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Laxifloranone, a New Phloroglucinol Derivative from *Marila laxiflora*[1]

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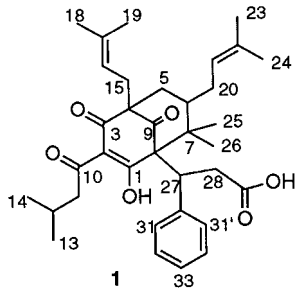
A new polyisoprenylated phloroglucinol derivative has been isolated from the twigs of *Marila laxiflora* and characterized on the basis of 1D and 2D NMR spectra. Laxifloranone (**1**) shows moderate inhibition of the cytopathic effects of in vitro HIV infection.

As part of our interest in natural products with HIV-inhibitory activity,[1] we undertook the investigation of the organic extract of *Marila laxiflora* Rusby (Clusiaceae), which showed activity in the NCI's anti-HIV primary screen.[2] Previous studies in our laboratory of genera in the Clusiaceae family have traced the HIV-inhibitory activity of *Garcinia*, *Clusia*, *Symphonia*,[3] *Allanblackia*,[4] and *Vismia*[5] to a series of prenylated benzophenone derivatives. In an effort designed to identify other extracts from plants in the Clusiaceae[6] that may contain this class of compounds (dereplication), *M. laxiflora* was selected for further study. Bioassay-guided fractionation of *M. laxiflora* led, instead, to the isolation of a new prenylated phloroglucinol, laxifloranone (**1**). Prenylated phloroglucinol compounds have been previously isolated from *Garcinia*,[7] but differ from laxifloranone in having a hydrofuran ring rather than a cinnamic acid residue. Only one report of secondary metabolites from the genus *Marila* has appeared in the literature.[8]



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On the basis of HRFABMS and ^{13}C NMR data, the molecular formula of **1** was assigned as $C_{35}H_{46}O_6$. Both the ^{13}C and ^1H NMR spectra of **1** showed broad and overlapping signals in $CDCl_3$. A change of solvent to pyridine-d_5 resolved the ^{13}C NMR spectrum; however, resolution of the ^1H NMR spectrum could only be achieved by elevating the temperature to 60 °C. As in the guttiferones,[3] the broad, overlapping peaks suggested the existence of a keto−enol tautomerism, which could be resolved at higher temperatures.

Characteristic ^{13}C NMR resonances at δ 143.9 (C-30), 132.4 (C-31,31′), 128.2 (C-32,32′), and 127.0 (C-33) were indicative of a monosubstituted aromatic ring. Two vinyl protons, four vinylic methyl groups, and four allylic protons in the ^1H NMR spectrum suggested the presence of two isopent-2-enyl side chains. An additional keto isopentyl side chain was determined by COSY correlations from H-12 to H-13 and H-14 along with HMBC correlations from H-11 to C-10, C-12, and C-14. A ^{13}C NMR resonance at δ 174.4 (C-29), along with a band in the IR spectrum at 1714 cm^{-1}, showed evidence of a carboxylic acid, which was linked to C-28, as determined by an HMBC correlation. Resonances for an enolized 1,3-diketone system (δ 117.9, 197.2, 196.2), a nonconjugated ketone (δ 209.5), and two quaternary carbons (δ 62.3, 71.7), along with ^{13}C NMR signals for quaternary (δ 50.2), methine (δ 49.1), and methylene (δ 39.1) carbons, indicated a bicyclo[3.3.1]nonane ring system and suggested that laxifloranone was a polyisoprenyl phloroglucinol derivative related to the guttiferones.[3]

The substitution pattern around the nonane ring system was determined using HMBC correlations. A correlation from H-27 to C-1, C-8, C-9 and to C-30 indicated that the aromatic ring was linked to the nonane system at C-8. Additional correlations from H-27 to C-28 and C-29 revealed attachment of the carboxylic acid side chain to the remaining site at C-27. HMBC correlations from H-5a to C-15 and C-20 showed the connection of the isoprenyl side chains to C-4 and C-6. By elimination, a remaining C-1 keto isopentyl side chain was attached to C-2.

The relative stereochemistry of **1** was elucidated on the basis of NOESY and RESOLVE experiments. Observed NOE correlations between H-5a (δ 2.34) and the methyl protons at δ 1.32 (H-25) placed them in a 1,3-diaxial relationship to each other and defined a chair conformation for the cyclohexene ring. A coupling constant of 7.5 Hz for H-5a was consistent with axial−axial coupling between H-5a and H-6. This was supported by the large vicinal coupling (13.5 Hz) between the methylene protons at C-5 and a small coupling constant (2.0 Hz) for H-5b, indicating equatorial coupling to H-6 and location of the isoprenyl side chain in the axial position.[7] Further NOE correlations between H-20 and H-24, and H-16 and H-18 indicated that those methylene groups were cis to the methines in the two isoprene side chains.

To determine the stereochemistry at C-27, we tried to form a δ lactone between the carboxyl (C-29) and the enol at C-1. However, an attempted closure using the Mitsunobu reaction[9] failed, probably due to the rigidity of the bicyclic system and steric hindrance of the side chain, resulting, instead, exclusively in compound **2** in very high yield

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Scheme 1. Mitsunobu Reaction of Laxifloranone (**1**) and Product **2**

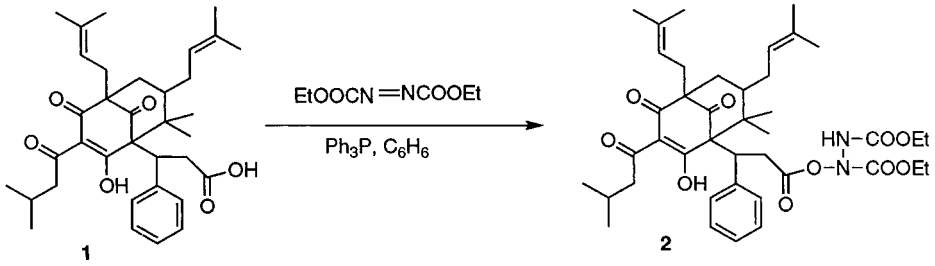

Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1** Recorded in Pyridine-d_5 at 500 MHz and 60 °C

C#	δ ^{13}C	δ ^{1}H (mult., J = Hz)	HMBC (H to C#)	NOE
1	197.2			
2	117.9			
3	196.2			
4	62.3			
5	39.1	a 2.34 (dd, 7.5, 14.5)	3, 4, 6, 15, 20	5b, 15, 24, 25
		b 2.24 (d 2.0 14.5)	3, 4, 6, 7, 9, 20	6, 15, 21
6	49.1	1.47 (m)	5, 8, 21, 25	5a, 20, 21, 25, 26
7	50.2			
8	71.7			
9	209.5			
10	202.8			
11	47.6	a 2.61 (dd, 6.5, 14.5)	10, 12, 14	11b, 13
		b 3.03 (dd, 6.5, 14.5)	10, 12, 13	11a, 12, 14
12	26.3	2.13 (m)	10, 11, 14	13
13	22.6	0.87 (3H, d, 6.5)	11, 12, 14	11a, 11b, 12, 32, 32′
14	22.9	0.91 (3H, d, 6.5)	11, 12, 13	11a, 11b, 12
15	31.2	2.81 (2H, m)	4, 5, 9, 16, 17	5a, 5b, 16, 19
16	121.0	5.51 (m)	15, 18, 19	5a, 15, 18, 24
17	134.2			
18	26.1	1.68 (3H, s)	16, 17, 19	14, 16
19	18.2	1.71 (3H, s)	16, 17, 18	5a, 15
20	30.0	2.21 (2H, m)	5, 6, 21, 22	5a, 21, 24, 26
21	125.2	4.92 (m)	6, 20, 22, 23, 24	5b, 26
22	132.5			
23	25.8	1.62 (3H, s)	21, 22, 24	13, 21, 24
24	17.8	1.47 (3H, s)	21, 22, 23	5a, 13, 14, 23, 25
25	28.3	1.32 (3H, s)	6, 7, 8, 26	5a, 27, 28a, 31, 31′
26	23.8	1.20 (3H, s)	6, 7, 8, 25	20, 27
27	44.2	4.83 (d, 10.5)	1, 8, 9, 28, 29, 30	6, 23, 25, 26, 28a, 28b, 31, 31′
28	41.3	a 3.18 (d, 15.5)	27, 29, 30	25, 27, 28b
		b 3.37 (dd, 10.5, 15.5)	27, 29, 30	27, 28a, 31, 31′
29	174.4			
30	143.9			
31,31′	132.4	7.88 (d, 5.0)	27, 31, 31′, 33	25, 27, 28, 32, 32′
32,32′	128.2	7.23 (dd, 7.0)	30, 32, 32′	13, 31, 31′
33	127.0	7.18 (t, 7.0)	31, 31′	32, 32′

(Scheme 1). HRFABMS and extensive NMR studies characterized this product.

A NOE was observed between H-27 (δ 4.99) and the axial methyl at C-7 (δ 1.31), in both compounds **1** and **2**. H-27 and H-28 showed strong downfield chemical shifts, as compared with model molecules, such as 3-phenylbutanoic acid[10] or 3-phenyl-5-oxo-hexanoic acid.[11] The coupling constants $J_{27-28} = 10.5$ Hz, $J_{27-28'} = 0$ Hz in **1**, which were preserved in **2**, and the NOE between H-27 and the aromatic H-31, pointed to a distinct, stable conformation of the C-27−C-29 moiety, rather than a freely rotating one. Based on these measurements and on examination of a Dreiding model, a relative configuration 27*R** is proposed. Such a configuration maintains H-27 in close proximity with the enolic OH, the carbonyl at C-9, and the aromatic ring, while H-28 is strongly influenced by the C-9 carbonyl, more than in the alternative *27S** configuration.

As with the guttiferones,[3,4] compound **1** exhibited only partial cytoprotection (80%) and moderate inhibition of the cytopathic effects of HIV-1 infection in a human T-lymphoblastoid cell line (CEM-SS),[12] with an EC_{50} value of 0.62 μg/mL and an $IC_{50} = 6.6$ μg/mL. Compound **2**, however, in which the carboxyl is blocked, was entirely inactive.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity Inova 500 in pyridine-d_5 (60 °C) at 500 MHz for ^{1}H and 125 MHz for ^{13}C. Optical rotations were measured on a Perkin−Elmer 241 polarimeter. IR spectra were recorded on a Perkin−Elmer Spectrum 2000 and UV spectra on a Beckman DU 64 spectrometer. MS spectra were measured with a JEOL SX102 spectrometer.

Plant Material. The plant material was collected in Guatemala (1988) by N. Trushell of The New York Botanical Gardens (NYBG), under contract to the National Cancer Institute. Taxonomic identification was provided by J. Castillo; a voucher specimen (Q65V719) is maintained at The NYBG.

Extraction and Isolation. Air-dried and ground twigs of *M. laxiflora* were extracted successively with CH_2Cl_2−MeOH (1:1) and MeOH. The combined extracts were dried in vacuo to give 64.48 g of crude extract. A portion of this extract (5.25 g) was subjected to a solvent−solvent partitioning scheme[13] that concentrated the anti-HIV activity in the hexane- (852

mg) and MeOtBu- (896 mg) soluble fractions. Size exclusion chromatography of the active MeOtBu material on Sephadex LH-20 (3 × 86 cm) with CH_2Cl_2−MeOH (1:1) provided an early eluting fraction (0.29 g) that was subjected to vacuum liquid chromatography (VLC) on diol media with a hexane−EtOAc gradient. Final purification was achieved by reversed-phase HPLC (Rainin Dynamax, C_{18}, 1 × 25 cm) using CH_3CN-H_2O [8:2 with 0.05% (v/v) TFA] to give 45 mg (0.07% yield) of laxifloranone (**1**).

Laxifloranone (1): yellow oil; $[\alpha]_D$ +23.6° (*c* 0.83, MeOH); UV(MeOH) λ_{max} 348 nm (ϵ 18 669); IR ν_{max} (film): 2964, 2927, 2360, 1714, 1668, 1539, 1456, 1299, 1234, 759, 702 cm^{-1}; HRFABMS *m*/*z* 563.3386, (MH^+ calcd for $C_{35}H_{46}O_6$, 563.3373); for ^1H NMR and ^{13}C NMR data, see Table.1

Mitsunobu Reaction of 1. A mixture of laxifloranone (**1**, 6.2 mg, 0.011 mmol), Ph_3P (18 mg, 0.069 mmol) and DEAD (diethyl azodicarboxylate) (12 mg, 0.069 mmol) was dissolved in C_6H_6 (6 mL) and stirred at room temperature for 24 h (although TLC indicated that **1** was consumed within 2 h). The solvent was removed, and the residue was chromatographed on a column of Sephadex LH-20 (elution with $CHCl_3$−CH_3OH 1:1). Highly purified **2** (6.6 mg) was obtained as an amorphous material.

Compound 2: IR ν_{max} (film) 3327 (br), 2964, 2928, 1745, 1663, 1374, 1238, 1074, 703 cm^{-1}; HRFABMS (FAB^+, CsI) *m*/*z* 853.3021 (MCs^+ calcd for $C_{41}H_{56}N_2O_9Cs$, 853.3025); ^1H NMR (C_5D_5N at 60 °C) δ 7.86, 7.23, 7.14 (aromatic protons), 5.51 (br m, H-16), 4.99 (br d, J = 9 Hz, H-27), 4.92 (br m, H-21), 4.19 (br m, H-28,28′ and OCH_2), 3.12 (br m, H-11), 2.81 (t, J = 6.5 Hz, H-15,15′), 2.70 (m, H-11′), 2.35 (dd, J = 14, 7 Hz, H-5), 2.25 (br d, J = 14 Hz, H-5′), 2.25 (H-20, 20′), 2.17 (br m, H-12), 1.71 (3H, br s, H-19), 1.69 (3H, br s, H-18), 1.64 (3H, br s, H-23), 1.50 (m, H-6), 1.49 (3H, br s, H-24), 1.31 (3H, s, H-25), 1.23 (3H, s, H-26), 1.13 (6H, t, J = 7 Hz, $OCH_2C\underline{H}_3$), 0.98 (3H, d, J = 6.5 Hz, H-14), 0.94 (3H, d, J = 6.5 Hz, H-13); ^{13}C NMR (C_5D_5N at 60 °C, partial) δ 209.2 (C-9), 196.1 (C-3), 172.7 (C-29), 143.8 (C-30), 134.2 (C-17), 132.3 (C-22,31,31′), 128.1 (C-32,32′), 126.9 (C-33), 125.1 (C-21), 120.7 (C-16), 71.9 (C-8), 63.9 (C-4), 63.6, 61.7 ($O\underline{C}H_2CH_3$), 49.9 (C-7), 49.0 (C-6), 47.5 (C-11), 43.4 (C-28), 43.2 (C-27), 38.8 (C-5), 31.0 (C-15), 29.9 (C-20,25), 26.1 (C-12), 25.9 (C-18), 25.7 (C-23), 23.7 (C-26), 22.9 (C-14), 22.5 (C-13), 18.0 (C-19), 17.7 (C-24), 14.5, 14.1 (OCH_2-$\underline{C}H_3$).

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