Carapanosins A–C from Seeds of Andiroba (Carapa guianensis, Meliaceae) and Their Effects on LPS-Activated NO Production

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Academic Editor: Derek J. McPhee
Received: 7 February 2017; Accepted: 15 March 2017; Published: 22 March 2017

Abstract: Two new phragmalin-type limonoids, Carapanosins A and B (1 and 2), and a new gedunin-type limonoid, Carapansin C (3), together with five known limonoids (4–8) were isolated from the oil of Carapa guianensis AUBLET (Meliaceae) seeds, a traditional medicine in Brazil and Latin American countries. Their structures were elucidated on the basis of spectroscopic analyses using 1D and 2D NMR techniques and HRFABMS. Compounds 1–8 were evaluated for their effects on the production of NO in LPS-activated mouse peritoneal macrophages. The NO inhibitory assay suggested that Compounds 3, 6, and 8 may be valuable as potential inhibitors of macrophage activation.

Keywords: Carapa guianensis; Meliaceae; seed oil; limonoid; Carapanosins A–C

1. Introduction

Limonoids have mainly been found in Meliaceae and Rutaceae plants, and are modified triterpenoids that originate from a precursor with 4,4,8-trimethyl-17-furylsteroids that typically contains four highly oxidized (A, B, C, and D) rings. Meliaceae plants are distributed in tropical regions throughout the world [1]. Carapa guianensis AUBLET (Meliaceae) is a popular medicinal plant known as “Andiroba” in Brazil, and is in the same family as mahogany. Andiroba is a tall rainforest tree that grows up to 40 m in height. It is in the same family as mahogany and has been called Brazilian mahogany or bastard mahogany due to their similarities. The andiroba tree produces a brown, ligneous, quadrilateral nut that is approximately 3 to 4 in. in diameter and has the appearance of a chestnut. The nut from andiroba contains several oil-rich kernels and seeds that are composed of an ~60% pale yellow oil. The seed oil of andiroba was previously reported to exhibit highly efficient analgesic [2], anti-bacterial [3], anti-inflammatory [4], anti-cancerous [5], anti-tumor, anti-fungal [6], and anti-allergic properties [7] and was also found to be effective against wounds, bruises, herpes ulcers, rheumatism, ear infections, and insect bites as a repellent [8,9]. We previously reported Carapanolides A and B [10], guianolide A and B [11], Carapanolides C–I [12], Carapanolides J–L [13], Carapanolides M–S [14], and Carapanolides T–X [15] in the seed oil of andiroba. Our continuing research on the seed oil of andiroba revealed the structures of two new phragmalin-type limonoids, Carapanosins A (1) and B (2), a new gedunin-type limonoid, Carapansin C (3), and five known limonoids (4–8). We herein describe the isolation and structural elucidation of the new limonoids as well as their inhibitory effects of NO production.
2. Results and Discussion

The oil from *C. guianensis* seeds was subjected to silica gel column chromatography, medium-pressure liquid chromatography (MPLC), and reverse phase HPLC in order to obtain the new limonoids 1–3 and known limonoids 4–8. Known compounds were identified as Carapanolide H (4) [12], Swietephragmin G (5) [16], Swietephragmin D (6) [16], 17-epi-17-hydroxyazadiradione (7) [17], and 17-β-hydroxyazadiradione (8) [17] by comparisons with spectroscopic data of the literature.

Carapanosin A (1), a colorless crystal, had the molecular formula of C$_{36}$H$_{42}$O$_{16}$ ([M + H]$^+$, calcld. 731.2551) as determined by HRFABMS. The IR absorption bands indicated the existence of hydroxy group ($\nu_{\text{max}}$ 3647 cm$^{-1}$) and several carbonyl groups (1751, 1700 and 1652 cm$^{-1}$).

The UV spectrum showed a furan ring and an αβ-unsaturated δ-lactone at $\lambda_{\text{max}}$ 208 nm (log $\varepsilon$ 3.52) and 235.5 nm (log $\varepsilon$ 3.54). 1H- and 13C-NMR spectra (Table 1) exhibited signals assignable to three tertiary methyl groups [δ$_{\text{H}}$ 0.89, 1.34, 1.47 (each s)], two acetyl groups [δ$_{\text{H}}$ 1.58, 2.05 (each 3 H, s); δ$_{\text{C}}$ 20.1, 20.8 (each q)], a propanoyl [δ$_{\text{H}}$ 1.10 (3 H, t), 2.31 (dq), 2.42 (m); δ$_{\text{C}}$ 8.9 (q), 27.8 (t), 174.0 (s)], a methyl ester [δ$_{\text{H}}$ 3.78 (3 H, s); δ$_{\text{C}}$ 52.5 (q), 173.6 (s)], two sp$^3$ methylenes, six sp$^3$ methines including five oxymethines [δ$_{\text{H}}$ 4.09 (d), 4.57 (s), 4.99 (dd), 5.29 (s), 5.71 (s); δ$_{\text{C}}$ 68.8, 68.9, 71.4, 78.7, 83.8 (each d)], and seven sp$^3$ quaternary carbons including four oxycarbons [δ$_{\text{C}}$ 78.4, 83.0, 84.5, 85.2 (each s)], and the last three displacements have already been quoted above for the orthoester. I suggest seven sp$^3$ quaternary carbons including one with a hydroxyl attached, rather than an oxygen bridge [δ$_{\text{C}}$ 78.4], an αβ-unsaturated δ-lactone [δ$_{\text{H}}$ 6.06 (1 H, s); δ$_{\text{C}}$ 122.1 (d), 159.6 (s)], and a furan ring [δ$_{\text{H}}$ 6.54 (dd), 7.42 (t), 7.55 (brs)]. In the 1H-1H COSY spectrum, cross peaks were observed between H-5–H-6, H$_2$-11–H-12, H-22–H-23, and H$_2$-2″′–H$_3$-3‴‴, as shown in boldface in Figure 1.

In the HMBC spectrum (Figure 1), cross peaks were observed from H-3 [δ$_{\text{H}}$ 4.57 (s)]/C-2 [δ$_{\text{C}}$ 78.4 (s)], C-4, C-5, C-1′ [δ$_{\text{C}}$ 171.1 (s)]; H-6 [δ$_{\text{H}}$ 4.09 (d)]/C-4, C-5, C-7 [δ$_{\text{C}}$ 173.6 (s)]; H-12 [δ$_{\text{H}}$ 4.99 (dd)]/C-11, C-13, C-14 [δ$_{\text{C}}$ 162.7 (s)], C-17 [δ$_{\text{C}}$ 78.7 (d)], C-18, C-1′′′′ [δ$_{\text{C}}$ 172.3 (s)]; H-15 [δ$_{\text{H}}$ 6.06 (s)]/C-8 [δ$_{\text{C}}$ 83.0 (s)], C-13, C-14, C-16 [δ$_{\text{C}}$ 159.6 (s)]; H-17 [δ$_{\text{H}}$ 5.29 (s)]/C-12 [δ$_{\text{C}}$ 68.8 (d)], C-13, C-14, C-20 [δ$_{\text{C}}$ 121.7 (s)], C-21 [δ$_{\text{C}}$ 141.7 (d)], C-22 [δ$_{\text{C}}$ 110.2 (d)]; Me-18 [δ$_{\text{H}}$ 1.47 (s)]/C-12, C-13, C-14, C-17; Me-19 [δ$_{\text{H}}$ 1.34 (s)]/C-1 [δ$_{\text{C}}$ 84.5 (s)], C-5, C-9 [δ$_{\text{C}}$ 85.2 (s)], C-10; Me-28 [δ$_{\text{H}}$ 0.89 (s)]/C-3 [δ$_{\text{C}}$ 83.8 (d)], C-4, C-5, C-29, H$_2$-1″′ [δ$_{\text{H}}$ 3.78 (s)]/C-7[δ$_{\text{C}}$ 173.6 (s)]. The relative structure of 1 was determined on the basis of NOESY correlations (Figure 1). Intense NOESY correlation between H-3 and Me-28, and H-29$_{\text{pro-R}}$ and H-29$_{\text{pro-S}}$; between H-5β and H-6, H-12, H-30β, and Me-28; between H-6 and H-30β; between H-12 and H-5β, H-17β, and H-30β; and between Me-19 and H-6, H-29$_{\text{pro-R}}$, and Me-32 revealed an acetyl group at C-3 in the β orientation, C-12, a hydroxyl group at C-2, and a 2-methylpropanoyl group at C-30 in the α orientation. In addition, significant NOEs were observed between H-6 [δ$_{\text{H}}$ 6.07 (brs)] and H-11β, H-12β and H-17β; therefore, C-6 was presumed to be in an R-configuration, which was consistent with Carapanolide N$_{14}$. 

Figure 1. Key HMBC, COSY, and NOESY correlations for 1.
Table 1. $^1$H-NMR and $^{13}$C-NMR Data of Compounds 1 and 2.

| Position | 1 $^1$H (J, Hz) | 2 $^1$H (J, Hz) | 2 $^1$H (J, Hz) | HMBC |
|----------|-----------------|-----------------|-----------------|------|
|          | $^1$H a         | $^{13}$C b      | $^1$H a         | $^{13}$C b      |
|          | 84.5 (s)        | 84.1 (s)        | 83.8 (d)        | 85.3 (d)        |
|          | 78.4 (s)        | 83.4 (s)        | 43.9 (s)        | 44.5 (d)        |
|          | 5.19 s          | 44.2 (d)        | 2.47 brd        | 44.7 (d)        |
|          | 71.4 (d)        | 71.2 (d)        | 4, 9, 10, 29    | 4, 5, 10, 29   |
|          | 173.6 (s)       | 169.2 (s)       | 83.0 (s)        | 83.5 (s)        |
|          | 199 (s)         | 199 (s)         | 85.2 (s)        | 86.1 (s)        |
|          | 4.57 s          | 48.7 (s)        | 44.2 (s)        | 48.8 (s)        |
|          | 78.4 (s)        | 83.4 (s)        | 31.9 (t)        | 32.4 (t)        |
|          | 43.9 (s)        | 44.5 (d)        | 2.00 t (14.1)   | 9, 10, 12, 13   |
|          | 71.4 (d)        | 71.2 (d)        | 2.35 dd (14.1, 4.1) | 2, 8, 9, 12 |
|          | 68.8 (d)        | 68.5 (d)        | 4, 5, 9, 10    | 17, 18        |
|          | 4.09 dd (12.1, 10.9) | 4.99 dd (13.5, 4.2) | 4.21 (s)        | 4.29 (s)        |
|          | 122.1 (d)       | 124.2 (d)       | 12, 13, 14, 18 | 20, 22, 23    |
|          | 199 (s)         | 199 (s)         | 121.7 (s)       | 121.0 (s)      |
|          | 174.6 (s)       | 171.0 (s)       | 141.7 (d)       | 142.1 (d)      |
|          | 173.6 (s)       | 171.0 (s)       | 110.2 (d)       | 110.2 (d)      |
|          | 174.6 (s)       | 171.0 (s)       | 143.2 (d)       | 143.0 (d)      |
|          | 174.6 (s)       | 171.0 (s)       | 15.7 (q)        | 15.3 (q)       |
|          | 174.6 (s)       | 171.0 (s)       | 2.75 (d) (10.8) | 3.74 (t)       |
|          | 174.6 (s)       | 171.0 (s)       | 1.75 d (10.8)   | 3.75 (t)       |
|          | 174.6 (s)       | 171.0 (s)       | 3.21 (t) (14.7, 4.2) | 1.75 s        |
|          | 174.6 (s)       | 171.0 (s)       | 2.05 s (3 H, s) | 2.20 s         |
|          | 174.6 (s)       | 171.0 (s)       | 1.16 t (7.3)    | 1.16 t (7.3)   |
|          | 174.6 (s)       | 171.0 (s)       | 1.31 s (1 H, m) | 1.31 s (1 H, m) |
|          | 174.6 (s)       | 171.0 (s)       | 1.20 s (1 H, m) | 1.20 s (1 H, m) |

Carapanosin B (2), a colorless amorphous, had the molecular formula of C$_{38}$H$_{44}$O$_{17}$ (m/z 773.2659 [M + H]$^+$, calc. 773.2657) as determined by HRFABMS. The IR spectrum showed the presence of hydroxyl, ester groups, and an αβ-unsaturated δ-lactone at $\nu_{\text{max}}$ 3566, 1734, and 1663 cm$^{-1}$; and the UV spectrum indicated the presence of a furan ring and an αβ-unsaturated δ-lactone at $\lambda_{\text{max}}$ 213 nm (log $\varepsilon$ 3.84) and 237.5 nm (log $\varepsilon$ 3.62). The $^1$H- and $^{13}$C-NMR spectra (Table 1) displayed signals due to three tertiary methyls ($\delta$H 0.92, 1.31, 1.59 (each 3 H, s)), three acetyl groups ($\delta$H 2.08 (3 H, s), $\delta$C 169.0 (s); $\delta$H 2.20 (3 H, s), $\delta$C 171.7 (s); $\delta$H 1.55 (3 H, s), $\delta$C 170.4 (s)), a propanoyl group ($\delta$H 1.16 (3 H, t), 2.43 (1 H, dq), 2.50 (1 H, m), $\delta$C 173.9 (s)), a methyl ester ($\delta$H 3.74 (3 H, s), $\delta$C 169.2 (s)), a methylene component...
[δH 2.00 (1 H, t), 2.35 (1 H, dd)], five sp3 methines including four oxymethines [δH 4.94 (dd), 5.19 (s), 5.91 (s), 6.31 (brd)], seven sp3 quaternary carbons including five oxycarbons [δC 83.4, 83.5, 84.1, 86.1 (each s)], an α,β-unsaturated δ-lactone [δH 6.62 (1 H, s), δC 124.2 (d), 152.6 (s), 163.4 (s)], and a furan ring [δH 6.56 (dd), 7.40 (t), 7.45 (brs)]. The 1H and 13C-NMR spectra (Table 1) of 2 were very similar to those of 1, so 2 is estimated to be phragmalin-1,8,9-orthoacetate, except for the absence of a hydroxy group and presence of an acetyl group at C-6 [δH 6.31 (brd), 7.40 (t), 7.45 (brs)]. In the NOESY spectrum, significant NOEs were observed between H-6 and H-11α, and Me-19, so the configuration of H-6 was determined to have the same R as Compound 1 and Carapanolide N [14], and its relative structure was established, as shown in Figure 2.

Carapanosin C (3) was obtained as a colorless crystal, m.p. 236–239 °C. Its molecular formula was determined to be C28H34O7 (m/z 483.2388 [M + H]+, calcd. 483.2383). The IR absorption bands indicated the existence of a hydroxy, an ester, an α,β-unsaturated six-membered ring ketone, and α,β-unsaturated δ-lactone at νmax 3566, 1734, 1699, 1668 cm−1, and the UV absorption band indicated a λmax 238.5 nm (log ε 3.74). 1H- and 13C-NMR spectra (Table 2) revealed the presence of five methyls [δH 1.08, 1.09, 1.16, 1.25, 1.36 (each 3 H, s)], a secondary acetoxy group [δH 1.98 (3 H, s), 5.25 (t); δC 169.6 (s)], αβ-unsaturated six-membered ring ketone [δH 5.87 and 7.06 (each 1 H, d), δC 203.8 (s)], an αβ-unsaturated δ-lactone [δH 5.64 (1 H, s), δC 111.0 (d), 163.4 (s), 170.3 (s)], an acetal carbon [δC 104.0 (s)] [16], and a β-substituted furan ring [δH 6.48 (dd), 7.43 (t), 7.58 (brs)], suggesting

Figure 2. Chemical structures for Compounds 1–8 and nimolicinol (9).
a gedunin-type limonoid. In the HMBC spectrum, the following correlations were observed: Me-18 [δH 1.16 (s)]/C-12, C-13, C-14 [δC 170.3 (s)], and C-17 [δC 104.0 (s)]; Me-19 [δH 1.25 (s)]/C-1 [δC 156.4 (d)], C-5, C-9, and C-10; Me-30 [δH 1.36 (s)]/C-7 [δC 73.2 (d)], C-8, C-9, and C-14 [δC 170.3 (s)]. The 1H-1H COSY spectrum (H-1–H-2; H-5–H-6–H-7; H-9–H-11–H-12; H-22–H-23) revealed the positions of substituents (Figure 3). These results suggested the planer structure of 3 was as the same as that of 9. However, major differences were detected in the 1H- and 13C-NMR spectra between 3 and 9. These differences between 3 and 9 were particularly prominent in C-12 (δC 23.2 in 3; δC 37.2 in 9), C-9 (δC 37.2 in 3; δC 45.5 in 9), and C-22 (δC 125.0 in 3; δC 110.1 in 9), and slight differences were observed in C-5 (δC 43.5 in 3; δC 40.5 in 9), C-6 (δC 23.0 in 3; δC 25.0 in 9), C-10 (δC 40.4 in 3; δC 42.1 in 9), and C-13 (δC 42.0 in 3; δC 44.5 in 9). The relative configuration of 3 was mainly established by a NOESY experiment (Figure 3). Cross-peaks were observed Me-30/H-7β [δH 5.25 (t)], H-15, and Me-19; H-21/H-12α, H-12β, and Me-18; and Me-18/H-9α, H-12α, H-15, H-21, and H-23. Compound 3 (17β-hydroxy-14,15-deoxy-gedunin) has not yet been isolated.

Figure 3. Key HMBC, COSY and NOESY correlations of Carapanosin C (3).

Table 2. 1H (600 MHz) and 13C (150 MHz) NMR spectroscopic data for Compound 3.

| Position | 1H a (J, Hz) | 13C b | Position | 1H a (J, Hz) | 13C b |
|----------|-------------|-------|----------|-------------|-------|
| 1        | 7.06 d (10.3) | 156.4 (d) | 14       | 5.64 s | 170.3 (s) |
| 2        | 5.87 d (10.3) | 128.5 (d) | 15       |         | 110.1 (d) |
| 3        | 203.8 (s)     | 16     | 19       | 1.25 s  | 18.8 (q)  |
| 4        | 43.7 (s)      | 17     | 20       |         | 125.0 (s) |
| 5        | 2.16 dd (12.6, 4.1) | 45.5 (d) | 18       | 1.16 s  | 23.3 (q)  |
| 6        | α            | 1.97 m  | 19       |         |         |
|          | β            | 1.99 m  |          |         |         |
| 7        | 5.25 t (2.9)  | 73.2 (d) | 21       | 7.58 brs | 142.9 (d) |
| 8        | 44.4 (s)      | 22     | 22       | 6.48 dd (1,8, 0.9) | 125.0 (d) |
| 9        | 2.20 dd (11.4, 8.5) | 37.2 (d) | 23       | 7.43 t (1.8) | 141.6 (d) |
| 10       | 40.4 (s)      | 28     | 28       | 1.08 s  | 26.8 (q)  |
| 11       | α            | 2.00 m  | 29       | 1.09 s  | 21.1 (q)  |
|          | β            | 1.86 ddd (14.1, 11.4, 1.7) | 15.1 (t) | 30 | 1.36 s  | 24.1 (q)  |
| 12       | α            | 2.30 dt (14.1, 9.9) | 23.2 (t) | 1' | 169.6 (s) |
|          | β            | 1.60 m  | 2' | 1.98 s  | 20.7 (q)  |

a Measured at 600 MHz in CDCl3. b Measured at 150 MHz in CDCl3. Assignment are based on HMBC spectrum.
Macrophages may be a potential therapeutic target for inflammatory diseases [20]. Activated macrophages release pro-inflammatory mediators, such as NO, reactive oxygen species, interleukin-1 beta, tumor necrosis factor-alpha, and other inflammatory mediators, which play important roles in biological defense. However, the overexpression of these mediators has been implicated in diseases such as osteoarthritis, rheumatoid arthritis, and diabetes because the increased production of pro-inflammatory mediators has been shown to induce severe or chronic inflammation [21]. Eight limonoids, and L-NMMA, an inducible nitric oxide synthase (iNOS) inhibitor, were evaluated for their inhibitory effects on NO production (Figure 4). All tested compounds did not exhibit cytotoxicity (Cell viability 92.7%–100.4% at 30 μM). Of these, Compounds 3, 6, and 8 exhibited stronger inhibitory activity on NO production (IC_{50} 3: 13.7 μM; 6: 4.9 μM; 8: 10.8) than L-NMMA (IC_{50} 23.9 μM). On the other hand, Compounds 4 and 7 showed moderate activity on NO production (IC_{50} 4: 25.5 μM; 7: 28.9 μM).

![Figure 4](image-url)  
Inhibitory activities on NO production and cytotoxicities of Compounds 3, 4, 6–8 and L-NMMA. Each value represents the mean ± the standard error (S.E.) of four determinations. Significant differences from the vehicle control (0 μM) group shown as *: p < 0.05 and **: p < 0.01 in the NO inhibitory assay.

3. Experimental

3.1. General Experimental Procedures

Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. IR spectra were recorded on a PerkinElmer 1720X FTIR spectrophotometer (Perkin-Elmer Inc., Wellesley, MA, USA). UV spectra were measured on a HITACHI U-2000 spectrometer using EtOH as a solvent. 1H- and 13C-NMR spectra were obtained on an Agilent vnmrs 600 spectrometer (Agilent Technologies, Santa Clara, CA, USA) with standard pulse sequences, operating at 600 and 150 MHz, respectively. CDCl₃ was used as the solvent and TMS as the internal standard.

FABMS were recorded on a JEOL JMS-7000 mass spectrometer (JEOL, Tokyo, Japan). Column chromatography was performed over silica gel (70–230 mesh; Merck, Darmstadt, Germany), while medium pressure liquid chromatography (MPLC) was conducted with silica gel (230–400 mesh, Merck). HPLC was carried out using an ODS column [Cosmosil 5C₁₈-MS column (Nacalai Tesque, Inc., Kyoto,
3.2. Isolation of Compounds 1–3

Preliminary silica gel column chromatography was performed to separate the seed oil (1.1 kg) of *Carapa guianensis* AUBLER into 8 fractions: Fraction A (Fractions 1–76, 900 g) was eluted with CHCl₃, B (Fractions 77–110, 12.0 g) with CHCl₃, C (Fractions 111–125, 21.0 g) with CHCl₃/EtOAc = 5:1, D (Fractions 126–155, 10.9 g) with CHCl₃/EtOAc = 5:1, E (Fractions 156–170, 1.4 g) with CHCl₃/EtOAc = 2:1, F (Fractions 171–180, 2.4 g) with EtOAc, G (Fractions 181–195, 2.9 g) with EtOAc, and H (Fractions 196–208, 0.7 g) with EtOAc/MEOH = 5:1. Fraction E (1.4 g) was rechromatographed on a silica gel (70–230 mesh, 100 g) column using n-hexane/EtOAc = 1:1 to yield Residue E7 (426 mg). Residue E7 (426 mg) was rechromatographed on a silica gel (70–230 mesh, 100 g) column using n-hexane/EtOAc = 2:1 to yield Residue E11 (125 mg), E12 (33 mg), and E13 (43 mg). Residue E11 was separated by HPLC (ODS, 70% MeOH) to yield Compounds 1 (3.4 mg), 2 (2.9 mg), and 3 (2.7 mg).

**Carapanosin A (1):** Colorless amorphous solid; m.p. 140–142 °C; [α]₂₀¹°D −74.6° (c 0.32, CHCl₃); UV (EtOH) λ_max (log ε): 208 (3.52), 235.5 (3.54); IR (cm⁻¹, KBr): 3647, 1751, 1700, 1652; FAB-MS m/z (rel.int.): 731 [M + H]⁺ (100), 671 (12), 95 (17); HR-FAB-MS m/z 731.2551 [M + H]⁺ (C₃₈H₄₅O₁₆, calcd. 731.2551).

**Carapanosin B (2):** Colorless amorphous. [α]₂₀¹°D 64.0° (c 0.05, EtOH); UV (EtOH) λ_max (log ε): 237.5, 213 (log ε 3.62, 3.84); IR (cm⁻¹, KBr): 3566, 1734, 1663, 1039. FAB-MS m/z (rel.int.): 773 [M + H]⁺ (49), 715 (65), 699 (77), 43 (100); HR-FAB-MS m/z 773.2659 [M + H]⁺ (C₃₈H₄₅O₁₇, calcd. 773.2657).

**Carapanosin C (3):** Colorless crystal; m.p. 236–239 °C; [α]₂₀¹°D +80.5° (c 0.13, EtOH); UV (EtOH) λ_max (log ε): 238.5 (log ε 3.74); IR (cm⁻¹, KBr): 3566 (OH), 1734, 1699, 1668, 1240, 1171; FAB-MS m/z (rel.int.): 505 [M + Na]⁺ (50), 483 [M + H]⁺ (77), 465 (23), 423 (52), 405 (14), 328 (25), 176 (37), 95 (100); HR-FAB-MS m/z: 483.2388 [M + H]⁺ (C₂₈H₃₅O₁₇, calcd. for 483.2383).

3.3. Cell Cultures

RAW264.7 cells (mouse macrophages) (obtained from DS Pharma Biomedical Co., Ltd. (Osaka, Japan)) were grown in DMEM. The medium was supplemented with 10% FBS and antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin). The cells were incubated at 37 °C in a 5% CO₂ humidified incubator.

3.4. Determination of RAW264.7 Cell Proliferation

RAW264.7 cell proliferation was examined in accordance with a method reported previously [22]. Briefly, RAW264.7 cells (5 × 10⁴ cells in 100 µL) were seeded onto a 96-well microplate, and incubated for 24 h. DMEM containing test samples (100 µL total volume, a final concentration of 30, 10, 3, or 1 µM) dissolved in DMSO (final concentration of 0.2%) was added. After treatment for 24 h, MTT solution
was added. After a 3 h incubation, 20% sodium dodecyl sulfate in 0.1 M HCl was added to dissolve the formazan produced in the cells. The absorbance of each well was read at 570 nm using a microplate reader. The optical density of vehicle control cells was assumed to be 100%. Values are expressed as the mean ± standard error of the mean (S.E.M.). One-way ANOVA, followed by Dunnett’s test, was used for statistical analysis. Significant differences from the vehicle control (0 µM) group shown as *: \( p < 0.05 \) and **: \( p < 0.01 \).

4. Conclusions

Two new phragmalin-type limonoids, Carapanosins A (1) and B (2) as well as a gedunin-type limonoid, Carapanosin C (3) were isolated from the seeds of *Carapa guianensis*. Their structures were elucidated by spectroscopic analyses. In the NO inhibitory assay, Compounds 3, 6, and 8 exhibited similar NO inhibitory activities (IC\(_{50}\) 3: 13.7 µM; 6: 4.9 µM; 8: 10.8 µM) to L-NMMA (IC\(_{50}\) 23.9 µM) without cytotoxicity. These results suggest that Compounds 3, 6, and 8 have potential as anti-inflammatory disease agents.

Acknowledgments: We thank Akira Yoshino for the collection and identification of the plant material. We also thank Mihoyo Fujitake for MS measurement.

Author Contributions: R. Tanaka prepared the manuscript. K. Higuchi, T. Miyake, S. Ohmori, Y. Tani, contributed to the isolation and structural elucidation. K. Minoura measured NMR spectra. T. Kikuchi performed the evaluation of bioactivities. T. Yamada supervised the whole research project.

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

References

1. Editorial Committee of Flora of China. *Flora Reipublicae Popularis Sinicae*; Science Press: Beijing, China, 1997; Volume 43.
2. Penido, C.; Costa, K.A.; Pennafort, R.J.; Costa, M.F.S.; Pereira, J.F.G.; Siani, A.C.; Henrique, M.G.M.O. Anti-allergic effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on allergen-induced vascular permeability and hyperalgesia. *Inflamm. Res.* 2005, 54, 295–303. [CrossRef] [PubMed]
3. Pereira da Silva, V.; Oliveira, R.R.; Figueiredo, M.R. Isolation of Limonoids from seeds of *Carapa guianensis* Aublet (Meliaceae) by high-speed countercurrent chromatography. *Phytochem. Anal.* 2009, 20, 77–81. [CrossRef] [PubMed]
4. Penido, C.; Conte, F.P.; Chagas, M.S.; Rodrigues, C.A.; Pereira, J.F.; Henrique, M.G.M.O. Antiinflammatory effects of natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet on zymosan-induced arthritis in mice. *Inflamm. Res.* 2006, 55, 457–464. [CrossRef] [PubMed]
5. Nakanishi, K. Phytochemical survey of Malaysian plants. Preliminary chemical and pharmacological screening. *Chem. Pharm. Bull.* 1965, 13, 882–890. [CrossRef] [PubMed]
6. Ferreira, M.C.; Vieira, M.L.A.; Zani, C.L.; Alves, T. M.A.; Junior, P.A.S.; Murta, S.M.F.; Romanha, A.J.; Gil, L.H.G.; Carvalho, A.G.O.; Zilli, J.E.; et al. Molecular phylogeny, diversity, symbiosis and discover of bioactive compounds of endophytic fungi associated with the medicinal Amazonian plant *Carapa guianensis* Aublet (Meliaceae). *Biochem. Syst. Ecol.* 2015, 59, 36–44. [CrossRef]
7. Ferraris, F.K.; Rodrigues, R.; da Silva, V.P.; Figueiredo, R.; Penido, C.; Henrique, M.G.M.O. Modulation of T lymphocyte and eosinophil functions in vitro by natural tetranortriterpenoids isolated from *Carapa guianensis* Aublet. *Int. Immunopharmacol.* 2011, 11, 1–11. [CrossRef] [PubMed]
8. Miranda Junior, R.N.; Dolabela, M.F.; da Silva, M.N.; Povoa, M.M.; Maia, J.G. Antiplasmodial activity of the andiroba (*Carapa guianensis* Aubl., Meliaceae) oil and its limonoid-rich fraction. *J. Ethnopharmacol.* 2012, 142, 679–683. [CrossRef] [PubMed]
9. Prophiro, J.S.; da Silva Mario, A.N.; Kanis, L.A.; da Rocha, L.C.B.P.; Duque-Luna, J.E.; da Silva, O.S. First report on susceptibility of wild Aedes aegypti (Diptera: Culicidae) using *Carapa guianensis* (Meliaceae) and *Copaifera sp.* (Leguminosae). *Parasitol. Res.* 2012, 110, 699–705. [CrossRef] [PubMed]
10. Inoue, T.; Nagai, Y.; Mitooka, A.; Ujike, R.; Muraoka, O.; Yamada, T.; Tanaka, R. Carapanolides A and B: Unusual 9,10-seco-mexicanolides having a 2R,9S-oxygen bridge from the seeds of *Carapa guianensis*. *Tetrahedron Lett.* 2012, 53, 6685–6688. [CrossRef]
11. Inoue, T.; Matsui, Y.; Kikuchi, T.; In, Y.; Yamada, T.; Muraoka, O.; Matsunaga, S.; Tanaka, R. Guianolides A and B, new carbon skeletal limonoids from the seeds of *Carapa guianensis*. *Org. Lett.* **2013**, *15*, 3018–3021. [CrossRef] [PubMed]

12. Inoue, T.; Matsui, Y.; Kikuchi, T.; In, Y.; Muraoka, O.; Yamada, T.; Tanaka, R. Carapanolides C–I from the seeds of andiroba (*Carapa guianensis*, Meliaceae). *Fitoterapia* **2014**, *96*, 56–64. [CrossRef] [PubMed]

13. Matsui, Y.; Kikuchi, T.; Inoue, T.; Muraoka, O.; Yamada, T.; Tanaka, R. Carapanolides J–L from the seeds of *Carapa guianensis* (andiroba) and their effects on LPS-activated NO production. *Molecules* **2014**, *19*, 17130–17140. [CrossRef] [PubMed]

14. Inoue, T.; Matsui, Y.; Kikuchi, T.; Yamada, T.; In, Y.; Muraoka, O.; Ninomiya, K.; Morikawa, T.; Tanaka, R. Carapanolides M–S from the seeds of andiroba (*Carapa guianensis*, Meliaceae) and triglyceride metabolism-promoting activity in high glucose-pretreated HepG2 cells. *Tetrahedron* **2015**, *71*, 2753–2760. [CrossRef]

15. Miyake, T.; Ishimoto, S.; Ishimatsu, N.; Higuchi, K.; Minoura, K.; Kikuchi, T.; Yamada, T.; Muraoka, O.; Tanaka, R. Carapanolides T–X from the seeds of *Carapa guianensis* (andiroba). *Molecules* **2015**, *20*, 20955–20966. [CrossRef] [PubMed]

16. Abdelgaleil, S.A.M.; Doe, M.; Morimoto, Y.; Nakatani, M. Rings B,D-seco limonoids from the leaves of Swietenia mahogany. *Phytochemistry* **2006**, *67*, 452–458. [CrossRef] [PubMed]

17. Kraus, W.; Cramer, R. 17-Epi-azadiradion uno 17-β-hydroxy-azadiradion, zwei neue Inhaltsstoffe aus *Azadirachta indica* A. Juss. *Tetrahedron Lett.* **1978**, *27*, 2395–2398. [CrossRef]

18. Siddiqui, S.; Faizi, S.; Siddiqui, B.-S. Studies on the chemical constituents of *Azadirachta indica* A. Juss (Meliaceae) part I: Isolation and structure of a new tetranortriterpenoid—Nimolicinol. *Heterocycles* **1984**, *22*, 295–298. [CrossRef]

19. Siddiqui, S.; Faizi, S.; Siddiqui, B.-S.; Ghaiasuddin, H.E. Constituents of Azadirachta isolation and structure elucidation of a new antibacterial tetranortriterpenoid, mahmoodin, and new protolimonoid, naheedin. *J. Nat. Prod.* **1992**, *55*, 303–310. [CrossRef] [PubMed]

20. Netea, M.G.; Joosten, L.A.B. Master and commander: Epigenetic regulation of macrophages. *Cell Res.* **2016**, *26*, 145–146. [CrossRef] [PubMed]

21. Chamessian, A.; van de Ven, T.; Buchheit, T.; Hsia, H.L.; McDuffie, M.; Gamazon, E.R.; Walsh, C.; Bruehl, S.; Buckenmaier, C.T., III; Shaw, A. Differential expression of systemic inflammatory mediators in amputees with chronic residual limb pain. *Pain* **2017**, *158*, 68–74. [CrossRef] [PubMed]

22. Yamada, T.; Muroga, Y.; Jinno, M.; Kajimoto, T.; Usami, Y.; Numata, A.; Tanaka, R. New class azaphilone produced by a marine fish-derived Chaetomium globosum. The stereochemistry and biological activities. *Bioorg. Med. Chem.* **2011**, *19*, 4106–4113. [CrossRef] [PubMed]

**Sample Availability**: Samples of the compounds are not available from the authors.

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