Carapanolides T–X from *Carapa guianensis* (Andiroba) Seeds

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Abstract: Two new mexicanolide-type limonoids, carapanolides T–U (1–2), and three new phragmalin-type limonoids, carapanolides V–X (3–5), were isolated from the seeds of *Carapa guianensis* (andiroba). Their structures were determined on the basis of 1D- and 2D-NMR spectroscopy.

Keywords: Carapa guianensis; Meliaceae; andiroba; seeds; limonoid; mexicanolide; phragmalin; NO production

1. Introduction

Limonoids, a series of structurally-diverse and highly-oxygenated tetranortriterpenes, are mainly found in the Meliaceae family and have been attracting attention from biogenetic and synthetic perspectives [1–3]. *Carapa guianensis* Aublet (Meliaceae), known locally as andiroba, is a tall tropical tree that is widely distributed in the Amazonas State of Brazil, and its wood is extensively used as commercial timber. The towering tree of andiroba grows up to 40 m in height. Extracts from its bark, flowers and seeds have been used for centuries by the Amazonian people and exhibit various repellent [4], analgesic [5], anti-bacterial [6], anti-inflammatory [7], wound healing [8], anti-malarial [9], anti-allergic [10] and anti-plasmoidal [11] activities, in addition to acute and subacute toxicities [12]. Our previous study on the components of the seed oil of *Carapa guianensis* revealed the structures of two new unusual 9,10-seco-mexicanolide-type limonoids, carapanolides A and B [13], two novel carbon skeletal limonoids, guianolides A and B [14], and carapanolides C–I [15], carapanolides J–L [16] and carapanolides M–S [17]. We herein describe the isolation and structural determination of five novel limonoids, carapanolides T–X (1–5). The structures of 1–5 were determined on the basis of NMR spectroscopy, including 1D and 2D (¹H, ¹H-COSY, NOESY, HSQC, HMBC) NMR and HRFABMS.

2. Results and Discussion

Carapanolide T (1) (Figure 1) was obtained as a colorless amorphous solid, and its molecular formula was established as C₃₁H₄₀O₁₀ ([M + H]⁺; m/z 573.2704, calcd. for 573.2697) by HRFABMS, implying 12 on the degrees of unsaturation. IR and UV spectra showed the presence of hydroxy groups at νₚₚₚ 3462 cm⁻¹, ester groups at νₚₚₚ 1727 cm⁻¹ and αβ-unsaturated δ-lactone at λₚₚₚ 230 nm (log ε 3.85). ¹H- and ¹³C-NMR data indicated that eight out of the 12 units of...
unsaturation came from three carbon-carbon double bonds and three ester carbonyls, including a lactone carbonyl and ketone.

Therefore, the remaining degrees of unsaturation required 1 to be pentacyclic. The ¹H- and ¹³C-NMR spectra of 1 (Table 1) indicated the presence of four tertiary methyls (δ_H 0.69, 0.86, 1.23, 1.27 (each s)), a 2-methyl propanoyl (δ_H 1.25 and 1.27 (each 3H, d), 2.71 (1H, sept); δ_C 19.1 and 19.2 (each q), 34.3 (d), 176.2 (s)), methyl ester (δ_H 3.71 (s); δ_C 52.2 (q), 173.6 (s)), four methylenes (δ_C 20.7 (t), 32.9 (t), 33.7 (t), 45.0 (t)), four sp³ methines, including two oxymethines (δ_H 4.80 (s), 5.16 (s)), a furan ring (δ_H 6.49 (dd), 7.44 (t), and 7.51 (brs)), an α,β-unsaturated δ-lactone (δ_H 5.16 (s), 6.18 (s); δ_C 78.8 (d), 116.3 (d), 167.5 (s)), five sp³ quaternary carbons, including two oxycarbons (δ_C 72.8 (s), 76.7 (s)), and a ketone (δ_C 216.8 (s)). An analysis of the ¹H-¹H COSY spectrum of 1 revealed the partial structures shown in bold face in Figure 2.

In the HMBC spectrum (Figure 2), cross-peaks were observed from Me-18 (δ_H 1.27 (s)) to C-12, C-13, C-14 (δ_C 164.7 (s)) and C-17 (δ_C 78.8 (d)), from Me-19 (δ_H 1.23 (s)) to C-1 (δ_C 216.8 (s)), C-5, C-9, and C-10, from H-5 (δ_H 3.36 (dd)) to C-4, C-6, C-7 (δ_C 173.6 (s)) and C-10, from H-30 (δ_H 2.51 and 3.55 (each d)) to C-1, C-2 (δ_C 76.7 (s)), C-3 (δ_C 85.5 (d)), C-8 (δ_C 72.8 (s)) and C-9 and from H-15 (δ_H 6.18 (s)) to C-8, C-13, C-14 (δ_C 164.7 (s)) and C-17. In the ¹H-¹H COSY spectrum, (H-5–H-2–H-9–H-11–H-12;
H-22–H-23; H-3′′–H-3′′′ and H-3‴″‴) revealed the partial structure shown in Figure 2. Significant NOE interactions (Figure 2) were observed from H-3 (δ_H 4.80 (s))/Me-28, Me-29; H-5 (δ_H 3.36 (dd))/H-11β, Me-28, H-30β; 2-OH (δ_H 4.05 (s))/H-12α, Me-19; 8-OH (δ_H 2.81 (s))/H-9, H-12α, Me-18; H-17 (δ_H 5.16 (s))/H-5, H-11β, H-12β and H-30β, which indicated the α-orientation of H-3, H-9, Me-18, Me-19, 2-OH and 8-OH and the β-orientation of H-5 and H-17. Therefore, the relative structure of 1 was established as shown in Figure 1.

Figure 2. Key HMBC, COSY and NOESY correlations for carapanolide T (1).

Carapanolide U (2) was isolated as colorless needles and shown to have the molecular formula C_{32}H_{40}O_{10} (m/z: 585.2701 [M + H]^+ (calcd for 585.2700) by HRFABMS. IR and UV spectra revealed the presence of hydroxy and ester groups and an αβ-unsaturated δ-lactone at ν_{max} 3537, 1748 and 1719 cm⁻¹, and λ_{max} at 230 nm (log ε 4.11). The 1H- and 13C-NMR spectra of 2 (Table 1) were very similar to those of 1, except for the absence of the 2-methylpropanoyl group at C-3 and the presence of the tigroyl group at C-3. The relative structure of 2 was determined as shown in Figure 1.

Carapanolide L (3) was obtained as colorless crystals, and its molecular formula was established as C_{35}H_{38}O_{14} ([M + H]^+; m/z 683.2335, calcd. for 683.2340) by HRFABMS, implying 17 on the index of hydrogen deficiency. IR and UV spectra revealed the presence of ester groups and an αβ-unsaturated δ-lactone at ν_{max} 1748 and 1719 cm⁻¹ and λ_{max} at 226 nm (log ε 3.73). 1H- and 13C-NMR data indicated that eight out of the 17 units of unsaturation came from three carbon-carbon double bonds and five ester carbonyls, including two lactone carbonyls. Therefore, the remaining degrees of unsaturation required 3 to be non-acyclic. The 1H- and 13C-NMR spectra of 3 (Table 2) indicated the presence of two tertiary methyls (δ_H 1.02, 1.14 (each s)), two acetylts (δ_H 2.04 (s), 2.17 (s); δ_C 20.8 (q), 21.8 (q), 169.1 (s), 170.1 (s)), an n-propanoyl group (δ_H 1.07 (3H, t), 2.25 (1H, m), 2.29 (1H, m); δ_C 8.5 (q), 27.4 (t), 173.3 (s)), an orthoacetyl group (δ_H 1.68 (3H, s); δ_C 20.9 (q), 120.1 (s)), four methylenes, including an oxymethylene (δ_H 4.34 and 4.86 (each 1H, d), four sp³ methines, including three oxymethines (δ_H 5.10 (s), 5.27 (s) and 5.78 (s)), a furan ring (δ_H 6.44 (dd), 6.44 (t) and 7.52 (brs)), seven sp³ quaternary carbons, including four oxy carbons (δ_C 82.6, 83.9, 84.2, 84.6 (each s)), and two lactone carbonyl groups (δ_C 163.0 and 171.0 (each s)). An analysis of the 1H-1H COSY spectrum of 3 revealed the partial structures shown in bold face in Figure 3. In the HMBC spectrum (Figure 3), cross-peaks were observed from Me-18 (δ_H 1.14 (s)) to C-12, C-13, C-14 (δ_C 159.6) and C-17 (δ_C 80.4), from Me-28 (δ_H 1.02 (s)) to C-3 (δ_C 81.3), C-4, C-5 and C-29, from H-30 (δ_H 5.78 (s)) to C-1 (δ_C 84.6 (s)), C-2 (δ_C 84.2 (s)), C-3, C-8 (δ_C 82.6 (s)) and C-9 (δ_C 83.9 (s)) and from H-15 (δ_H 6.05 (s))
to C-8, C-13, C-14 and C-16 (δC 163.0 (s)). Therefore, the planar structure of 3 was established as phragmalin-1,8,9-orthoacetate [13], and the positions of the two acetyl and an n-propyl group were located at C-2, C-3 and C-30, respectively, by detailed 1H-1H COSY and HMBC correlations (Figure 3). In the NOESY spectrum, significant NOEs (Figure 3) were observed between H-3 and H-29 pro-S, H-30 and Me-28, between H-5β (δH 2.72 (dd)) and H-12β, Me-28 and H-30 between H-15 (δH 6.05 (s)) and H-17β and H-30, between H-17β (δH 5.10 (s)) and H-12β, H-15, H-22 and H-30β and between Me-18 (δH 1.14 (s)) and H-11α, H-12α and Me-32. Therefore, the relative structure of 3 was established as shown in Figure 1.

Table 1. 1H- (600 MHz) and 13C- (150 MHz) NMR spectroscopic data of compounds 1 and 2.

| Position | 1 | 2 |
|----------|---|---|
|          | 1H a (J, Hz) | 13C b | 1H a (J, Hz) | 13C b |
| 1        | 216.8 |      | 216.7 |
| 2        | 76.7  |      | 77.0  |
| 3        | 4.80 s | 85.5 | 4.88 s | 85.8 |
| 4        | 39.7  |      | 40.0  |
| 5        | 3.36 dd (9.4, 1.5) | 41.8 | 3.39 dd (7.9, 1.1) | 41.8 |
| 6 α      | 2.34 dd (17.3, 1.5) | 32.9 | 2.37 m | 32.9 |
| 6 β      | 2.38 dd (17.3, 9.4) | 2.34 m |          |
| 7        | 173.6 |      | 173.6 |
| 8        | 72.8  |      | 72.9  |
| 9        | 1.85 dd (13.3, 6.0) | 60.2 | 1.87 t (5.6) | 60.1 |
| 10       | 47.9  |      | 47.8  |
| 11 α     | 1.48 m | 20.7 | 1.67 m | 20.7 |
| 11 β     | 1.52 m | 1.53 m |          |
| 12 α     | 1.28 m | 33.7 | 1.34 m | 33.8 |
| 12 β     | 2.00 ddd (14.1, 6.8, 3.6) | 2.02 m |          |
| 13       | 38.5  |      | 38.5  |
| 14       | 164.7 |      | 164.6 |
| 15       | 6.18 s | 116.3 | 6.16 s | 116.3 |
| 16       | 167.5 |      | 167.6 |
| 17       | 5.16 s | 78.8 | 5.18 s | 79.8 |
| 18       | 1.27 s | 23.2 | 1.28 s | 23.2 |
| 19       | 1.23 s | 18.3 | 1.24 s | 18.4 |
| 20       | 119.8 |      | 119.8 |
| 21       | 7.51 brs | 141.7 | 7.51 br s | 141.7 |
| 22       | 6.49 dd (2.1, 0.9) | 110.4 | 6.49 m | 110.4 |
| 23       | 7.44 t (2.1) | 143.1 | 7.44 t (1.4) | 143.1 |
| 28       | 0.69 s | 22.3 | 0.70 s | 22.4 |
| 29       | 0.86 s | 22.6 | 0.88 s | 22.6 |
| 30 α     | 2.51 d (16.0) | 45.0 | 2.52 dd (14.9, 1.2) | 45.0 |
| 30 β     | 3.55 d (16.0) | 3.58 d (14.9) |          |
| 3'       | 176.2 |      | 167.2 |
| 3''      | 2.71 sept (6.7) | 34.3 |          | 128.1 |
| 3'''     | 1.25 d (6.7) | 19.1 | 6.96 q (7.1) | 138.8 |
| 3''''    | 1.27 d (6.7) | 19.2 | 1.88 d (7.1) | 14.7 |
| 3''''''  | 1.92 s |          | 12.4 |
| 7*       | 3.71 s | 52.2 | 3.70 s | 52.1 |
| 2-OH     | 4.05 s |          |          |
| 8-OH     | 2.81 s |          |          |

a Measured at 600 MHz in CDCl₃. b Measured at 150 MHz in CDCl₃.
Carapanolide W (4) was obtained as colorless crystals, and its molecular formula was established as C_{34}H_{40}O_{14} ([M + H]^+; m/z 673.2492, calc. for 673.2496) by HRFABMS. IR and UV spectra revealed the presence of hydroxy groups and ester groups and an \( \alpha\beta \)-unsaturated \( \delta \)-lactone at \( \nu_{\text{max}} \) 3657, 1728 and 1698 cm\(^{-1} \) and \( \lambda_{\text{max}} \) at 230 nm (log \( \epsilon \) 3.73). The \(^1\)H- and \(^13\)C-NMR spectra of 4 (Table 2) indicated the presence of three tertiary methyl groups (\( \delta^H 0.74, 1.32, 1.48 \) (each s)), an acetyl (\( \delta^H 2.09 \) (s); \( \delta^C 21.7 \) (q), 169.1 (s)), an \( n \)-propanoyl group (\( \delta^H 1.16 \) (3H, t), 2.45 (2H, m); \( \delta^C 8.9 \) (q), 28.1 (t), 173.8 (s)), methyl ester (\( \delta^H 3.71 \) (3H, s); \( \delta^C 52.3 \) (q), 173.9 (s)), three methylene groups, five \( sp^3 \) methine groups, including four oxymethine groups (\( \delta^H 3.86 \) (dd), 5.22 (s), 5.35 (s), 5.90 (s)), a furan ring (\( \delta^H 6.61 \) (dd), 7.53 (br s), 7.64 (t)), seven \( sp^3 \) quaternary carbons, including four oxycarbons (\( \delta^C 83.5 \) (s), 83.7 (s), 84.2 (s), 86.1 (s)), three ester carboxyls (\( \delta^C 169.1 \) (s), 173.8 (s), 173.9 (s)) and an \( \alpha\beta \)-unsaturated \( \delta \)-lactone (\( \delta^H 6.62 \) (s), \( \delta^C 123.7 \) (d), 163.5 (s)). Cross-peaks were observed in the HMBC spectrum, from Me-18 (\( \delta^H 1.48 \) (s)) to C-12 (\( \delta^C 66.6 \) (d)), C-13, C-14 (\( \delta^C 153.8 \) (s)) and C-17 (\( \delta^C 78.8 \) (d)), from Me-19 (\( \delta^H 1.32 \) (s)) to C-1 (\( \delta^C 84.2 \) (s)), C-5, C-9 (\( \delta^C 86.1 \) (s)) and C-10, from H-15 (\( \delta^H 6.62 \) (s)) to C-8 (\( \delta^C 83.7 \) (s)), C-13, C-14 and C-16 (\( \delta^C 163.5 \) (s)) and from H-30 (\( \delta^H 5.35 \) (s)) to C-1 (\( \delta^C 84.2 \) (s)), C-2 (\( \delta^C 83.5 \) (s)), C-3 (\( \delta^C 85.0 \) (d)), C-8 and C-9. The singlet oxymethine proton at \( \delta^H 5.22 \) was assigned to C-3 through HMBC correlations to C-1, C-2, C-5, C-28, C-29, C-30 (\( \delta^C 74.3 \) (d)) and C-3’ (\( \delta^C 169.1 \) (s)), while another singlet proton at \( \delta^H 5.35 \), showing HMBC correlations to C-1, C-2, C-8, C-9, C-14 (\( \delta^C 153.8 \) (s)) and C-30’ (\( \delta^C 173.8 \) (s)), was assigned to C-30. An oxymethine proton at \( \delta^H 5.22 \) (dd) was assigned to C-12 through HMBC correlations to C-9, C-11, C-13, C-14, C-17 (\( \delta^C 78.8 \) (d)) and C-18. In an analysis of the \(^1\)H-\(^1\)H COSY spectrum of 4, H-5–H-6; H-22–H-23; H-30’–H-30’’ revealed the partial structure. The relative configuration of 4 was established on the basis of NOE interactions. Significant NOE interactions were observed from H-5 (\( \delta^H 2.11 \) (m))/H-11\( \beta \), H-30, Me-28; H-30 (\( \delta^H 5.35 \) (s))/H-3, H-5, H-15; H-17 (\( \delta^H 5.90 \) (s))/H-5, H-12\( \beta \), H-15, H-21, H-22, which indicated the \( \alpha \)-orientation of H-3, Me-18 and Me-19 and the \( \beta \)-orientation of H-5, H-12, H-17 and H-30. Therefore, the relative structure of 4 was established as shown in Figure 1.
Table 2. $^1$H- (600 MHz) and $^{13}$C- (150 MHz) NMR spectroscopic data of compounds 3–5.

| Position | $^1$H a (J, Hz) | $^{13}$C b | $^1$H a (J, Hz) | $^{13}$C b | $^1$H a (J, Hz) a | ε c b |
|----------|----------------|------------|----------------|------------|------------------|-------|
| 1        | 84.6          | 84.2       | 84.2           | 85.1       | 171.0            | 169.4 |
| 2        | 84.2          | 83.5       | 83.5           | 79.6       | 173.9            |       |
| 3        | 5.27          | 81.3       | 5.22           | s          | 4.59             |       |
| 4        | 46.4          |            | 44.6           | 45.5       | s                |       |
| 5        | 2.72          | dd (4.7, 3.2) | 33.8         | m          | 39.9             |       |
| 6 α      | 2.64          | dd (17.0, 3.2) | 68.6         | m          | 33.7             | 71.5  |
| 6 β      | 2.39          | dd (17.0, 4.7) | 2.34         | m          | 5.98             |       |
| 7        | 171.0         |            | 173.9          |           | 1.43 (s)         | 71.7  |
| 8        | 82.6          |            | 83.7           |           | 4.49             | 69.4  |
| 9        | 83.9          |            | 86.1           |           | d (2.3)          |       |
| 10       | 46.9          |            | 48.1           |           | 1.64             |       |
| 11 α     | 2.19          | m          | 25.6           | m          | 34.6             | 69.4  |
| 11 β     | 2.33          | m          | 2.21           | dd (14.7, 4.1) | 66.6   | 71.7  |
| 12 α     | 1.50          | m          | 26.5           | 3.86       | 4.48             |       |
| 12 β     | 1.64          | m          | 37.6           |           | d (2.3)          |       |
| 13       | 159.6         |            | 153.8          |           | 2.79             | 42.3  |
| 14       | 6.05          | s          | 121.0          | s          | dd (18.7, 10.4) | 26.9  |
| 15       | 6.05          | s          | 121.0          | s          | dd (18.7, 10.4) | 26.9  |
| 16       | 163.0         |            | 163.5          |           | 2.22             |       |
| 17       | 5.10          | s          | 80.4           | s          | 78.8             | 76.9  |
| 18       | 1.14          | s          | 18.7           | 1.48       | 13.0             | 15.8  |
| 19 α     | 4.86          | d (14.0)   | 31.4           | s          | 15.5             | 13.8  |
| 19 β     | 4.34          | d (14.0)   | 119.4          |           | 121.4            |       |
| 20       | 7.52          | br s       | 141.4          | 7.53       | 44.8             | 141.0 |
| 21       | 6.44          | dd (1.7, 0.6) | 109.7       | dd (1.7, 0.9) | 109.6   | 110.9 |
| 22       | 7.44          | t (1.7)    | 143.3          | t (1.7)    | 142.4            | 143.1 |
| 23       | 1.02          | s          | 14.2           | 0.74       | 14.5             | 15.4  |
| 24 pro-R | 1.78          | d (11.6)   | 39.2           | 1.72       | 39.8             | 39.9  |
| 24 pro-S | 2.38          | d (11.6)   | 1.96           | d (11.5)   | 2.06             |       |
| 30       | 5.78          | s          | 68.1           | 5.35       | 74.3             | 69.8  |
| 31       | 120.1         |            | 119.7          |           | 6.01             |       |
| 32       | 1.68          | s          | 20.9           | 1.70       | 16.5             | 21.1  |
| 32'      | 2.17          | s          | 21.8           |           | 1.76             |       |
| 3'       | 170.1         |            | 169.1          |           | 169.1            |       |
| 3''      | 169.1         |            | 169.1          |           | 169.6            |       |
| 3'''     | 20.8          | s          | 20.8           | 2.09       | 21.7             | 21.2  |
| 4''      | 3.71          | s          | 52.3           | 3.69       | 53.1             |       |
| 5''      | 170.1         |            | 173.8          |           | 173.8            |       |
| 5'''     | 2.22          | s          | 21.4           |           | 169.6            |       |
| 6''      | 1.70          | s          | 20.1           |           | 169.7            |       |
| 7''      | 1.70          | s          | 20.1           |           | 169.6            |       |
| 8''      | 172.5         |            | 172.5          |           | 172.5            |       |

a Measured at 600 MHz in CDCl₃. b Measured at 150 MHz in CDCl₃.

Carapanolide X (5) was isolated as a colorless amorphous solid and had the molecular formula C₃₈H₆₈O₁₈ ([M + H]⁺; m/z 791.2765, calcd. for 791.2763) as determined by HRFABMS. The IR spectrum showed the presence of a hydroxyl at νmax 3352 cm⁻¹ and ester groups at νmax 1742 cm⁻¹. $^1$H- and $^{13}$C-NMR spectra (Table 2) indicated the presence of three methyls (δH 1.10, 1.23, 1.43 (each 3H, s)), three acetyl groups (δH 1.70, 2.18, 2.22 (each 3H, s)), a propanoyl group (δH 1.09 (3H, t), 2.38 (2H, dq), δC 172.5 (s)), a methoxycarbonyl group (δH 3.69 (3H, s), δC 53.1 (q), 169.4 (s)), sp³ methylene (δC 26.9 (t)), δ-lactone (δC 5.98 (1H, s), δC 76.9 (d), 170.8 (s)), a tertiary hydroxyl group (δC 79.6 (s)), seven sp³ methines, including five oxymethines (δH 4.48 (d), 4.49 (d), 4.59 (s), 5.98 (s)), seven sp³ quaternary carbons, including four oxycarbons (δC 79.6 (s), 84.8 (s), 85.1 (s), 86.0 (s), and a furan ring (δH 6.46 (dd), 7.00 (t), 7.48 (brs)). In the HMBC spectrum, cross-peaks were observed between Me-18 (δH 1.43 (s)) and C-12 (δC 71.7 (d)), C-13, C-14 and C-17 (δC 76.9 (d)), between Me-19...
(δ\textsubscript{H} 1.23 (s)) and C-1 (δ\textsubscript{C} 85.1 (s)), C-5, C-9 (δ\textsubscript{C} 86.0 (s)) and C-10, between Me-28 (δ\textsubscript{H} 1.10 (s)) and C-1, C-3, C-4, C-5 and C-29 and between H-3 (δ\textsubscript{H} 4.59 (s)) and C-1 and C-2 (δ\textsubscript{C} 79.6 (s)), C-4, C-5, C-28, C-29, C-30 (δ\textsubscript{C} 69.8 (d)) and C-3\textsuperscript{1} (δ\textsubscript{C} 169.6 (s)), between H-5 (δ\textsubscript{H} 3.30 (brs)) and C-1, C-3, C-4, C-6 (δ\textsubscript{C} 71.5 (d)), C-7 (δ\textsubscript{C} 169.4 (s)), C-10, C-19, C-28 and C-29, between H-6 (δ\textsubscript{H} 5.98 (brs)) and C-4, C-5, C-7 and C-10 and between H-30 (δ\textsubscript{H} 6.01 (s)) and C-1, C-2, C-3, C-8 (δ\textsubscript{C} 84.8 (s)), C-9 and C-30\textsuperscript{1} (δ\textsubscript{C} 172.5 (s)).

In the 	extsuperscript{1}H-	extsuperscript{1}H COSY spectrum, (H-5–H-6; H-11–H-12; H-14–H-2–15; H-22–H-23 and H-30\textsuperscript{11}–H-30\textsuperscript{111}) was observed. Therefore, the positions of two hydroxyls, a propanoyl and a methoxycarbonyl were located at C-2, C-6, C-30 and C-7, while three acetyl groups were located at C-3, C-11 and C-12, respectively (Figure 4). In the NOESY experiments, cross peaks (Figure 4) were observed between H-3 (δ\textsubscript{H} 4.59 (s)) and H-29 pro-S, H-30 and Me-28, between H-5 (δ\textsubscript{H} 3.30 (brs)) and Me-28 and H-30, between H-6 (δ\textsubscript{H} 5.98 (brs)) and H-5 β and H-11, between H-11 (δ\textsubscript{H} 4.49 (d)) and H-5β and H-12, between H-12 (δ\textsubscript{H} 4.48 (d)) and H-5β, H-11 and H-17β, between H-30β (δ\textsubscript{H} 6.01 (s)) and H-11 and H-12 and between Me-19 and H-6 and Me-32. Therefore, the propanoyl group at C-30 and acetoxy groups at C-11 and C-12 were all α, while the acetoxy group at C-3 had a β orientation. The configuration of C-6 was presumed to be R, which was the same as that of carapanolide N [17].

Macrophages may be a potential therapeutic target for inflammatory diseases [18]. Activated macrophages release pro-inflammatory mediators, such as NO, reactive oxygen, 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 [18]. In the present study, four limonoids and \textsuperscript{N}\textsuperscript{G}-monomethyl-L-arginine acetate (L-NMMA), an inducible nitric oxide synthase (iNOS) inhibitor, were evaluated for their inhibitory effects on NO production in LPS-stimulated RAW264.7 cells (Figure 5). To determine safe concentrations, the cytotoxicities of these limonoids against RAW 264.7 were assessed by the MTT assay. In the NO inhibitory assay, Compounds 1 and 2 exhibited comparable NO inhibitory activities (IC\textsubscript{50} 1: 22.0 µM; 2: 23.3 µM) to L-NMMA (IC\textsubscript{50} 23.9 µM). Of these, 2 did not show cytotoxicities at 1–30 µM. Compound 1 exhibited low cytotoxicity at 30 µM, but not at the effective concentration, namely 10 µM. Compounds 3–5 did not exhibit inhibitory effects on macrophage activation (IC\textsubscript{50} >30 µM). These...
results suggested that Compounds 1 and 2 have potential as anti-inflammatory disease agents. In a previous study, we revealed inhibitory activities on NO production of a phragmalin-type limonoid, such as carapanolide J (IC$_{50}$ 37.4 µM) [16], and gedunin-type limonoids, such as 7-deacetoxy-7-oxogedunin (IC$_{50}$ 12.8 µM), 6α-acetoxygedunin (IC$_{50}$ 7.9 µM), 6α-hydroxygedunin (IC$_{50}$ 19.1 µM), 6α-acetoxy-7α-deacetoxy-7α-hydroxygedunin (IC$_{50}$ 9.4 µM), gedunin (IC$_{50}$ 4.6 µM) and 7-deacetoxy-7-hydroxygedunin (IC$_{50}$ 8.4 µM), [19]. Compounds 1 and 2 exhibited stronger inhibitory activities on NO production than carapanolide J, however weaker than the gedunin-type limonoids. These data suggest that gedunin-type limonoids are more effective for the inhibition of NO production than phragmalin-type ones in general.

Figure 5. Inhibitory activities on NO production and cytotoxicities of Compounds 1–5 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 and # $p < 0.05$ and ## $p < 0.01$ in the cytotoxicity assay.

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 (Perkin-Elmer Inc., Wellesley, MA, USA). $^1$H- and $^{13}$C-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. CDCl$_3$ was used as the solvent and TMS as the internal standard. FABMS
were recorded on a JEOL-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 run on a JASCO PU-1586 instrument (JASCO, Tokyo, Japan) equipped with a differential refractometer (RI 1531). Fractions obtained from column chromatography were monitored by TLC (silica gel 60 F254, Merck).

3.2. Plant Material

The oil of (2.03 kg) Carapa guianensis AUBLET (Meliaceae) was collected in the Amazon, Brazil, in March 2013, and was kindly provided by Mr. Akira Yoshino (who is a representative 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 1–5

The seed oil of Carapa guianensis AUBLET (Meliaceae) (2.03 kg) was dissolved in CHCl3, and the CHCl3 solution was subjected to CC (silica gel 14 kg), affording 7 fractions: Fraction A (fraction (Fr.) No. 1–85, 1.512 kg) was eluted with n-hexane-CHCl3 = 1:1; B (Fr. No. 86–179, 229.1 g) was eluted with CHCl3; C (Fr. No. 180–20, 29.3 g) was eluted with CHCl3–EtOAc = 5:1; D (Fr. No. 221–225, 13.2 g) was eluted with CHCl3–EtOAc = 2:1; E (Fr. No. 226–265, 84.5 g) was eluted with CHCl3–EtOAc = 2:1; F (Fr. No. 266–290, 25.3 g) was eluted with EtOAc; G (Fr. No. 291–315, 72.8 g) was eluted with EtOAc:MeOH = 5:1; and H (Fr. No. 316–333, 45.4 g) was eluted with MeOH. Residue D was rechromatographed over a silica gel column (CC) (230–400 mesh, 300 g) eluted with n-hexane–EtOAc (1:1) to give 13 fractions: D1 (Fr. No. 1–35, 1.52 g), D2 (Fr. No. 36–49, 0.81 g), D3 (Fr. No. 50–88, 0.70 g), D4 (Fr. No. 89–115, 0.53 g), D5 (Fr. No. 116–130, 0.60 g), D6 (Fr. No. 131–140, 0.52 g), D7 (Fr. No. 141–205, 0.47 g), D8 (Fr. No. 206–215, 0.51 g), D9 (Fr. No. 216–220, 0.42 g), D10 (Fr. No. 221–240, 0.40 g), D11 (Fr. No. 241–250, 1.11 g) and D12 (Fr. No. 251–313, 1.36 g). Fraction D(6) was subjected to CC (230–400 mesh, 40 g) eluted with n-hexane–EtOAc (3:1) to give an amorphous solid (24.1 mg) that was purified by HPLC (ODS, 75% MeOH, at 25 °C, flow rate 4.0 mL min⁻¹, UV = 220 nm, column 250 × 20 mm i.d., 5 μm) to give Compounds 2 (6.2 mg) and 3 (1.8 mg). Fraction D(8) was subjected to CC (230–400 mesh, 40 g) eluted with n-hexane–EtOAc (3:1) to give an amorphous solid (34.0 mg), which was subsequently subjected to CC (230–400 mesh, 40 g) eluted with n-hexane–EtOAc (3:1) to give an amorphous solid that was purified by HPLC (ODS, 75% MeOH, at 25 °C, flow rate 4.0 mL min⁻¹, UV = 220 nm, column 250 × 20 mm i.d., 5 μm) to give Compounds 1 (7.50 mg) and 4 (3.8 mg). Fraction D(9) was subjected to CC (230–400 mesh, 30 g) eluted with n-hexane–EtOAc (3:1) to give an amorphous solid (25.5 mg), which was subsequently separated by HPLC (ODS, 70% MeOH, at 25 °C, flow rate 4.0 mL min⁻¹, UV = 220 nm, column 250 × 20 mm i.d., 5 μm) to give Compound 5 (6.3 mg).

3.4. Analytical Data

**Compound 1**: Colorless crystals; mp 83–85 °C; [α]D 25 +16.6° (c 0.1, CHCl3); HRFABMS m/z: 573.2719 [M + H]⁺ (C31H42O10, calc 573.2720); UV (EtOH) λmax nm (log ε): 230 (3.85); IR (KBr) νmax cm⁻¹: 3462 (OH), 2970, 1727 (O=C=O), 1649 (C=C-C=O), 1461; 1H- and 13C-NMR, see Table 1. FABMS m/z (relative intensity (rel. int.)): 573 ([M + H]⁺, 100), 555 (11), 485 (20).

**Compound 2**: Colorless crystals; mp 133–136 °C; [α]D 25 −18.1° (c 0.1, EtOH); HRFABMS m/z: 585.2701 [M + H]⁺ (C32H41O10, calc 585.2700); UV λmax (EtOH) nm (log ε): 230 (4.11); IR (KBr) νmax cm⁻¹: 3537 (OH), 1748, 1719; 1H- and 13C-NMR, see Table 1. FABMS m/z (rel. int.): 607 (3) [M + Na]⁺, 585 (6), [M + H]⁺, 567 (6), 485 (7), 83 (100).

**Compound 3**: Colorless crystals; mp 181–184 °C; [α]D 25 −74° (c 0.4, EtOH); HRFABMS m/z: 683.2335 [M + H]⁺ (C35H50O14, calc 683.2340); UV λmax (EtOH) nm (log ε): 226 (3.73); IR (KBr) νmax cm⁻¹:
Compound 4: Colorless crystals; mp 144–146 °C; \([\alpha]^{25}_D +43.3^\circ\) (c 0.1, CHCl\(_3\)); HRFABMS \(m/z\): 791.2492 \([M + H]^+\) (C\(_{34}\)H\(_{41}\)O\(_{14}\), calcd for 791.2496); UV \(\lambda_{\text{max}}^{\text{(EtOH)}}\) nm (log \(\varepsilon\)): 208 (1.26), IR (KBr) \(\nu_{\text{max}}\) cm\(^{-1}\): 3352 (OH), 1742 (O-C=O); \(^1\)H- and \(^13\)C-NMR, see Table 2. FABMS \(m/z\) (rel. int.): 791 \([M + H]^+\) (55), 735 (5), 95 (54), 43 (100), 329 (14), 176 (47).

3.5. Determination of RAW264.7 Cell Proliferation

RAW264.7 cell proliferation was examined according to a method reported previously [20] with few modifications. Briefly, RAW264.7 cells (5 \(\times\) 10\(^4\) cells in 100 \(\mu\)L) were seeded onto 96-well microplates and incubated for 24 h. D-MEM (100 \(\mu\)L) containing test samples (final concentration of 100, 30, 10 or 3 \(\mu\)M) dissolved in DMSO (final concentration 0.2%) was added. After the cells had been treated for 24 h, 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 [16] with slight modifications. Briefly, RAW264.7 cells (5 \(\times\) 10\(^4\) cells in 100 \(\mu\)L) were seeded onto 96-well microplates and incubated for 24 h. D-MEM (100 \(\mu\)L) containing test samples (final concentration of 100, 30, 10 or 3 \(\mu\)M) dissolved in DMSO (final concentration 0.2%) and LPS (final concentration of 5 \(\mu\)g/mL) were added. After cells had been treated for 24 h, 50 \(\mu\)L of 0.1% \(N\)-(1-naphtyl)ethylenediamine in \(H_2O\) and 50 \(\mu\)L 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

Two novel mexicanolide-type limonoids, carapanolides T–U (1–2), as well as three novel phragmalin-type limonoids, carapanolides V–X (3–5), were isolated from the seeds of Carapa guianensis (andiroba). Their structures were determined by spectroscopic analyses. Compounds 1 and 2 were mexicanolide-type limonoids that had OH in C-2 and C-8. Compounds 3–5 were phragmalin-type limonoids that were highly oxidized.

In the NO inhibitory assay, Compounds 1 and 2 exhibited similar NO inhibitory activities (IC\(_{50}\) 1: 22.0 \(\mu\)M; 2: 23.3 \(\mu\)M) against L-NMMA (IC\(_{50}\) 23.9 \(\mu\)M). Compound 1 did not exhibit cytotoxicity at 1–30 \(\mu\)M, while Compound 2 exhibited low cytotoxicity at 30 \(\mu\)M, but not at an effective concentration at 10 \(\mu\)M. These results suggest that Compounds 1 and 2 have potential as anti-inflammatory disease agents.

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