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# Guianolides A and B, New Carbon Skeletal Limonoids from the Seeds of *Carapa guianensis*

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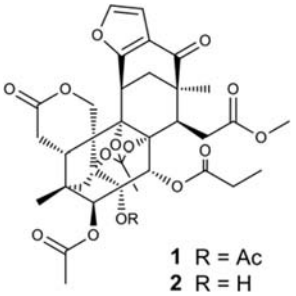
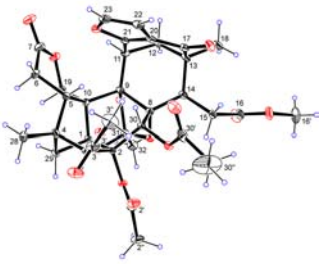
## ABSTRACT



**Two novel limonoids, named guianolides A (1) and B (2), were isolated from the seeds of *Carapa guianensis* AUBLET (Meliaceae). Their structures were established by spectroscopic analyses and X-ray crystallography. Guianolides A (1) and B (2) featured an unprecedented carbon skeleton via the formation of a C-11−C-21 bond.**

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] In recent years, a number of limonoids have been isolated by several research groups, such as phyllanthoids A and B,[2] aphanamixoid A,[3] walsucochinoids A and B,[4] chukrasone A,[5] Tabulvelutin A,[6] cipaferens A−D,[7] and walsogynes B−G.[8] Andiroba (*Carapa guianensis*, Meliaceae) is a tall tropical tree with fragment flowers, sometimes reaching up to 50 m tall. Andiroba is one of the largest leafed trees in the rain forests of South America. The woody four cornered nut has four cells, with each cell containing two to three seeds with oil-rich kernels. Seed oil from *C. guianensis* is used as

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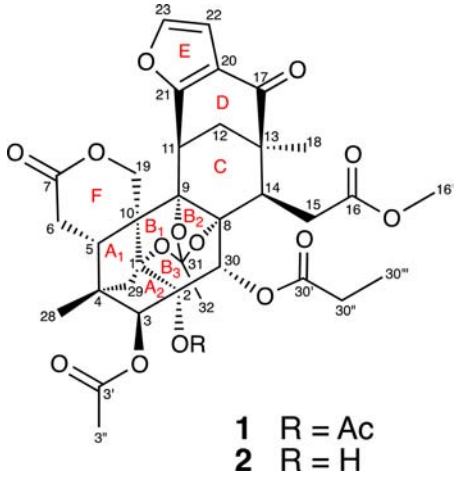
a repellent[9] and has analgesic,[10] antibacterial,[11] anti-inflammatory,[12] anticancerous,[13] and antiallergic[14] activities. It is also effective against arthritis and rheumatism.[15]

We recently reported the isolation and structure elucidation of carapanolides A and B, two novel 9,10-*seco*-2*R*,9*S*-epoxymexicanolide-type limonoids from the seeds of *C. guianensis*.[16] In the current study, two new carbon skeletal limonoids, named guianolides A (**1**) and B (**2**), were isolated from the seed oil of *C. guianensis*. Guianolides A (**1**) and B (**2**) featured an unprecedented carbon skeleton via the formation of the C-11−C-21 bond in phragmalin-1,8,9-orthoacetate. We describe herein the structures and cytotoxic activities of **1** and **2** against three tumor cell lines, P388, L1210, and HL-60.

Medium-pressure liquid chromatography (MPLC) and reverse phase HPLC were used to obtain the two new limonoids, **1** and **2**.




Guianolide A (**1**)[17] was obtained as a colorless crystal. Its molecular formula was determined to be $C_{36}H_{40}O_{15}$ (*m*/*z* 713.2445 $[M+H]^+$, calcd 713.2445) based on HRFABMS due to 17 degrees of unsaturation. The IR absorption bands indicated the existence of several carbonyl groups ($\nu_{max}$ 1749 $cm^{-1}$) and an $\alpha,\beta$-unsaturated six-membered ring ketone ($\nu_{max}$ 1682 $cm^{-1}$). The UV spectrum of **1** showed $\lambda_{max}$ 210 and 260 nm. The $^{13}C$ NMR spectrum of **1** suggested

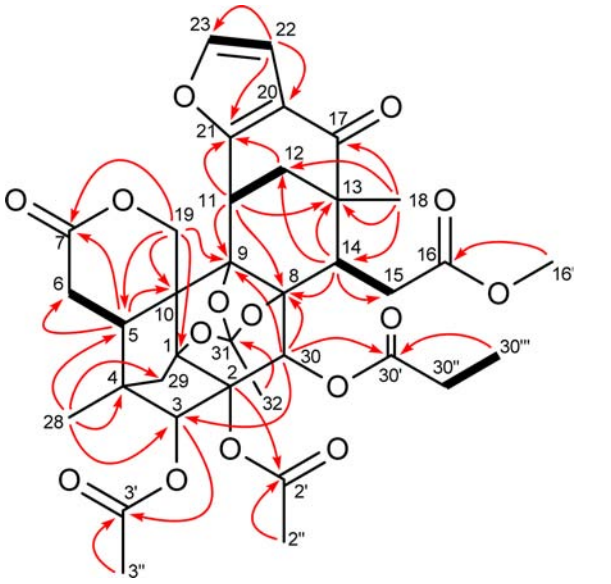


**Figure 1.** Key $^1H-^1H$ COSY (bold —) and HMBC (→(red)) correlations of **1**.

that 8 out of 17 degrees of unsaturation came from two carbon−carbon double bonds and six carbonyls; thus, the remaining 9 degrees of unsaturation indicated **1** to be nonacyclic. The $^1H$ and $^{13}C$ NMR spectra (Table 1) showed the presence of two acetyls [$\delta_H$ 1.89, 2.08 (each 3H, s)], a propanoyl [$\delta_H$ 1.10 (3H, t), 2.18 (2H, m); $\delta_C$ 170.6], a methyl ester [$\delta_H$ 3.71 (3H, s); $\delta_C$ 51.9 (q), 174.2 (s)], a $\delta$-lactone [$\delta_H$ 4.82, 4.89 (each 1H, d); $\delta_C$ 172.3 (s)], a ketone [$\delta_C$ 193.2 (s)], an orthoacetate [$\delta_H$ 1.81 (3H, s); $\delta_C$ 21.4 (q), 86.0 (s), 87.1 (s), 87.6 (s), 120.6 (s)], a tertiary methyl [$\delta_H$ 1.05 (3H, s)], five methylenes, five $sp^3$ methines including two oxymethines [$\delta_H$ 5.13 (s); $\delta_C$ 80.5 (d); $\delta_H$ 5.40 (s); $\delta_C$ 68.9 (d)], seven $sp^3$ quaternary carbons including four oxycarbons, a disubstituted olefin [$\delta_H$ 6.98 (d), 7.43 (d); $\delta_C$ 109.0 (d), 142.1 (d)], and a tetrasubstituted olefin [$\delta_C$ 123.6, 161.9 (each s)]. When **1** was compared with usual phragmalin-type limonoids, it lacked a methylene and one more methine in the DEPT and HSQC spectra. Analysis of the $^1H-^1H$ COSY spectrum (H-5−$H_2$-6; H-11−$H_2$-12; H-14−$H_2$-15; H-22−H-23; $H_2$-30‴−$H_3$-30‴) of **1** revealed the partial structures shown in boldface in Figure 1.

The planar structure of **1** was constructed by the detailed analysis of 1D and 2D NMR data, especially the HMBC spectrum. The A, B, and F rings were readily established as phragmalin-1,8,9-orthoacetate when compared with those of andirolides E, F, O, and P,[18,19] which were isolated from the flowers of *C. guianensis*, and the linkage of the substituents was confirmed by the HMBC spectrum (Figure 1). In the HMBC spectrum, two acetyl groups were attached to

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(17) Guianolide A (**1**): Colorless crystals (in *n*-hexane/$CHCl_3$); mp 143−145 °C; HRFABMS at *m*/*z*: 713.2445 $[M+H]^+$ (calcd for 713.2445, $C_{36}H_{41}O_{15}$); $[\alpha]_D^{22}$ +106.2° (*c* 0.100, $CHCl_3$); UV ($CH_3CN$) $\lambda_{max}$ (log $\varepsilon$) 260 (3.36), 210 (3.72); CD [0.000400 M, $CH_3CN$] $\lambda_{max}(\Delta\varepsilon)$: 228 (0), 262 (43.07), 289 (11.5), 330 (30.3), 358 (0); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3608, 2951, 2359, 1749, 1682, 1439; $^1H$ and $^{13}C$ NMR data, see Table 1.

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**Table 1.** $^{1}H$ and $^{13}C$ NMR Data for **1** and **2** in $CDCl_3$

| | **1** | | **2** | |
|---|---|---|---|---|
| | $\delta_C{}^a$ | $\delta_H$ (mult; $J$, Hz)$^a$ | $\delta_C{}^a$ | $\delta_H$ (mult; $J$, Hz)$^a$ |
| 1 | 86.0 (s) | | 86.2 (s) | |
| 2 | 86.8 (s) | | 80.8 (s) | |
| 3 | 80.5 (d) | 5.13 s | 83.2 (d) | 4.48 s |
| 4 | 45.5 (s) | | 44.8 (s) | |
| 5 | 35.1 (d) | 1.66 (t, 5.6) | 36.4 (d) | 1.78 (t, 5.6) |
| 6α | 31.3 (t) | 2.41 (dd, 16.5, 5.6) | 31.5 (t) | 2.41 (dd, 16.4, 5.6) |
| 6$\beta$ | | 3.14 (dd, 16.5, 5.6) | | 2.98 (dd, 16.4, 5.6) |
| 7 | 172.3 (s) | | 172.2 (s) | |
| 8 | 87.6 (s) | | 89.0 (s) | |
| 9 | 87.1 (s) | | 87.8 (s) | |
| 10 | 47.5 (s) | | 47.2 (s) | |
| 11 | 34.3 (d) | 4.00 (t, 3.2) | 34.4 (d) | 4.04 (t, 3.2) |
| 12α | 41.2 (t) | 2.35 (dd, 13.1, 3.2) | 40.9 (t) | 2.33 (dd, 13.2, 3.2) |
| 12$\beta$ | | 2.14 (dd, 13.1, 3.2) | | 2.14 (dd, 13.2, 3.2) |
| 13 | 45.9 (s) | | 45.9 (s) | |
| 14 | 51.5 (d) | 2.92 (m) | 51.0 (d) | 2.91 (m) |
| 15A | 30.1 (t) | 2.34 (m) | 29.7 (t) | 2.36 (m) |
| 15B | | 2.93 (m) | | 2.89 (m) |
| 16 | 174.2 (s) | | 174.1 (s) | |
| 17 | 193.2 (s) | | 193.2 (s) | |
| 18 | 21.9 (q) | 1.05 s | 21.9 (q) | 1.04 s |
| 19α | 68.8 (t) | 4.82 (d, 13.2) | 68.8 (t) | 4.66 (d, 13.2) |
| 19$\beta$ | | 4.89 (d, 13.2) | | 5.05 (d, 13.2) |
| 20 | 123.6 (s) | | 123.8 (s) | |
| 21 | 161.9 (s) | | 161.8 (s) | |
| 22 | 109.0 (d) | 6.98 (d, 1.7) | 109.0 (d) | 6.95 (d, 1.7) |
| 23 | 142.1 (d) | 7.43 (d, 1.7) | 142.1 (d) | 7.43 (d, 1.7) |
| 28 | 14.7 (q) | 0.93 s | 14.5 (q) | 0.94 s |
| $29_{pro\text{-}R}$ | 38.2 (t) | 2.38 (d, 11.7) | 37.2 (t) | 2.16 (d, 11.5) |
| $29_{pro\text{-}S}$ | | 1.66 (d, 11.7) | | 1.75 (d, 11.5) |
| 30 | 68.9 (d) | 5.40 s | 69.0 (d) | 5.01 s |
| 31 | 120.6 (s) | | 120.7 (s) | |
| 32 | 21.4 (q) | 1.81 s | 21.5 (q) | 1.82 s |
| 2′ | 169.9 (s) | | | |
| 2″ | 21.9 (q) | 2.08 s | | |
| 3′ | 169.1 (s) | | 169.9 (s) | |
| 3″ | 20.7 (q) | 1.89 s | 20.9 (q) | 1.86 s |
| 16′ | 51.9 (q) | 3.71 s | 52.1 (q) | 3.70 s |
| 30′ | 170.6 (s) | | 171.4 (s) | |
| 30″A | 27.4 (t) | 2.18 m | 27.9 (t) | 2.29 m |
| 30″B | | 2.18 m | | 2.29 m |
| 30‴ | 8.4 (q) | 1.10 (t, 7.0) | 8.7 (q) | 1.12 (t, 7.4) |
| 2-OH | | | | 2.88 s |

$^a$ $^{1}H$ NMR spectra were recorded at 600 MHz and $^{13}C$ NMR spectrum at 150 MHz.

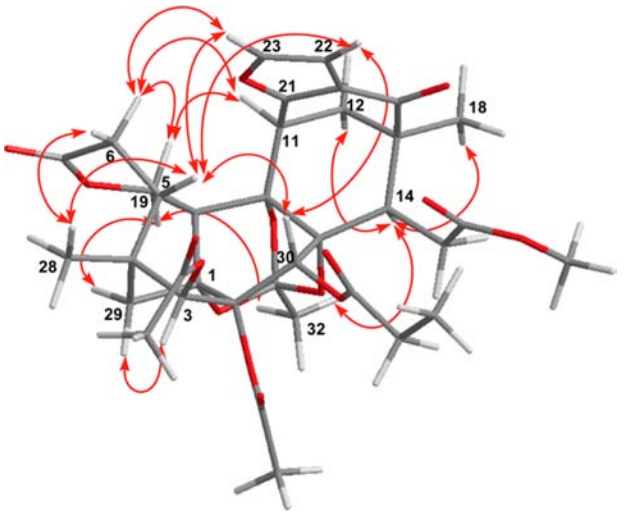



**Figure 2.** Key NOE correlations (↔) of **1**.

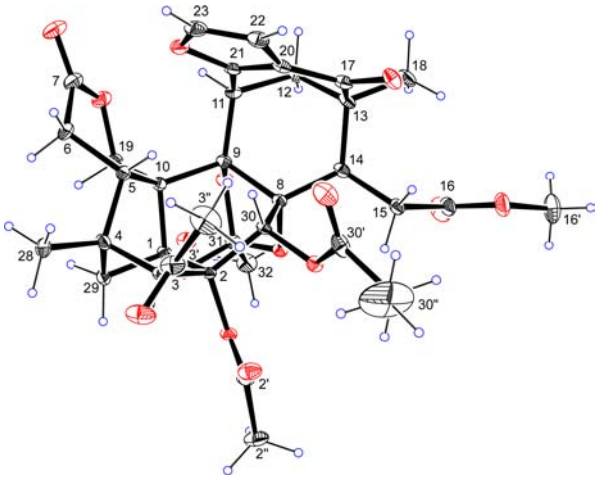
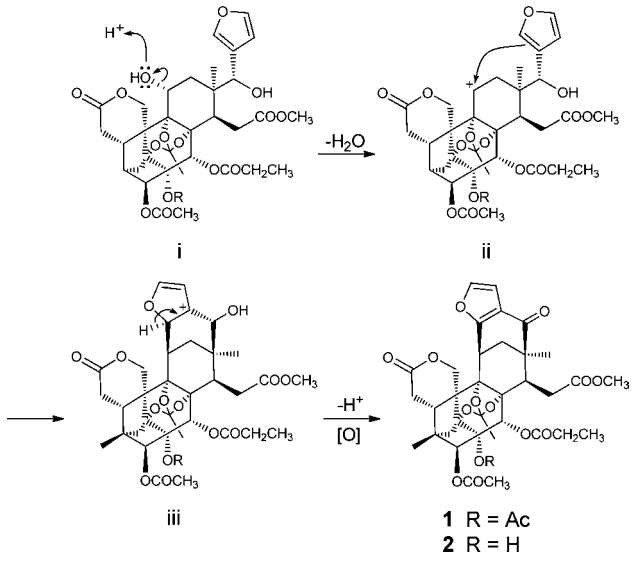



**Figure 3.** Single-crystal X-ray structure of **1**.

C-2 and C-3 by the correlation between H-3 ($\delta_H$ 5.13) and the carbonyl of the acetyl group at C-3′ ($\delta_C$ 169.1) and a quaternary oxygenated carbon at C-2 ($\delta_C$ 86.8); between Me-28 ($\delta_H$ 0.93) and C-3 ($\delta_C$ 80.5), C-4, C-5, and C-29; between an acetyl Me at C-2″ ($\delta_H$ 2.08) and the carbonyl of the acetyl group at C-2′ ($\delta_C$ 169.9); and between $H_2$-29 ($\delta_H$ 1.66, 2.38) and C-1 ($\delta_C$ 86.0), C-2, C-3, C-4, C-5, and C-10, and a propanoyl ester group was attached to C-30 ($\delta_C$ 68.9) by the correlation between H-30 and the carbonyl of the propanoyl group at C-30′ ($\delta_C$ 170.6). An 1,8,9-orthoacetate was confirmed by the HMBC spectrum, between $H_2$-19 ($\delta_H$ 4.82, 4.89) and C-1, C-5, C-7 ($\delta_C$ 172.3), C-9 ($\delta_C$ 87.1), and C-10; between H-14 ($\delta_H$ 2.92) and C-8 ($\delta_C$ 87.6); and between orthoacetyl Me ($\delta_H$ 1.81) and C-31 ($\delta_C$ 120.6). Most of the scaffolds of C−E rings were mainly established by comprehensive analysis of the HMBC spectrum, by the correlation between $H_2$-12 [($\delta_H$ 2.14, 2.35 (each 1H, dd)] and the methine signal ($\delta_C$ 34.3) at C-11, C-13, C-14, and C-21 ($\delta_C$ 161.9); between H-11 ($\delta_H$ 4.00, 1H, t) and C-8, C-12, C-13, and C-21; between H-22 ($\delta_H$ 6.98) and C-20 ($\delta_C$ 123.6, s), C-21, and C-23 ($\delta_C$ 142.1, d); and between Me-18 ($\delta_H$ 1.05, s) and C-12, C-13, C-14, and C-17 ($\delta_C$ 193.2). Thus, the above-mentioned results indicated a unique linkage between C-11 and C-21; therefore, the C−E rings were revealed as an uncommon connected ring system.

The relative stereostructure of **1** was determined by the NOESY spectrum (Figure 2). The NOESY cross-peaks of Me-32/H-14, H-19α; H-14/H-12α, Me-18 indicated that H-14, Me-18, and Me-32 presented an α-orientation. The NOESY correlation of H-11/H-6$\beta$, H-19$\beta$; H-6$\beta$/H-23 indicated that H-11 was also in the α-direction. On the

**Scheme 1.** Plausible Biogenetic Pathway for **1** and **2**




other hand, the cross-peaks of Me-28/H-5; H-5/H-22, H-23, and H-30; H-22/H-30 indicated that H-5, Me-28, and H-30 adopted a $\beta$-orientation. In addition, H-29$_{pro\text{-}R}$/H-19$\alpha$; H-29$_{pro\text{-}S}$/H-3 indicated that H-3 adopted an $\alpha$-orientation. Therefore, the C and D rings presented a *cis* form and were bent at a 90° angle, and the E ring was in the same plane as the D ring. Thus, the E-ring was located spatially on the upper part of the B-ring. Fortunately, single-crystal X-ray diffraction analysis was successfully conducted to confirm the proposed structure. In addition, **1** included the two molecules of $CHCl_3$ as a crystal solvent and, therefore, allowed unambiguous assignment of the absolute configuration of **1** (Figure 3).

Guianolide B (**2**)[20] was assigned the molecular formula $C_{34}H_{38}O_{14}$ (*m*/*z* 671.2339 $[M+H]^+$, calcd 671.2339) by HRFABMS. The $^1H$ and $^{13}C$ NMR spectra (Table 1) of **2** were very similar to those of **1**, except for the absence of an acetyl group at C-2 and the presence of an OH group [$\delta_H$ 2.88 (1H, s), $\delta_C$ 80.8 (s)]. HMBC correlations were observed at 2-OH/C-3 and C-32. The NOESY experiment revealed that the relative structure of **2** had the same conformation as **1**.

Guianolides A (**1**) and B (**2**) featured an unprecedented carbon skeleton via the formation of the C-11−C-21 bond of phragmalin-1,8,9-orthoacetate. A possible biosynthetic pathway of **1** and **2** was postulated in Scheme 1. The biosynthetic precursor of **1** and **2** has been proposed to be 11-hydroxy-phragmalin-1,8,9-orthoacetate **i**, which was dehydroxylated enzymatically to give **ii**. **ii** then underwent an intermolecular Friedal−Crafts type reaction, followed by oxidation into **1** and **2** (Scheme 1).[21,22]

As a primary screen for cancer cell growth inhibition, compounds **1** and **2** were examined using the murine P388 and L1210, and human HL-60 leukemia cell lines. Compound **1** showed weak activity against the P388 cell line ($IC_{50}$ 33.7 $\mu$M), but **2** was inactive against all cell lines.

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**Supporting Information Available.** Experimental procedures and physical and spectroscopic data of guianolides A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(20) Guianolide B (**2**): Colorless amorphous; HRFABMS at *m*/*z*: 671.2339 $[M+H]^+$ (calcd for 671.2339, $C_{34}H_{39}O_{14}$); $[\alpha]_D^{22}$ +53.8° (*c* 0.100, $CHCl_3$); UV ($CH_3CN$) $\lambda_{max}$ (log $\varepsilon$) 261 (3.52), 215 (3.75); CD [0.000400 M, $CH_3CN$] $\lambda_{max}(\Delta\varepsilon)$: 226 (0), 261 (53.4), 287 (15.3), 317 (43.3), 324 (34.5), 330 (41.7), 339 (21.6), 359 (0); IR $\nu_{max}$ (KBr) $cm^{-1}$: 3435, 2923, 1754, 1731, 1678, 1510; $^1H$ and $^{13}C$ NMR data, see Table 1.

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