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Article

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 seeds oil of *Carapa guianensis* Aublet (andiroba), 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**—**3** were evaluated for their effects on the production of NO in LPS-activated mouse peritoneal macrophages. The NO inhibitory assay suggested that compound **2** may be valuable as potential 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. Limonoids in the plant kingdom occur mainly in the Meliaceae and Rutaceae families [1]. Andiroba is one of the Meliaceae plant in the rain forests of South America. Its woody four cornered nut has four cells, each of which contains two to three seeds with oil-rich kernels. Extracts from its bark, flowers, 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]. On the other hand, Andiroba oil is a rich source of essential fatty acids including oleic, palmitic, stearic, and linoleic acids. It yields up to 65% unsaturated fatty

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acids and can contain approximatoly 9% linoleic acid. In Latin America, pharmacies offer the oil often mixed with copaiba oil and honey for internal healing. Andiroba oil transports healing alpha-hydroxyl acids and other ingredients as they moisturize and protect the skin.

Our previous study 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], and Carapanolides J—L [11], Carapanolides M—S [12], Carapanolides T—X [13], Carapanosins A – C [14] in the seed oil of andiroba. Recently, we reported the absolute structure of Guianolactones A and B from the seed oil of *Carapa guianasis* (Meliaceae) [15]. Our recent study on the components of the seed oil of *Carapa guianasis* revealed the structures of a new unusual 19-*nor*-phragmalin-type limonoid, named Carapanosin D and two novel mexicanolide-type limonoids, named Carapanosins E and F. We herein described the isolation and structural determination of three new limonoids, and the effects of **1**—**3** on the production of NO in LPS-activated mouse peritoneal macrophages. The structures of **1**—**3** were determined on the basis of NMR spectroscopy, including 1D and 2D (^{1}H, ^{1}H-COSY, NOESY, HSQC, HMBC) NMR, and FABMS.

2. Results and Discussion

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

C. guianensis seeds were separated by SiO_2 column chromatography, medium-pressure liquid chromatography (MPLC), and reverse phase HPLC in order to obtain the novel limonoids **1**—**3**.

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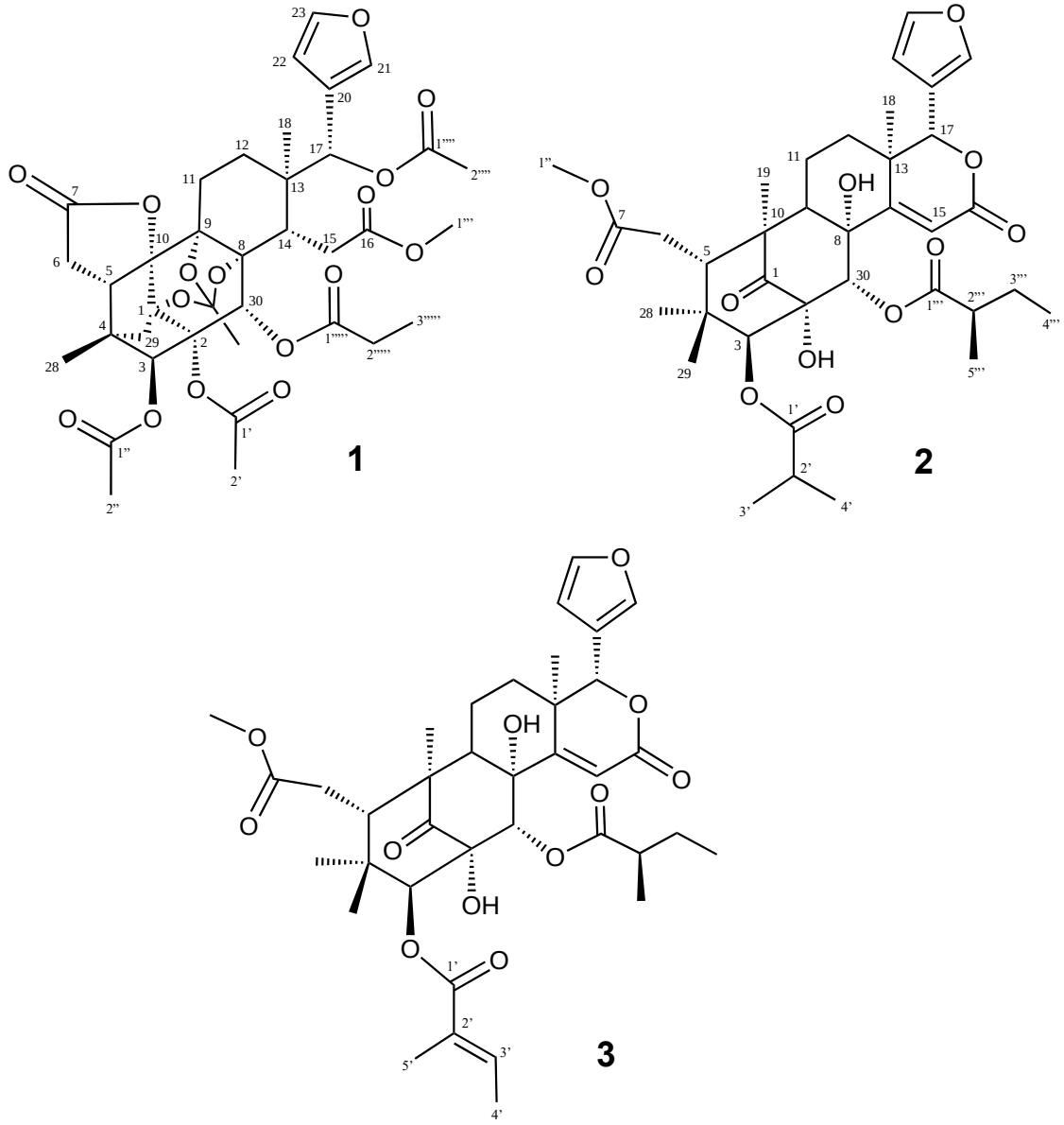

Fig. 1. Structures of compounds **1**—**3** from the seeds of *C. guianensis*.

Carapanosin D (**1**), a colorless crystal, was assigned the molecular formula $C_{37}H_{44}O_{16}$ (*m/z* 745.2693 $[M + H]^+$, calcd 745.2707) based on HRFABMS, indicating the presence of 16 degrees of unsaturation came from two carbon—carbon double bonds and six carbonyls; thus, the remaining 8 degrees of unsaturation indicated **1** to be octacyclic. The IR spectrum showed 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 methyl groups [δ_H 0.98, 1.23 (each s)]; three acetyl groups [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 group [δ_H 1.20 (3H, t),

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

Position		1			Position		1		
		^{1}H [a] (*J*, Hz)	^{13}C [b]	HMBC			^{1}H [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	a	2.52 d (19.3)	30.0 (t)	4, 5, 7, 10	28		0.98	14.5 (q)	
6	b	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	a	1.82 m	24.7 (t)	8, 9, 10, 12, 13	1'			170.1 (s)	
	b	1.84 m			2'		2.15 s	21.6 (q)	1'
12	a	1.05 ddd (1.4, 7.1, 14.4)	31.5 (t)	9, 11, 13, 14, 17	1"			169.6 (s)	
	b	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	a	2.84 dd (4.1, 16.5)	30.4 (t)	8, 13, 14, 16	2""		1.96 s	21.26 (q)	1""
	b	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.

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)], 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)], 1,8,9-orthoester, a furan ring [δ_H 6.40 (dd), 7.37 (t), 7.68 (brd)], and a lactone [δ_C 174.2 (s)]. Analysis of the ^{1}H-^{1}H COSY spectrum of **1** revealed the partial structure shown in bold face in Figure 2. In the HMBC spectrum, cross-peaks were observed 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

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[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 1.78 and 1.91 (each d)]/C-1, C-2, C-3, C-4, C-5, and C-10; between H-30 [δ_H 5.94 (s)]/C-2, C-3, C-8, C-9, C-14, and C-1’’’’’ [δ_C 172.0 (s)] (Figure 2). The above NMR data of **1** were similar with those of andirolide O [16], the exclusive difference being lack of C-19 methylene in andirolide O, 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 19-*nor* limonoid, and E ring have a γ-lactone, C-16—C-17 was opened and the presence of a CH_2COOCH_3 and a $CHOCOCH_3$ at C-15 and C-17, respectively. Thus, the structure 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 NOEs 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; between CH_3-18 and H-11α, 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 et al., who described Tabulvelutin A as an unique 7,10-γ-lactone, Carapanosin D (**1**) is the second example of 19-*nor*-phragmalin [17].

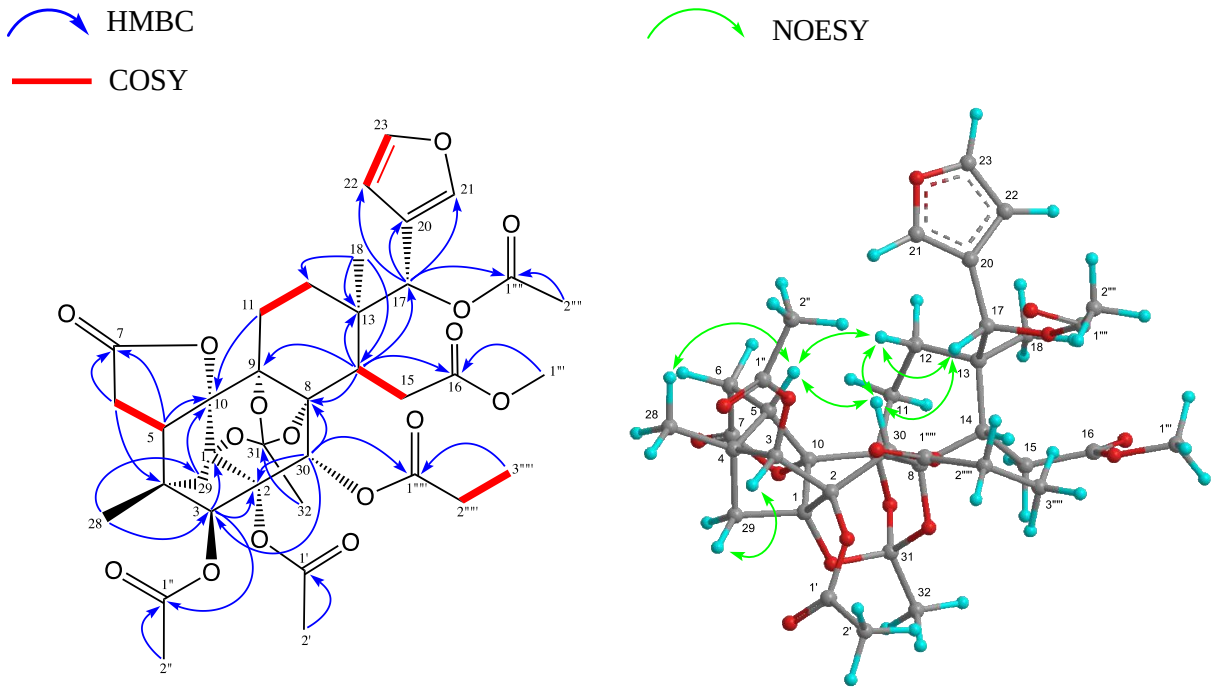

Figure 2. Key HMBC, COSY, and NOESY correlations of Carapanosin D (**1**).

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Carapanosin E (**2**) was obtained as a colorless amorphous crystal. Its molecular formula was determined to be $C_{36}H_{48}O_{12}$ (*m/z* 673.3224 $[M + H]^+$, calcd 673.3224) by HRFABMS. The IR spectrum indicated the existence 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 group [δ_H 1.20 (3H, d), 1.27 (3H, d), 2.86 (1H, sept); δ_C 175.5 (s)], 2-methylbutanoyl group [δ_H 0.87 (3H, t), 1.12

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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)
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 $CDCl_3$. Assignment are based on HMBC spectrum.

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(3H, d), 1.46 m), 1.64 (1H, m), 2.43 (1H, m); δ_C174.4 (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 hydroxyl groups which disappears 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** would be suggested mexicanolide-type limonoid. In the HMBC spectrum, the following correlations were observed: 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. The ^{1}H-^{1}H COSY spectrum (H-5–H-6; H-9–H_2-11–H_2-12; H-22–H-23; H_3-3'–H-2' –H_3-4'; H_3-5''''–H-2''''–H_2-3''''-H_3-4'''') revealed the positions of substituents (Figure 3). These results suggested the plain structure of **2** was shown in Figure 1. The relative configuration of **2** was mainly established by a NOESY experiment. 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-O*H*; 8-O*H*/2-O*H*; 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.

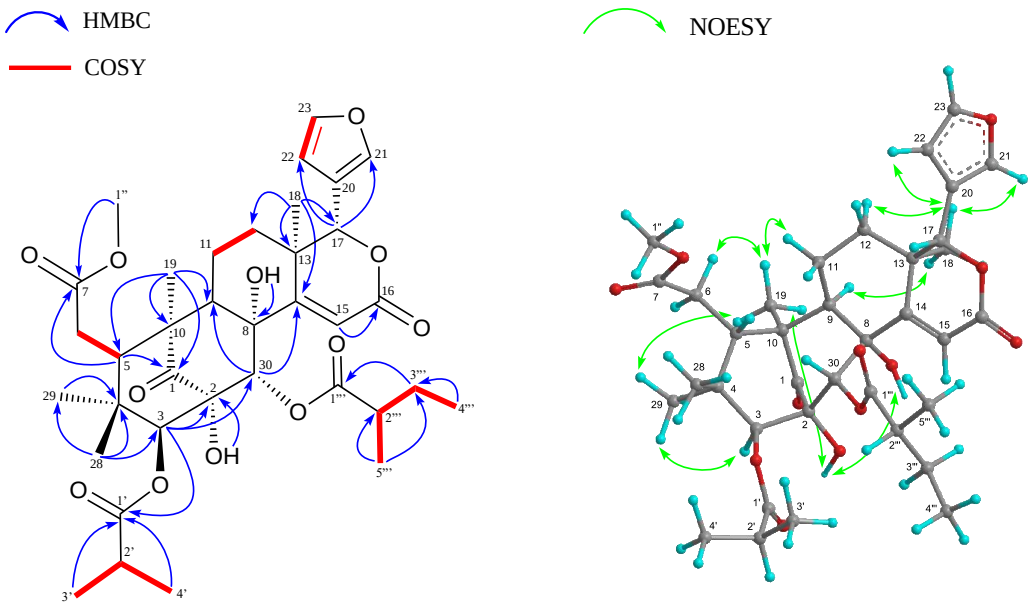

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Figure 3. Key HMBC, COSY, and NOESY correlations of Carapanosin E (**2**).

Carapanosin F (**3**) had the molecular formula $C_{37}H_{48}O_{12}$ (*m/z* 673.3224 $[M+H]^+$, calcd 673.3224) by HRFABMS. The UV, IR spectra showed αβ-unsaturated δ-lactone and hydroxyl, ester, and six-membered ring ketone [UV λ_{max} (CH_3CN) nm (log ε): 232 (3.82); IR ν_{max} cm^{-1} (KBr): 3462, 1727, 1707]. The ^1H and ^{13}C NMR spectra 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) is involved in blood pressure regulation and blood flow distribution, whereas its overexpression may induce tissue injury, multiple organ dysfunction, and death, as well as systemic inflammatory responses in sepsis, such as hypotension, cardiodepression, and vascular hyporeactivity [17]. In the present study, three limonoids and L-NMMA, an inducible nitric oxide synthase (iNOS) inhibitor, were evaluated for the its inhibitory effects on NO production in LPS-stimulated RAW264.7 cells. To determine safe concentrations, the cytotoxicities of these limonoids

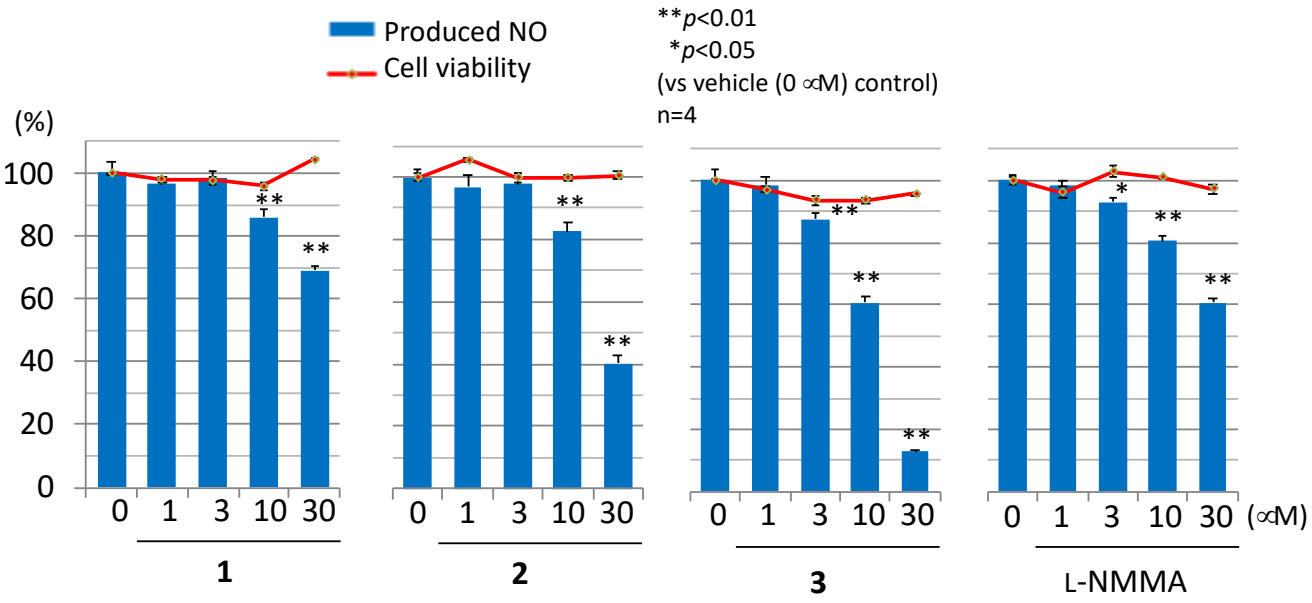

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Figure 4. Inhibitory activities on NO production and cytotoxicities of Compounds **1**–**3** and L-NMMA. Each value represents the mean the standard error (S.E.) of four determinations. Significant differences from the vehicle control (0 μM) group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

against RAW 264.7 were assessed by the MTT assay. Compound **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) to the positive control, L-NMMA (IC_{50} of NO produced 47.6 μM). These results suggested that compounds **2** and **3** may be valuable as potential inhibitors of NO production.

3. Experimental Section

3.1. General procedures

Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected. Optical rotations were measured using a JASCO DIP-1000 digital polarimeter. IR spectra were recorded using a Perkin-Elmer 1720X FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian INOVA 500 spectrometer with standard pulse sequences, operating at 500 and 125 MHz, respectively. $CDCl_3$ was used as the solvent and TMS, as the internal standard. FABMS were recorded on a JEOL-7000 mass spectrometer (70 eV). Column chromatography was carried out over silica gel (70-230 mesh, Merck) and MPLC was carried out with silica gel (230-400 mesh, Merck). HPLC was run on a JASCO PU-1586 instrument equipped with a differential refractometer (RI 1531). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F_{254}, Merck).

3.2. Plant material

The oil of (2.03 kg) *Carapa guianensis* AUBLET (Meliaceae) was collected in the Amazon, Brazil, in March, 2013. 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 *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), afford in 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,

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2.9 g), and H (Fr. No. 196-208, 0.7 g) [15]. Fraction D was rechromatographed over a silica gel column (CC) (230–400 mesh, 200 g) eluted with *n*-hexane–AcOEt (1:1) to give 8 fractions: D1 (Fr. No. 1–35, 4.52 g), D2 (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), D(8) (Fr. No. 206–215, 0.24 g). Fraction D(4) was subjected to CC (230–400 mesh, 100 g) eluted with *n*-hexane–EtOAc (3:1) to give an amorphous solid (34.1 mg) that was separated by HPLC (ODS, 75% MeOH) to give compounds **2** (6.2 mg) and **3** (1.79 mg). Fraction D(5) was subjected to CC (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).

3.4. Analytical Data

Carapanosin D (**1**): Colorless amorphous; $[\alpha]_D^{20}$ -9.5˚ (*c* 0.1, EtOH); HRFABMS *m/z*: 745.2693 $[M + H]^+$ ($C_{37}H_{44}O_{16}$, calcd for 745.2707); IR (KBr) ν_{max} cm^{-1}; 2975, 1747(O-C=O), 1633; ^1H and ^{13}C NMR, 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; mp 96-98˚C; $[\alpha]_D^{26}$ -25.8˚ (*c* 0.1, $CHCl_3$); HRFABMS *m/z*: 673.3224 $[M + H]^+$ ($C_{36}H_{49}O_{12}$, calcd for 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; ^1H and ^{13}C NMR, see Table 2. FABMS *m/z* (rel. int.): 673 (27) $[M + H]^+$, 57 (100).

Carapanosin F (**3**): Colorless amorphous solid; mp 83-85˚C; $[\alpha]_D^{26}$ +16.6˚ (*c* 0.1, $CHCl_3$); HRFABMS *m/z*: 685.3224 $[M + H]^+$ ($C_{37}H_{49}O_{12}$, calcd for 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; ^1H and ^{13}C NMR, see Table 2. FABMS *m/z* (rel. int.): 685 (11) $[M + H]^+$, 83 (100).

3.5. Cell Cultures

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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% CO_2 humidified incubator.

3.6. Determination of RAW264.7 cell proliferation

RAW264.7 cell proliferation was examined according to a method reported previously [17] with little modifications. Briefly, RAW264.7 cells (5×10^4 cells in 100 μL) were seeded onto 96-well microplates, and incubated for 24h. D-MEM (100 μL) containing test samples (final concentration of 100, 30, 10, or 3 μM) dissolved in DMSO (final concentration 0.2 %) was added. After the cells had been treated for 24h, the MTT solution was added. After 3 h of incubation, 20% sodium dodecyl sulfate (SDS) in 0.1 M HCl was added to dissolve the formazan produced by 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%.

3.6. Inhibitory assay of NO production

An inhibitory assay of nitric oxide production was performed according to a method reported previously [14] with slight modifications. Briefly, RAW264.7 cells (5×10^4 cells in 100 μL) were seeded onto 96-well microplates, and incubated for 24h. D-MEM (100 μL) containing test samples (final concentration of 100, 30, 10, or 3 μM) dissolved in DMSO (final concentration 0.2 %) and LPS (final concentration of 5 μg / mL) were added. After cells had been treated for 24 h, 50 μL of 0.1 % *N*-(1-naphtyl)ethylenediamine in H_2O and 50 μL of 1 % sulfanilamide in 5 % phosphoric acid were added. After being incubated for 30 min, 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%.

4. Conclusions

A novel *nor*-phragmalin-type limonoid, named Carapanosin D (**1**) and two novel mexicanolide-type limonoids, named Carapanosins E and F (**2** – **3**) were isolated from the seeds of *Carapa guianensis* (andiroba). Their structures were determined by spectroscopic analyses. Compound **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) to the

positive control, L-NMMA (IC_{50} of NO produced 47.6 μM). These results suggested that compounds **2** and **3** may be valuable as potential inhibitors of NO production.

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