliced and extracted three times with MeOH (40 L each) for three days. The extract was concentrated under reduced pressure at 35 °C, and the residue (386 g) was partitioned between CH_2Cl_2 and H_2O (1:1) to provide the CH_2Cl_2-soluble fraction (fraction A; 87 g). Fraction A (87 g) was purified by CC (3.9 kg of SiO_2, 70–230 mesh; CH_2Cl_2/MeOH gradient) to produce 12 fractions: A1–A12. Fraction A3 (7.5 g) was subjected to CC (340 g of SiO_2, 230–400 mesh; CH_2Cl_2/acetone 30:1–0:1, 900 mL fractions) to give 11 subfractions: A3-1–A3-11. Fraction A3-4 (340 mg) was purified by MPLC (silica column, CH_2Cl_2/acetone 8:1–0:1) to produce eight subfractions (each 250 mL, A3-4-1–A3-4-8). Fraction A3-4-4 (46 mg) was purified by preparative TLC (silica gel, $CHCl_3$/MeOH, 10:1) to obtain a mixture of β-sitostenone (**12**) and stigmasta-4,22-dien-3-one (**13**) (8.5 mg). Fraction A5 (6.9 g) was subjected to CC (365 g of SiO_2, 230–400 mesh; CH_2Cl_2/MeOH 15:1–0:1, 950 mL fractions) to form ten subfractions: A5-1–A5-10. Fraction A5-3 (625 mg) was purified by CC (28 g of SiO_2, 230–400 mesh, $CHCl_3$/acetone (7:1–0:1), 250 mL fractions) to give nine subfractions: A5-3-1–A5-3-9. Fraction A5-3-5 (88 mg) was further purified by preparative TLC (SiO_2; CH_2Cl_2/acetone 8:1) to yield a mixture of 6β-hydroxystigmast-4-en-3-one (**14**) and 6β-hydroxystigmasta-4,22-dien-3-one (**15**) (3.7 mg). Fraction A7 (7.3 g) was subjected to CC (330 g of SiO_2, 230–400 mesh; $CHCl_3$/MeOH 10:1–0:1, 800 mL fractions) to give nine subfractions: A7-1–A7-9. A part (142 mg) of fraction A7-2 was further purified by preparative TLC (SiO_2; CH_2Cl_2/MeOH 15:1) to form (–)-deoxypodophyllotoxin (**8**) (7.2 mg). A part (133 mg) of fraction A7-3 was further purified by preparative TLC (SiO_2; $CHCl_3$/MeOH 12:1) to yield (–)-yatein (**10**) (5.1 mg). A part (136 mg) of fraction A7-4 was further purified by preparative TLC (SiO_2; $CHCl_3$/MeOH 9:1) to obtain 2-*O*-methyl-7-oxolaetine (**3**) (5.3 mg). A part (118 mg) of fraction A7-6 was further purified by preparative TLC (SiO_2; CH_2Cl_2/MeOH 5:1) to produce 7-oxonorisocorydine (**7**) (6.5 mg). Fraction A7-7 (650 mg) was purified by MPLC (silica column, CH_2Cl_2/MeOH 9:1–0:1) to form seven subfractions (each 170 ml, A7-7-1–A7-7-7). A part (112 mg) of fraction A7-7-4 was further purified by preparative TLC (SiO_2; $CHCl_3$/acetone 5:1) to form 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**) (5.5 mg). A part (125 mg) of fraction A7-7-5 was purified by preparative TLC (SiO_2; CH_2Cl_2/acetone 4:1) to obtain dehydropodophyllotoxin (**9**) (6.9 mg). A part (122 mg) of fraction A7-8 was purified by preparative TLC (SiO_2; CH_2Cl_2/EtOAc 2:1) to yield *N-trans*-feruloylmethoxytyramine (**11**) (4.9 mg). Fraction A8 (7.2 g) was subjected to CC (325 g of SiO_2, 230–400 mesh; CH_2Cl_2/MeOH 8:1–0:1, 850 mL fractions) to give ten subfractions: A8-1–A8-10. Fraction A8-2 (530 mg) was purified by MPLC (silica column, $CHCl_3$/MeOH 7:1–0:1) to form six subfractions (each 180 ml, A8-2-1–A8-2-6). Fraction A8-2-4 (83 mg) was further purified by preparative TLC (SiO_2; CH_2Cl_2/acetone 6:1) to obtain hernandonine (**4**) (8.2 mg). Fraction A8-5 (135 mg) was further purified by preparative TLC (SiO_2; CH_2Cl_2/MeOH 4:1) to obtain oxohernagine (**6**) (7.1 mg). Fraction A8-6 (135 mg) was further purified by preparative TLC (SiO_2; $CHCl_3$/MeOH 3:1) to yield oxohernangerine (**5**) (6.5 mg). Fraction A9 (6.4 g) was subjected to CC (290 g of SiO_2, 230–400 mesh; $CHCl_3$/MeOH 6:1–0:1, 1 L fractions) to obtain eight subfractions: A9-1–A9-8. A part (142 mg) of fraction A9-3 was purified by preparative TLC (SiO_2; $CHCl_3$/MeOH 5:1) to obtain 3-hydroxyhernandonine (**1**) (4.5 mg). A part (105 mg) of fraction A9-5 was purified by preparative TLC (SiO_2; CH_2Cl_2/MeOH 4:1) to obtain oxohernangerine (**5**) (5.9 mg).

3-Hydroxyhernandonine (**1**): yellow needles; m.p. 268–270 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 220 (3.90), 268 (3.79), 284 (3.78), 343 (3.46), 362 (3.47) nm; IR (KBr): υ_{max} = 3315 (OH), 1652 (C=O), 1060, 969 (OCH_2O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.20 (2H, s, OCH_2O-10,11), 6.28 (2H, s, OCH_2O-1,2),

6.52 (1H, s, D_2O exchangeable, OH-3), 7.08 (1H, d, *J* = 8.5 Hz, H-9), 8.10 (1H, d, *J* = 5.0 Hz, H-4), 8.28 (1H, d, *J* = 8.5 Hz, H-8), 8.88 (1H, d, *J* = 5.0 Hz, H-5); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 101.3 (OCH_2O-1,2), 101.7 (OCH_2O-10,11), 108.6 (C-9), 114.9 (C-11b), 118.7 (C-8), 119.1 (C-4), 122.3 (C-11a), 122.9 (C-11c), 123.9 (C-3a), 127.6 (C-7a), 139.2 (C-2), 145.2 (C-5), 145.8 (C-11), 148.4 (C-3), 149.6 (C-1), 150.4 (C-10), 157.3 (C-6a), 182.5 (C-7); ESI-MS: *m*/*z* = 358 $[M + Na]^+$; HR-ESI-MS: *m*/*z* = 358.0325 $[M + Na]^+$ (calcd for $C_{18}H_9NO_6Na$, 358.0328).

4′-O-Demethyl-7-O-methyldehydropodophyllotoxin (**2**): colorless needles; m.p. 273–275 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 223 (4.47), 262 (4.58), 321 (3.98), 355 (3.69) nm; IR (KBr): υ_{max} = 3452 (OH), 1764 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.88 (6H, s, OMe-3′ and OMe-5′), 4.09 (3H, s, OMe-7), 5.52 (2H, s, H-9), 5.65 (1H, br s, D_2O exchangeable, OH-4′), 6.09 (2H, s, OCH_2O), 6.57 (2H, s, H-2′, and H-6′), 7.07 (1H, s, H-5), 7.57 (1H, s, H-2); ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 56.0 (OMe-3′), 56.0 (OMe-5′), 59.9 (OMe-7), 66.4 (C-9), 98.4 (C-2), 101.8 (OCH_2O), 103.9 (C-5), 107.7 (C-2′), 107.7 (C-6′), 119.5 (C-8′), 125.8 (C-8), 127.8 (C-6), 130.4 (C-1), 132.2 (C-1′), 133.6 (C-4′), 137.7 (C-7′), 148.5 (C-7), 148.9 (C-4), 148.9 (C-3′), 148.9 (C-5′), 150.0 (C-3), 169.3 (C-9′); ESI-MS: *m*/*z* = 433 $[M + Na]^+$; HR-ESI-MS: *m*/*z* = 433.0898 $[M + Na]^+$ (calcd for $C_{22}H_{18}O_8Na$, 433.0899).

2-O-Methyl-7-oxolaetine (**3**): yellow needles; m.p. > 300 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 221 (4.49), 266 (4.33), 362 (4.00), 427 (3.95) nm; IR (KBr): υ_{max} = 1652 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.93 (3H, s, OMe-1), 6.21 (2H, s, OCH_2O-10,11), 7.08 (1H, d, *J* = 8.4 Hz, H-9), 7.21 (1H, s, H-3), 7.77 (1H, d, *J* = 5.2 Hz, H-4), 8.24 (1H, d, *J* = 8.4 Hz, H-8), 8.86 (1H, d, *J* = 5.2 Hz, H-5); ESI-MS: *m*/*z* = 358 $[M + Na]^+$; HR-ESI-MS: *m*/*z* = 358.0692 $[M + Na]^+$ (calcd for $C_{19}H_{13}O_5Na$, 358.0691).

Hernandonine (**4**): yellow needles; m.p. > 350 °C (CH_2Cl_2-MeOH); UV (MeOH): λ_{max} (log ε) = 222 (4.50), 265 (4.34), 295 (sh, 3.90), 312 (sh, 3.63), 364 (4.02), 428 (3.97) nm; IR (KBr): υ_{max} = 1651 (C=O), 1062, 971 (OCH_2O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.20 (2H, s, OCH_2O-10,11), 6.28 (2H, s, OCH_2O-1,2), 7.08 (1H, d, *J* = 8.5 Hz, H-9), 7.21 (1H, s, H-3), 7.74 (1H, d, *J* = 5.0 Hz, H-4), 8.29 (1H, d, *J* = 8.5 Hz, H-8), 8.85 (1H, d, *J* = 5.0 Hz, H-5); ESI-MS: *m*/*z* = 342 $[M + Na]^+$.

Oxohernangerine (**5**): yellow prisms; m.p. 257–258 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 211 (4.55), 252 (4.46), 268 (sh, 4.42), 316 (3.87), 362 (4.04), 408 (4.02), 477 (3.55) nm; IR (KBr): υ_{max} = 3415 (OH), 1642 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.68 (3H, s, OMe-11), 6.32 (2H, s, OCH_2O-1,2), 7.24 (1H, d, *J* = 8.4 Hz, H-9), 7.27 (1H, s, H-3), 7.76 (1H, d, *J* = 5.2 Hz, H-4), 8.37 (1H, d, *J* = 8.4 Hz, H-8), 8.86 (1H, d, *J* = 5.2 Hz, H-5); ESI-MS: *m*/*z* = 344 $[M + Na]^+$.

Oxohernagine (**6**): yellow prisms; m.p. 253–255 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 213 (4.51), 274 (4.41), 361 (3.95), 403 (3.92) nm; IR (KBr): υ_{max} = 3424 (OH), 1650 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.54 (3H, s, OMe-1), 3.76 (3H, s, OMe-11), 4.11 (3H, s, OMe-2), 7.21 (1H, s, H-3), 7.22 (1H, d, *J* = 8.4 Hz, H-9), 7.77 (1H, d, *J* = 5.2 Hz, H-4), 8.31 (1H, d, *J* = 8.4 Hz, H-8), 8.86 (1H, d, *J* = 5.2 Hz, H-5); ESI-MS: *m*/*z* = 360 $[M + Na]^+$.

7-Oxonorisocorydine (**7**): yellow needles; m.p. 250–252 °C (EtOAc); UV (MeOH): λ_{max} (log ε) = 212 (4.49), 273 (4.40), 362 (3.94), 403 (3.90) nm; IR (KBr): υ_{max} = 3385 (OH), 1653 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 3.53 (3H, s, OMe-1), 4.03 (3H, s, OMe-10), 4.08 (3H, s, OMe-2), 7.14 (1H, d, *J* = 8.4 Hz, H-9), 7.23 (1H, s, H-3), 7.77 (1H, d, *J* = 5.2 Hz, H-4), 8.28 (1H, d, *J* = 8.4 Hz, H-8), 8.86 (1H, d, *J* = 5.2 Hz, H-5); ESI-MS: *m*/*z* = 360 $[M + Na]^+$.

(–)-Deoxypodophyllotoxin (**8**): colorless needles; m.p. 168–170 °C (MeOH); UV (MeOH): λ_{max} (log ε) = 212 (4.62), 291 (3.68) nm; IR (KBr): υ_{max} = 1765 (C=O), 1581, 1502, 1474 (aromatic ring C=C stretch), 1032, 941 (OCH_2O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.73 (3H, m, H-7, H-8, and H-8′), 3.07 (1H, m, H-7), 3.75 (6H, s, OMe-3′, and OMe-5′), 3.80 (3H, s, OMe-4′), 3.92 (1H, m, H-9), 4.46 (1H, m, H-9), 4.60 (1H, d, *J* = 3.5 Hz, H-7′), 5.93, 5.95 (each 1H, each d, *J* = 1.5 Hz, OCH_2O), 6.34 (2H, s, H-2′, and H-6′), 6.52 (1H, s, H-5), 6.67 (1H, s, H-2); ESI-MS: *m*/*z* = 421 $[M + Na]^+$.

Dehydropodophyllotoxin (**9**): colorless needles; m.p. 264–266 °C (CH_2Cl_2-MeOH); UV (MeOH): λ_{max} (log ε) = 262 (4.57), 311 (3.95), 321 (3.97) nm; IR (KBr): υ_{max} = 3421 (OH), 1762 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.83 (6H, s, OMe-3′, and OMe-5′), 3.95 (3H, s, OMe-4′), 5.37 (2H, br s, H-9), 5.68 (1H, br s, D_2O exchangeable, OH-7), 6.10 (2H, s, OCH_2O), 6.52 (2H, s, H-2′, and H-6′), 7.09 (1H, s, H-5), 7.49 (1H, s, H-2); ESI-MS: m/z = 433 $[M + Na]^+$.

(–)-Yatein (**10**): yellowish solid (MeOH); UV (MeOH): λ_{max} (log ε) = 212 (4.35), 230 (sh, 3.93), 287 (3.47) nm; IR (KBr): υ_{max} = 1764 (C=O), 1591, 1502, 1488 (aromatic ring C=C stretch), 1037, 925 (OCH_2O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.49 (1H, m, H-8), 2.53 (1H, m, H-7α), 2.58 (1H, m, H-8′), 2.62 (1H, dd, *J* = 13.2, 6.4 Hz, H-7β), 2.89 (1H, dd, *J* = 14.0, 6.2 Hz, H-7′α), 2.93 (1H, dd, *J* = 14.0, 5.2 Hz, H-7′β), 3.82 (6H, s, OMe-3′, and OMe-5′), 3.83 (3H, s, OMe-4′), 3.88 (1H, dd, *J* = 9.2, 7.6 Hz, H-9β), 4.18 (1H, dd, *J* = 9.2, 7.2 Hz, H-9α), 5.93, 5.94 (each 1H, each d, *J* = 1.2 Hz, OCH_2O), 6.36 (2H, s, H-2′, and H-6′), 6.46 (1H, d, *J* = 1.6 Hz, H-2), 6.47 (1H, dd, *J* = 7.6, 1.6 Hz, H-6), 6.69 (1H, d, *J* = 7.6 Hz, H-5); ESI-MS: m/z = 423 $[M + Na]^+$.

N-trans-Feruloylmethoxytyramine (**11**): white needles; m.p. 112–114 °C ($CHCl_3$-MeOH); UV (MeOH): λ_{max} (log ε) = 221 (3.61), 290 (2.86), 319 (3.34) nm; IR (KBr): υ_{max} = 3362 (OH), 1652 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.82 (2H, t, *J* = 6.8 Hz, H-11), 3.62 (2H, q, *J* = 6.8 Hz, H-10), 3.88 (3H, s, OMe-14), 3.92 (3H, s, OMe-3), 5.52 (1H, br t, *J* = 6.8 Hz, D_2O exchangeable, NH), 5.53 (1H, s, D_2O exchangeable, OH), 5.79 (1H, s, D_2O exchangeable, OH), 6.16 (1H, d, *J* = 15.6 Hz, H-8), 6.71 (1H, dd, *J* = 8.0, 1.6 Hz, H-17), 6.73 (1H, d, *J* = 1.6 Hz, H-13), 6.87 (1H, d, *J* = 8. Hz, H-16), 6.90 (1H, d, *J* = 8.4 Hz, H-5), 6.97 (1H, d, *J* = 1.6 Hz, H-2), 7.04 (1H, dd, *J* = 8.4, 1.6 Hz, H-5), 7.53 (1H, d, *J* = 15.6 Hz, H-7); ESI-MS: m/z = 366 $[M + Na]^+$.

Mixture of β-Sitostenone (**12**) and stigmasta-4,22-dien-3-one (**13**): colorless needles; m.p. 88–90 °C (MeOH); $[\alpha]_D^{25}$ = +85.8° (*c* 0.18, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 242 (4.21); IR (KBr): υ_{max} = 1685 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) of **12**: δ 0.70 (3H, s, H-18), 0.81 (3H, d, *J* = 6.8 Hz, H-27), 0.83 (3H, d, *J* = 6.8 Hz, H-26), 0.86 (3H, t, *J* = 7.2 Hz, H-29), 0.92 (3H, d, *J* = 6.4 Hz, H-21), 1.18 (3H, s, H-19), 5.71 (1H, s, H-4); ^{1}H-NMR ($CDCl_3$, 400 MHz) of **13**: δ 0.72 (3H, s, H-18), 0.79 (3H, d, *J* = 6.8 Hz, H-27), 0.82 (3H, t, *J* = 7.2 Hz, H-29), 0.83 (3H, d, *J* = 6.8 Hz, H-26), 1.02 (3H, d, *J* = 6.8 Hz, H-21), 1.18 (3H, s, H-19), 5.02 (1H, dd, *J* = 15.2, 8.8 Hz, H-23), 5.14 (1H, dd, *J* = 15.2, 8.8 Hz, H-22), 5.71 (1H, s, H-4); ESI-MS of **12**: m/z = 435 $[M + Na]^+$; ESI-MS of **13**: m/z = 433 $[M + Na]^+$.

Mixture of 6β-Hydroxystigmast-4-en-3-one (**14**) and 6β-hydroxystigmasta-4,22-dien-3-one (**15**): colorless needles; m.p. 208–209 °C (CH_2Cl_2-MeOH); $[\alpha]_D^{25}$ = + 29.7° (*c* 0.17, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 235 (4.11) nm; IR (KBr): υ_{max} = 3412 (OH), 1679 (C=O) cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) of **14**: δ 0.74 (3H, s, H-18), 0.81 (3H, d, *J* = 6.8 Hz, H-27), 0.84 (3H, d, *J* = 7.2 Hz, H-26), 0.87 (3H, t, *J* = 7.2 Hz, H-29), 0.92 (3H, d, *J* = 6.4 Hz, H-21), 1.38 (3H, s, H-19), 4.35 (1H, br s, H-6), 5.82 (1H, s, H-4); ^{1}H-NMR ($CDCl_3$, 500 MHz) of **15**: δ 0.76 (3H, s, H-18), 0.80 (3H, d, *J* = 6.8 Hz, H-27), 0.81 (3H, d, *J* = 6.8 Hz, H-26), 0.85 (3H, t, *J* = 7.2 Hz, H-29), 1.02 (3H, d, *J* = 6.8 Hz, H-21), 1.38 (3H, s, H-19), 4.35 (1H, br s, H-6), 5.03 (1H, dd, *J* = 15.2, 8.6 Hz, H-23), 5.15 (1H, dd, *J* = 15.2, 8.6 Hz, H-22), 5.82 (1H, s, H-4); ESI-MS of **14**: m/z = 451 $[M + Na]^+$; ESI-MS of **15**: m/z = 449 $[M + Na]^+$.

3.4. Biological Assay

The effect of the isolates on the neutrophil proinflammatory response was assessed by detecting the inhibition of elastase release and $O_2^{\bullet-}$ generation in fMLP/CB-activated neutrophils in a concentration-dependent manner.

3.4.1. Mensuration of Human Neutrophils

Human neutrophils from the venous blood of adult, healthy volunteers (20–27 years old) were isolated by a standard pattern of dextran sedimentation before centrifugation in a Ficoll Hypaque gradient and hypotonic lysis of the erythrocytes [27]. The purified neutrophils had >98% viable cells,

as detected by the trypan blue exclusion method [28], were resuspended in a calcium (Ca^{2+})-free HBSS buffer at pH 7.4 and were kept at 4 °C prior to use.

3.4.2. Mensuration of Superoxide Anion ($O_2^{\bullet-}$) Generation

The assay for the measurement of $O_2^{\bullet-}$ generation was based on the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome *c* [29,30]. In short, after supplementation with 1 mM Ca^{2+} and 0.5 mg/mL ferricytochrome *c*, neutrophils (6×10^5/mL) were equilibrated at 37 °C for 2 min and incubated with varied concentrations (10–0.01 μg/mL) of either DMSO (as a control) or tested compounds **1**–**15** (purity ≥ 98%) for 5 min. Cells were incubated with cytochalasin B (1 μg/mL) for 3 min before they were activated with 100 nM formyl-L-methionyl-L-leucyl-L-phenylalanine for 10 min. Changes in absorbance with the reduction of ferricytochrome *c* at 550 nm were constantly detected in a double-beam, six-cell positioner spectrophotometer with continuous stirring (Hitachi U-3010, Tokyo, Japan). Calculations were founded 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).

3.4.3. Mensuration of Elastase Release

The degranulation of azurophilic granules was measured by determining elastase release as reported previously [30,31]. Assays were carried out by applying MeO-Suc-Ala-Ala-Pro-Val-*p*-nitroanilide as the elastase substrate. In brief, 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 tested compounds for 5 min. Cells were treated with fMLP (100 nM)/CB (0.5 μg/mL), and the changes in absorbance at 405 nm were detected constantly in order to measure elastase release. The results were displayed as the percent of elastase release in the fMLP/CB-activated, drug-free control system.

3.4.4. Statistical Analysis

Results are represented as mean ± SEM, and comparisons were done by applying student's *t*-test. A probability of 0.05 or less was deemed significant. The software SigmaPlot was employed for the statistical analysis.

4. Conclusions

Fifteen compounds, including a new aporphine, 3-hydroxyhernandonine (**1**), and a new lignin, 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**), were isolated from the resinous wood of the root wood of *H. nymphaeifolia*. The structures of these isolates were elucidated according to spectroscopic data. Granule proteases (e.g., cathepsin G, elastase) and reactive oxygen species (ROS) [e.g., hydrogen peroxide, superoxide anion ($O_2^{\bullet-}$)] generated by human neutrophils gave rise to the pathogenesis of inflammatory diseases. The effects of the isolated compounds on proinflammatory responses were assessed by inhibiting fMLP/CB-induced elastase release and $O_2^{\bullet-}$ generation by neutrophils. The results of anti-inflammatory assays reveal that compounds **1**–**7** and **11** can obviously inhibit fMLP-induced elastase release and/or $O_2^{\bullet-}$ generation. Oxohernangerine (**5**) and 3-hydroxyhernandonine (**1**) were the most effective among the isolated compounds, with IC_{50} values of 2.65 ± 0.97 and 3.93 ± 0.48 μg/mL, respectively, against fMLP-induced $O_2^{\bullet-}$ generation and elastase release. Our research indicates *H. nymphaeifolia* and its isolated compounds (especially **1**–**7** and **11**) are worth further study and may be expectantly developed as candidates for the prevention or treatment of diverse inflammatory diseases.

Supplementary Materials: Supplementary materials are available online, Figures S1–S8: MS, 1D, and 2D-NMR spectra for 3-hydroxyhernandonine (**1**), Figures S9–S16: MS, 1D, and 2D-NMR spectra for 4′-*O*-demethyl-7-*O*-methyldehydropodophyllotoxin (**2**).

Author Contributions: C.-Y.W. and J.-J.C. performed the isolation and structure elucidation of the constituents and manuscript writing. C.-Y.W., S.-W.W., J.-W.Y., T.-L.H., M.-J.C., P.-J.S., T.-H.C., and J.-J.C. conducted the bioassay and analyzed the data. J.-J.C. planned, designed, and organized all of the research of this study and the preparation of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments: This research was supported by grants from the Ministry of Science and Technology, Taiwan (No. MOST 106-2320-B-010-033-MY3 and MOST 105-2320-B-010-040), awarded to J.-J. Chen. This work was also supported by the grants from Chang Gung Memorial Hospital (CMRPD1B0281~3, CMRPF1D0442~3, CMRPF 1F0011~3, CMRPF1F0061~3 and BMRP450). We are grateful to Ih-Sheng Chen for unselfishly providing us with plant material (root wood of *H. nymphaeifolia*).

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of the compounds are available from the authors.