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Carapanosins D—F from the Seeds of Andiroba (*Carapa guianensis*, Meliaceae) and Their Effects on LPS-Activated NO Production

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Abstract: A novel *nor*-phragmalin-type limonoid, named carapanosin D (**1**), and two novel mexicanolide-type limonoids, carapanosins E (2) and F (**3**), were isolated from the seed oil of andiroba (*Carapa guianensis* A_{ublet}), a traditional medicine in Brazil and Latin American countries. Their structures were unambiguously determined on the basis of spectroscopic analyses using one-dimensional (1D) and two-dimensional (2D) NMR techniques and High resolution Fast Atom Bombardment Mass Spectrometry (HRFABMS). Compounds **1**–**3** were evaluated for their effects on the production of nitric oxide (NO) in Lipopolysaccharide (LPS)-activated mouse peritoneal macrophages. The NO inhibitory assay suggested that compounds **2** and **3** have high potency as inhibitors of macrophage activation.

Keywords: *Carapa guianensis*; Meliaceae; andiroba; seeds; limonoids; *seco*-phragmalin; mexicanolide; carapanosins A–C; NO production

1. Introduction

Meliaceae plants are a well-known source of structurally diverse limonoids with a wide range of bioactivities, such as antimalarial and antifeedant. Limonoids in the plant kingdom occur mainly in the Meliaceae, Rutaceae, and Simaroubaceae families [1]. Andiroba is one of the Meliaceae plants in the rain forests of South America, and its woody four-cornered nut has four cells, each of which contains two to three seeds with oil-rich kernels. Limonoids, most of which are highly oxidized tetranortriterpenoids, can be classified in terms of *seco* form and cyclization patterns of rings A–D in the triterpene skeleton. Extracts from its flowers, bark, and seeds have been used for centuries by the Amazonian people and exhibit analgesic [2], anti-malarial [3], anti-inflammatory [4], anti-allergic [5], and anti-plasmoidal [6] activities, and also acute and subacute toxicities [7].

Our series of studies on the components of the seed oil of *C. guianensis* revealed the structures of carapanolides A and B [8], guianolide A and B [9], carapanolides C–I [10], carapanolides J–L [11], carapanolides M–S [12], carapanolides T–X [13], and carapanosins A–C [14] in the seed oil of andiroba. Last year, we reported the absolute structure of guianolactones A and B from the seed oil of *C. guianasis* (Meliaceae) [15]. Our recent study of the seed oil of *C. guianasis* revealed the structures of an unusual 19-*nor*-phragmalin-type limonoid, named carapanosin D and two novel mexicanolide-type limonoids, named carapanosins E and F. We herein describe the isolation and structural determination of three new limonoids and the effects of **1**–**3** on the production of nitric oxide (NO) in Lipopolysaccharide LPS-activated mouse peritoneal macrophages. The structures of **1**–**3** were determined on the basis of NMR spectroscopy, including one-dimensional (1D) and two-dimensional (2D) (^{1}H, ^{1}H-COSY, NOESY, HSQC, HMBC) NMR, and Fast Atom Bombardment (FABMS).

2. Results and Discussion

The seeds of *Carapa guianensis* were dissolved in MeOH, and the extract was separated by silica gel column chromatography (CC), medium-pressure liquid chromatography (MPLC), and reverse-phased HPLC to obtain three novel limonoids, **1**, **2**, and **3** (Figure 1).

Carapanosin D (**1**) was obtained as a colorless amorphous solid, and possesses a molecular formula of $C_{37}H_{44}O_{16}$ (*m*/*z* 745.2693 $[M + H]^+$, calcd. 745.2707) based on High resolution Fast Atom Bombardment Mass Spectrometry (HRFABMS). In accordance with the molecular formula, 16 degrees of unsaturation came from two carbon—carbon double bonds and six carbonyls; thus, the remaining eight degrees of unsaturation indicated **1** to be octacyclic. The IR absorption implied the presence of several carbonyl groups (ν_{max} 1747 and 1633 cm^{-1}). The ^1H and ^{13}C-NMR spectra (Table 1) exhibited signals assignable to two tertiary methyls [δ_H 0.98, 1.23 (each s)]; three acetyls [δ_H 1.96, 2.15, 2.30 (each 3H, s); δ_C 21.26, 21.33, 21.6 (each q), 169.3, 169.6, 172.0 (each s)], a propanoyl [δ_H 1.20 (3H, t), 2.26 (1H, m), 2.38 (1H, m); δ_C 21.3 (q), 28.0 (t), 172.0 (s)], a methyl ester [δ_H 3.69 (3H, s); δ_C 51.6 (q), 173.9 (s)], an 1,8,9-orthoacetyl group [δ_H 1.71 (3H, s), δ_C 20.6 (q), 84.0, 84.5, 85.3 (each s), 119.4 (s)] [16], four methylenes, five sp^3 methines including three oxymethine [δ_H 5.26 (s), δ_C 80.6 (d); 5.68 (s), δ_C 69.8 (d); 5.94 (s) δ_C 68.7 (d)], six sp^3 quaternary carbons including two oxycarbons [δ_C 86.1 (d), 86.4 (s)], a furan [δ_H 6.40 (dd), 7.37 (t), 7.68 (brd)], and a lactone [δ_C 174.2 (s)]. Analysis of the ^1H-^1H COSY spectrum of **1** revealed the partial structure shown in bold face in Figure 2. The HMBC connectivities between H_3-18 [δ_H 1.23 (s)]/C-12, C-13, C-14 and C-17 [δ_C 69.8 (d)]; between H-3 [δ_H 5.26 (s)]/C-1 [δ_C 84.6 (s)], C-2 [δ_C 86.1 (s)], C-4, C-5, C-28, C-29, and C-30 [δ_C 68.7 (d)]; between H_2-6 [δ_H 2.52 (d)], 2.68 (dd)]/C-4, C-5, C-7 [δ_C 174.2 (s)], and C-10 [δ_C 86.4 (s)]; between H-14 [δ_H 2.36 (dd)]/C-8 [δ_C 85.3 (s)], C-9 [δ_C 84.0 (s)], C-12, C-13, C-15, C-16 [δ_C 173.9 (s)], and C-30; between H-17 [δ_H 5.68 (s)] /C-12, C-13, C-14, C-20 [δ_C 122.5 (s)], C-21 [δ_C 142.0 (d)], and C-22 [δ_C 109.2 (d)]; between H_2-29 [δ_H 1.7 8 and 1.91 (each d)]/C-1, C-2, C-3, C-4, C-5, and C-10; and between H-30 [δ_H 5.94 (s)]/C-2, C-3, C-8, C-9, C-1 4, and C-1′′′′′ [δ_C 172.0 (s)] were obtained (Figure 2).

Table 1. ^1H- (600 MHz) and ^{13}C- (150 MHz) NMR spectroscopic data of compound **1**.

Position		1			Position		1		
		^1H [a] (*J*, Hz)	^{13}C [b]	HMBC			^1H [a] (*J*, Hz)	^{13}C [b]	HMBC
1			84.6 (s)		18		1.23	44.8 (q)	12, 13, 14, 17
2			86.1 (s)		20			122.5 (s)	
3		5.26 s	80.6 (s)	4, 5, 28, 30, 1″	21		7.68 brd (0.9)	142.0 (s)	17, 20, 22
4			44.6 (s)		22		6.40 dd (0.6, 1.7)	109.2 (d)	20, 23
5		2.82 d (10.1)	38.2 (d)	1, 3, 4, 6, 7, 10, 29	23		7.37 t (1.7)	143.2 (d)	20, 22
6	α	2.52 d (19.3)	30.0 (t)	4, 5, 7, 10	28		0.98	14.5 (q)	
	β	2.68 dd (10.1, 19.3)			29	*pro-R*	1.91	37.8 (t)	1, 2, 4, 5, 28
7			174.2 (s)		29	*pro-S*	1.78		
8			85.3 (s)		30		5.94 s	68.7 (d)	1, 2, 3, 8, 9, 14
9			84.0 (s)		31			119.4 (q)	
10			86.4 (s)		32		1.71 s	20.6 (q)	
11	α	1.82 m	24.7 (t)	8, 9, 10, 12, 13	1′			170.1 (s)	
	β	1.84 m			2′		2.15 s	21.6 (q)	1′
12	α	1.05 ddd (1.4, 7.1, 14.4)	31.5 (t)	9, 11, 13, 14, 17	1″			169.6 (s)	
	β	1.11 (2.9, 4.7, 14.4)			2″		2.30	21.33 (q)	1″
13			39.1 (s)		1‴		3.69 s	51.6 (q)	16
14		2.36 dd (7.6, 16.5)	47.6 (d)	8, 13, 15, 16, 17, 18, 30	1⁗			169.3 (s)	
15	α	2.84 dd (4.1, 16.5)	30.4 (t)	8, 13, 14, 16	2⁗		1.96 s	21.26 (q)	1‴
	β	2.20 m			1′′′′′			172.0 (s)	
16			173.9 (s)		2′′′′′		2.26, 2.38	28.0 (t)	1′′′′′
17		5.68 s	69.8 (d)	12, 13, 14, 20, 21, 22, 1‴	3′′′′′		1.20 t (7.3)	21.3 (q)	1′′′′′

[a] Measured at 600 MHz in $CDCl_3$. [b] Measured at 150 MHz in $CDCl_3$. Assignment are based on HMBC spectrum.

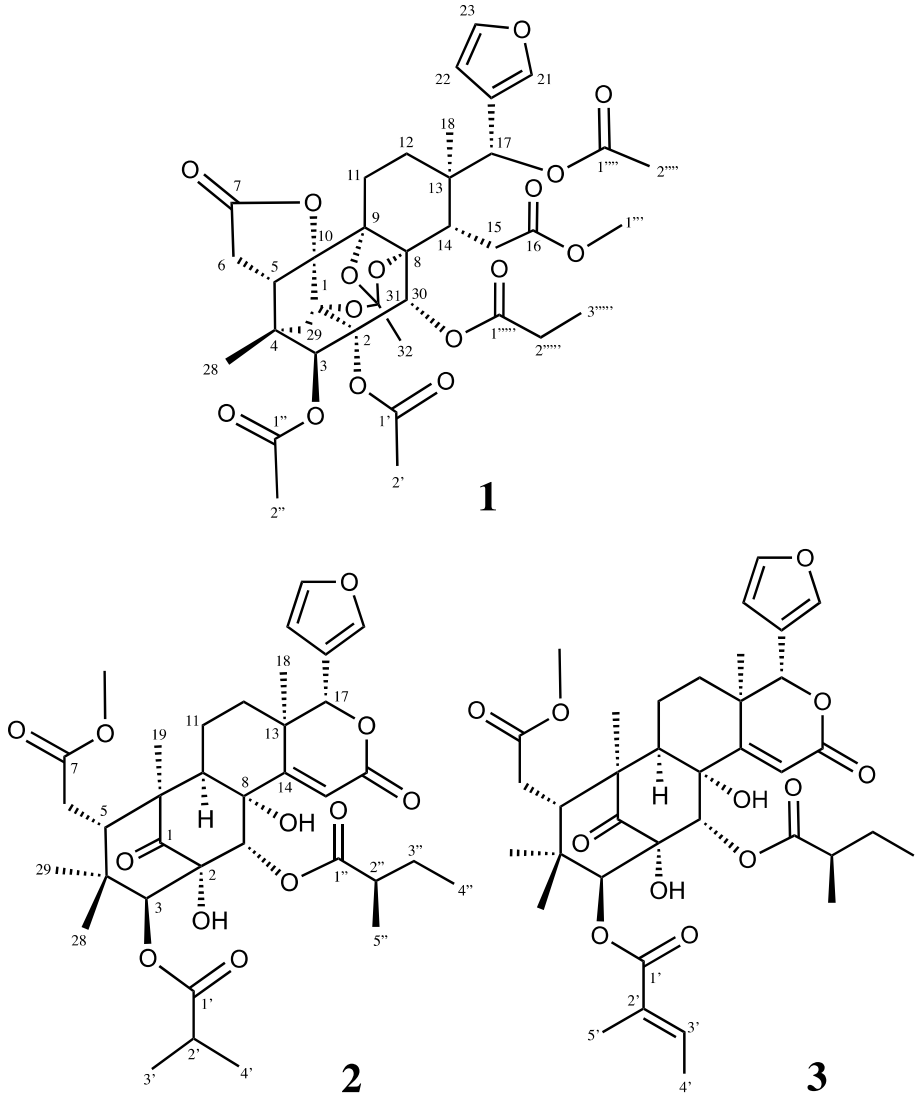

Figure 1. Structures of compounds **1**–**3** from the seeds of *C. guianensis*.

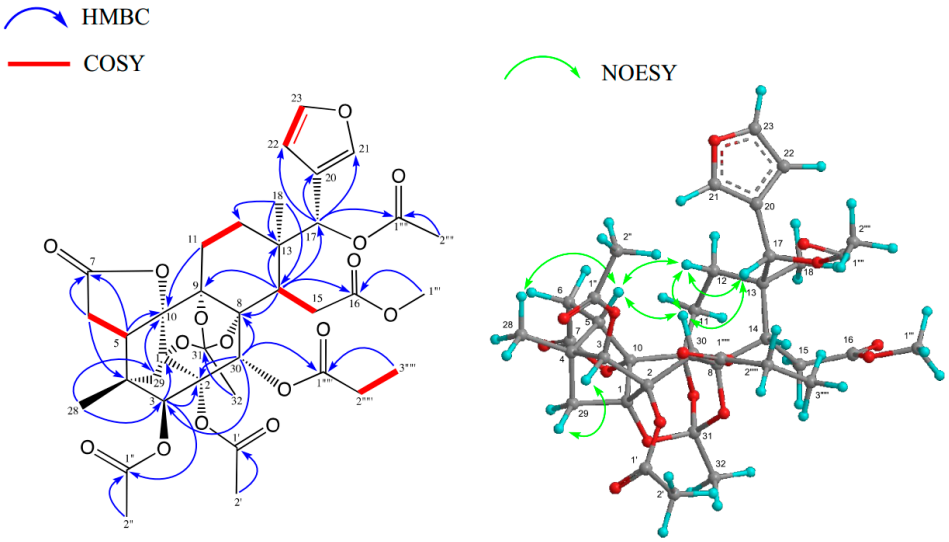

Figure 2. Key HMBC, COSY, and NOESY correlations of Carapanosin D (**1**).

The above NMR data of **1** were similar with those of andirolide O [17], the exclusive difference being lack of C-19 methylene in carapanosin D (**1**), which was confirmed by the HMBC correlations from H_2-6 and H_2-29 to the deshielded oxycarbon C-10 [δ_C 86.4 (s)], respectively. Therefore **1** would be a 19-*nor* limonoid, and the E ring has a γ-lactone. On the other hand, C-16–C-17 was opened and attached to methylester and acetate, respectively. Thus, the framework of **1** could be a C-19-*nor*, C-16,17-*seco*-phragmalin-1,8,9-orthoacetate. The relative configuration of **1** was determined by the NOESY spectrum, in which significant nuclear Overhauser effect (NOE) were observed between H-3 and H_2-29; between H-5 and H-12β, H-30, and CH_3-28; between H-14 and H-11β; between H-17 and H-12β, H-30; between H-30 and H-5, H-12β, H-15β, and H-17; and between CH_3-18 and H-11, H-12α, and H-22. Therefore, the relative structure of **1** was confirmed as shown in Figure 1. 19-*Nor*-phragmalin was first isolated from *Chukrasia tabularis* by Yin, J-L., et al., who described Tabulvelutin A as a unique 7,10-γ-lactone [18], carapanosin D (**1**) is the second example of 19-*nor*-phragmalin.

Carapanosin E (**2**) was obtained as a colorless amorphous crystal, and has a molecular formula of $C_{36}H_{48}O_{12}$ (*m*/*z* 673.3224 $[M + H]^+$, calcd. 673.3224) by HRFABMS. The IR absorptions implied the presence of hydroxy, ester, six-membered ring ketone, and αβ-unsaturated δ-lactone at ν_{max} 3489, 1727, 1710, and 1670 cm^{-1}. ^1H and ^{13}C-NMR spectra (Table 2) revealed the presence of four methyls [δ_H 0.83, 0.91, 1.09, 1.28 (each 3H, s)], 2-methylpropanoyl [δ_H 1.20 (3H, d), 1.27 (3H, d), 2.86 (1H, sept); δ_C 175.5 (s)], 2-methylbutanoyl [δ_H 0.87 (3H, t), 1.12 (3H, d), 1.46 m), 1.64 (1H, m), 2.43 (1H, m); δ_C 174.4 (s)], a methylester [δ_H 3.71 (3H, s); δ_C 52.3 (q), 173.8 (s)], an αβ-unsaturated δ-lactone [δ_H 6.34 (1H, s), δ_C 115.5 (d), 164.9 (s), 165.8 (s)], a six-membered ring ketone [δ_C 204.1 (s)], two tertiary hydroxyls that disappear by heavy water processing [δ_H 2.84, 4.08 (each 1H, s)], and a β-substituted furan ring [δ_H 6.47 (dd), 7.44 (t), 7.45 (d)], therefore, **2** could be suggested as a mexicanolide-type limonoid. The HMBC connectivities between H_3-18 [δ_H 1.28 (s)]/C-12, C-13, C-14 [δ_C 165.8 (s)], and C-17 [δ_C 78.9 (d)]; H_3-19 [δ_H 1.09 (s)]/C-1 [δ_C 204.1 (s)], C-5, C-9, and C-10; H-3 [δ_H 5.15 (s)]/C-1, C-2 [δ_C 86.3 (s)], C-4, C-5, C-28, C-29, and C-30 [δ_C 73.9 (d)]; H-15 [δ_H 6.34 (s)]/C-8 [δ_C 80.6 (s)], C-13, C-14, and C-16 [δ_C 164.9 (s)]; H-17 [δ_H 5.44 (s)]/C-12, C-13, C-16, C-20 [δ_C 120.3 (s)], C-21 [δ_C 141.6 (d)], and C-22 [δ_C 110.5 (d)]; H-30 [δ_H 6.51 (s)]/C-1, C-2, C-3 [δ_C 79.7 (s)], C-8, C-9, and C-14; 2-O*H* (δ_H 4.08 (s)/C-1, C-2, and C-30; 8-O*H* (δ_H 2.84 (s)/C-8, C-9, C-14, and C-30 were observed. In the ^1H-^1H COSY spectrum, five distinct spin sets of H-5–H-6; H-9–H_2-11–H_2-12; H-22–H-23; H_3-3′–H-2′–H_3-4′; and H_3′′′′-5–H-2′′′′–H_2-3′′′′–H_3-4′′′′ were observed (Figure 3). These results estimate the plain structure of **2** as shown in Figure 3. The relative configuration of **2** was mainly established by a NOESY experiment. It has strong cross-peaks of H_3-18/H-9α, H-12α, H-15, H-21, and H-23; H_3-19/H-6α, H-9α, H-11α, H_3-29, and 2-OH; 8-OH/2- OH; H-3/H-6α, and H_3-29, therefore, the relative structure was established as shown in Figure 1. The configuration of 2-methylbutanoyl group at C-30 was deduced to be *R* because the chemical shift value of Me-5′′′ [δ_H 1.12 (d, *J* = 7.2 Hz); δ_C 16.7 (q)] were in accordance with those of carapanolide F [δ_H 1.02 (d, *J* = 7.2 Hz); δ_C 16.0 (q)] [10], which was determined as *R* by a single-crystal X-ray diffraction analysis.

Carapanosin F (**3**) has the molecular formula $C_{37}H_{48}O_{12}$ (*m*/*z* 673.3224 $[M + H]^+$, calcd. 673.3224) as determined by HRFABMS. The UV, IR spectra showed αβ-unsaturated δ-lactone and hydroxyl, ester, and a six-membered ring ketone [UV λ_{max} (CH_3CN) nm (log ε): 232 (3.82); IR ν_{max} cm^{-1} (KBr): 3462, 1727, 1707]. NMR data were very similar to those of **2** except for a tigroyl group [δ_H 1.91 (s), 1.92 (d), 6.88 (m); δ_C 12.4 (q), 14.7 (q), 128.8 (s), 138.2 (d)] at C-3. NOESY spectrum revealed the relative stereochemistry of **3** to have the same conformation as **2**.

Physiological nitric oxide (NO) plays important roles in blood pressure regulation and blood flow distribution. However, its overexpression may cause multiple organ dysfunction, tissue injury, and systemic inflammatory responses in sepsis, such as hypotension, vascular hyporeactivity, and cardiodepression [19]. In this study, three limonoids and N^G-monomethyl-L-arginine acetate (L-NMMA), which is an inducible nitric oxide synthase (iNOS) inhibitor, were assayed for their inhibitory effects on NO production in LPS stimulated RAW 264.7 cells. Cytotoxicities of limonoids tested were evaluated by the [3-(4,5-dimethylthial-2-yl)-2,5-diphenyltetrazalium bromide] (MTT)

assay for determination of safe concentrations. Mexicanolide-type limonoids **2** and **3** exhibited stronger inhibitory activities (IC_{50} of NO produced **2**: 23.9 μM; **3**: 11.8 μM) than the positive control, L-NMMA (IC_{50} of NO produced 47.6 μM) without cytotoxicities (Figure 4). These results demonstrated that compounds **2** and **3** have potency as inhibitors of NO production. However, the effect of compounds **1** and **2** were inferior to gedunin type limonoids such as gedunin (IC_{50} of NO produced **2**: 4.6 μM), 6α-acetoxygedunin (IC_{50} 7.9 μM), 7-deacetoxy-7-hydroxygedunin (IC_{50} 8.7 μM), and 6α-acetoxy-7α-deacetoxy-7α-hydroxygedunin (IC_{50} 9.4 μM) from the flower oil of *C. guianensis* [20].

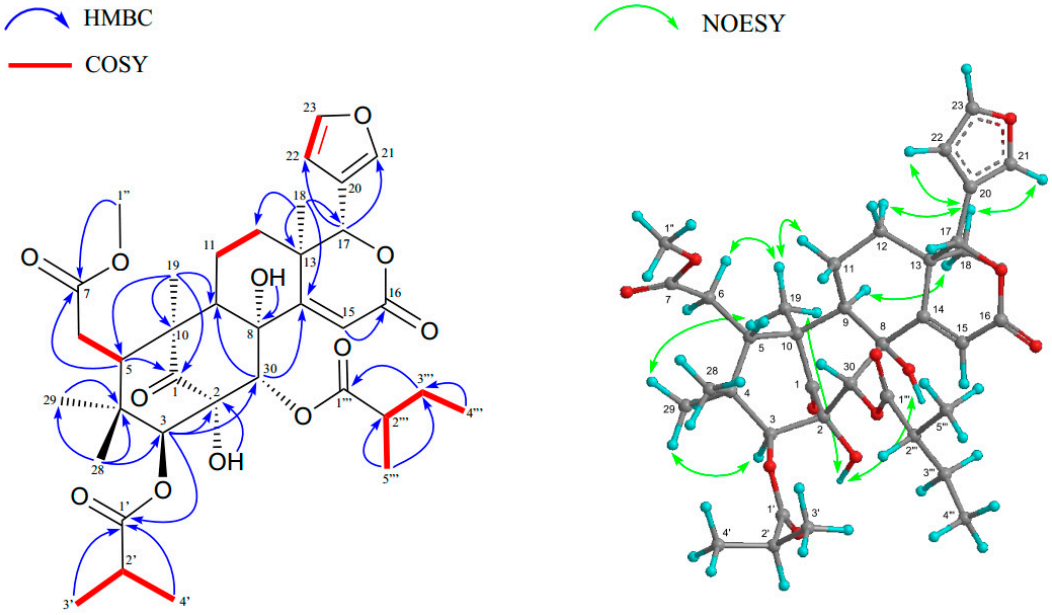

Figure 3. Key HMBC, COSY, and NOESY correlations of Carapanosin E (**2**).

Table 2. ^{1}H and ^{13}C-NMR spectroscopic data of compounds **2** and **3** (600 MHz, $CDCl_3$, 150 MHz).

Position		2		3	
		^{1}H [a] (*J*, Hz)	^{13}C [b]	^{1}H [a] (*J*, Hz)	^{13}C [b]
1			204.1 (s)		204.0 (s)
2			86.3 (s)		86.2 (s)
3		5.15 s	79.7 (d)	5.14 s	80.4 (d)
4			43.4 (s)		43.6 (s)
5		2.62 dd (6.7, 1.5)	38.6 (d)	2.68 t (1.0)	38.9 (d)
6	α	2.45 dd (18.2, 1.5)	32.9 (t)	2.39 t (1.0)	32.9 (t)
	β	2.34 dd (18.2, 6.7)		2.46 t (1.0)	
7			173.8 (s)		173.9 (s)
8			80.6 (s)		80.4 (s)
9		2.47 dd (12.9, 6.2)	65.7 (d)	2.45 m	65.4 (d)
10			55.1 (s)		55.7 (s)
11	α	1.71 m	19.9 (t)	1.72 m	20.0 (t)
	β	1.50 m		1.48 m	
12	α	1.56 m	30.1 (t)	1.54 m	30.2 (t)
	β	1.76 m		1.77 m	
13			39.3 (s)		39.3 (s)
14			165.8 (s)		166.0 (s)
15		6.34 s	115.5 (d)	6.22 s	115.4 (d)
16			164.9 (s)		164.8 (s)
17		5.44 s	78.9 (d)	5.43 s	78.9 (d)
18		1.28 s	21.2 (q)	1.27 s	21.3 (q)

Table 2. *Cont.*

Position		2		3	
		^{1}H [a] (*J*, Hz)	^{13}C [b]	^{1}H [a] (*J*, Hz)	^{13}C [b]
19		1.09 s	18.8 (q)	1.09 s	18.8 (q)
20			120.3 (s)		120.3 (s)
21		7.44 t (1.8)	141.6 (d)	7.45 dd (0.1, 0.2)	141.7 (d)
22		6.47 dd (1.8, 0.9)	110.5 (d)	6.47 dd (0.1)	110.5 (d)
23		7.45 d (0.9)	143.0 (d)	7.44 t (0.2)	143.0 (d)
28		0.83 s	25.0 (q)	0.92 s	21.3 (q)
29		0.91 s	21.4 (q)	0.86 s	25.5 (q)
30		6.51 s	73.9 (d)	6.36 s	74.4 (d)
1′			175.5 (s)		166.2 (s)
2′		2.86 sept (7.1)	34.3 (d)		128.8 (s)
3′		1.20 d (7.1)	18.1 (q)	6.88 q (7.1)	138.2 (d)
4′		1.27 d (7.1)	19.8 (q)	1.91 d (7.1)	12.4 (q)
5′				1.92 s	14.7 (q)
1″		3.71 s	52.3 (q)	3.72 s	52.3 (q)
1‴			174.4 (s)		174.1 (s)
2‴		2.43 m	40.8 (d)	2.39 m	40.7 (d)
3‴	A	1.46 m	26.5 (t)	1.43 dq (1.3, 1.2)	26.5 (t)
	B	1.64 m		1.60 dq (1.3, 1.2)	
4‴		0.87 t (7.2)	11.4 (q)	0.84 t (7.1)	11.3 (q)
5‴		1.12 d (7.2)	16.7 (q)	1.09 d (7.1)	16.7 (q)
2-OH		4.08 s		4.08 s	
8-OH		2.84 s		2.83 s	

[a] Measured at 600 MHz in $CDCl_3$. [b] Measured at 150 MHz in $CDCl_3$. Assignments are based on HMBC spectrum.

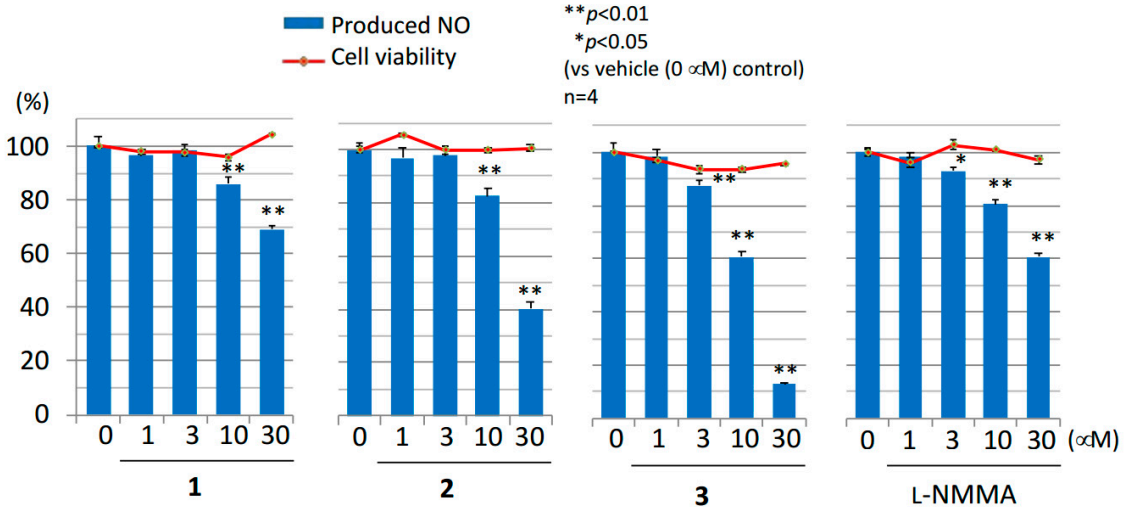

Figure 4. Inhibitory activities on nitic oxide (NO) production and cytotoxicities of Compounds **1**–**3** and N^{G}-monomethyl-L-arginine acetate (L-NMMA). Each value represents the mean and the standard error (S.E.) of four determinations. Significant differences from the vehicle control (0 μM) group shown as: * $p < 0.05$ and ** $p < 0.01$ in the NO inhibitory assay.

3. Experimental Section

3.1. General Procedures

Melting points were determined on a Yanagimoto micro-melting point apparatus (YANAKO Measuring Instrument Trading Corporation, Kyoto, Japan) and were uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter (JASCO Corporation, Tokyo, Japan).

IR spectra were recorded using a PerkinElmer 1720X FTIR spectrophotometer (PerkinElmer Japan Co. Ltd., Yokohama, Japan). All NMR experiments were measured with a Varian INOVA 600 spectrometer (Varian Medical Systems, Tokyo, Japan) with standard pulse sequences, operating at 600 and 150 MHz. $CDCl_3$ was used as the solvent and Tetramethylsilane (TMS) as the internal standard. FABMS were recorded on a JEOL-7000 mass spectrometer (70 eV) (JEOL Ltd., Tokyo, Japan). Column chromatography (CC) was carried out on silica gel 60 (70–230 mesh) (Merck Chemicals B.V., Tokyo, Japan) and MPLC was carried out with silica gel (230–400 mesh) (Merck Chemicals B.V., Tokyo, Japan). HPLC was completed using a JASCO PU-1586 instrument (JASCO Corporation, Tokyo, Japan) equipped with a differential refractometer (RI 1531). Fractions obtained from column chromatography were monitored by thin-layer chromatography (TLC) (silica gel 60 F_{254}) (Merck Chemicals B.V., Tokyo, Japan).

3.2. Plant Material

The seed oil (2.03 kg) of Andiroba (*Carapa guianensis* A_{ublet}, Meliaceae) was collected in the Amazon, Brazil, in March, 2013. It was kindly provided by Mr. Akira Yoshino (who is a representative person of the "NGO Green Heart love Amazon project"). A voucher specimen (CGS-01-2) was deposited in the Herbarium of the Laboratory of Medicinal Chemistry, Osaka University of Pharmaceutical Sciences.

3.3. Isolation of Compounds **1**–**3**

The seed oil of Andiroba (*Carapa guianensis* Aublet, Meliaceae) (2.03 kg) was dissolved in $CHCl_3$, and the $CHCl_3$ solution was subjected to CC (silica gel 14 kg), and affording 7 fractions: Fractions A (Fr. No. 1–76, 900 g), B (Fr. No. 77–110, 12.0 g), C (Fr. No. 111–125, 21.0 g), D (Fr. No. 126–155, 10.9 g), E (Fr. No. 156–170, 1.4 g), F (Fr. No. 171–180, 2.4 g), G (Fr. No. 181–195, 2.9 g), and H (Fr. No. 196–208, 0.7 g) [15]. Fraction D was rechromatographed over a silica gel open-column (230–400 mesh, 200 g) eluted with *n*-hexane–AcOEt (1:1) to give eight fractions: D(1) (Fr. No. 1–35, 4.52 g), D(2) (Fr. No. 36–49, 1.81 g), D(3) (Fr. No. 50–88, 1.40 g), D(4) (Fr. No. 89–115, 0.93 g), D(5) (Fr. No. 116–130, 0.60 g), D(6) (Fr. No. 131–140, 0.52 g), D(7) (Fr. No. 141–205, 0.47 g), and D(8) (Fr. No. 206–215, 0.24 g). Fraction D(4) was subjected to a silica gel open-column (230–400 mesh, 100 g) eluted with *n*-hexane–EtOAc (3:1) to give an amorphous solid (34.1 mg) that was purified by HPLC (ODS, 75% MeOH) to give compounds **2** (6.2 mg, Retention time: 53.3 min.) and **3** (1.79 mg, Retention time: 52.5 min.). Fraction D(5) was subjected to a silica gel open-column (230–400 mesh, 60 g) eluted with *n*-hexane–EtOAc (3:1) to give an amorphous solid (24.0 mg) that was purified by HPLC (ODS, 75% MeOH) to give compound **1** (4.5 mg, Retention time: 42.8 min.).

3.4. Analytical Data

Carapanosin D (**1**): Colorless amorphous solid; $[\alpha]_D^{20}$ −9.5° (*c* = 0.1, EtOH); HRFABMS *m*/*z*: calcd. for $C_{37}H_{44}O_{16}$, $[M + H]^+$: 745.2693; found 745.2707; IR (KBr) ν_{max} cm^{-1}; 2975, 1747(O–C=O), 1633; for ^1H and ^{13}C-NMR spectroscopic data, see Table 1; FABMS *m*/*z* (rel. int.): 745 (100), $[M + H]^+$, 685 $[M + H - HOAc]^+$ (72), 449 (33).

Carapanosin E (**2**): Colorless amorphous solid; m.p. 96–98 °C; $[\alpha]_D^{26}$ −25.8° (*c* 0.1, $CHCl_3$); HRFABMS: *m*/*z* calcd for $C_{36}H_{49}O_{12}$ $[M + H]^+$: 673.3224; found 673.3224; UV λ_{max} (CH_3CN) nm (log ε): 219 (3.76); IR (KBr) ν_{max} cm^{-1}: 3489 (OH), 2974, 1727 (O–C=O), 1710 (six membered ring ketone), 1670 (αβ-unsaturated δ-lactone and 1461; for ^1H and ^{13}C-NMR spectroscopic data, see Table 2; FABMS *m*/*z* (rel. int.): 673 (27) $[M + H]^+$, 57 (100).

Carapanosin F (**3**): Colorless amorphous solid; m.p. 83–85 °C; $[\alpha]_D^{26}$ +16.6° (*c* 0.1, $CHCl_3$); HRFABMS *m*/*z* calcd. for $C_{37}H_{49}O_{12}$ $[M + H]^+$: 685.3224; found 685.3224; UV λ_{max} (CH_3CN) nm (log ε): 232 (3.82), IR (KBr) ν_{max} cm^{-1}: 3462 (OH), 2970, 1727 (O–C=O), 1707 (six membered ring ketone), 1670

(αβ-unsaturated δ-lactone), 1549, and 1461; for ^{1}H and ^{13}C-NMR spectroscopic data, see Table 2; FABMS *m*/*z* (rel. int.): 685 (11) $[M + H]^+$, 83 (100).

3.5. Cell Cultures

RAW264.7 cells (mouse macrophages) purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan) were incubated in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) in a 5% CO_2 humidified incubator at 37 °C.

3.6. Determination of RAW264.7 Cell Proliferation

RAW264.7 cell proliferation was examined as described previously [13].

3.7. Inhibitory Assay of NO Production

An inhibitory assay of nitric oxide production was performed as describe previously [13].

4. Conclusions

A novel *nor*-phragmalin-type limonoid, named carapanosin D (**1**), and two new mexicanolide-type limonoids, named carapanosins E and F (**2**, **3**) were isolated from the seeds of *Carapa guianensis* (andiroba). Their structures were elucidated by extensive spectroscopic techniques. Carapanosin D (**1**) is the second example of 19-*nor*-phragmalin. Compounds **1**–**3** showed non-toxicities at 0–30 μM. Of these, compounds **2** and **3** showed superior inhibitory activities (IC_{50} of NO produced **2**: 23.9 μM; **3**: 11.8 μM) compared to the positive control, L-NMMA (IC_{50} of NO produced 47.6 μM). These results suggest that compounds **2** and **3** have high potency as inhibitors of macrophage activation.

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