



Chemical constituents of two *Mollinedia* species

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Abstract

A butanolide, marliolide, was isolated from the hexane extract of leaves of *Mollinedia marliae* (Monimiaceae) along with the long chain fatty alcohol hexacosanol. Phytol and a mixture of sitosterol and stigmasterol were also isolated from leaves of both *M. marliae* and *M. gilgiana*. *trans*-*N*-Feruloyltyramine, a ferulic acid derivative, is the major constituent of the stems of *M. gilgiana*. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

The genus *Mollinedia* belongs to the family Monimiaceae, order Laurales, sensu Dahlgren, 1980 (Dahlgren, 1980). This family comprises about 34 genera, five of them occurring in Brazil (Barroso, 1978). *Siparuna* and *Mollinedia* are the two major genera, outstanding in complexity and number of species (150 and 94, respectively) (Peixoto, 1979). The genus *Mollinedia* comprises ca. 70 species and is composed of small trees or shrubs of tropical and subtropical regions ranging from Mexico to the southern region of Brazil (Peixoto, 1983). In Brazilian folk medicine *M. brasiliensis* is reputed as a strong antispasmodic and useful for treating cramps (Corrêa, 1978). There is only one reference in the literature concerning the chemistry of this genus, which reports the isolation of a flavonoid, vitexin, from the leaves of *M. costaricensis*, and a benzyloquinoline alkaloid, mollinedine, from its roots (Lopez et al., 1988). In this work we report the isolation of a new γ -lactone, marliolide (**1**) from leaves of *M. marliae* A. Peixoto et V. Pereira, along with hexacosanol and phytol, the latter isolated also from leaves of *M. gilgiana* Perk. *trans*-*N*-Feruloyltyramine (**2**) is the major constituent of the stems of *M. gilgiana*. The two studied species are

endemic to the Atlantic Forest of Nova Friburgo, Rio de Janeiro State.

2. Results and discussion

The hexane extract of leaves of *M. marliae* was fractionated by silica gel column chromatography, affording a mixture of sitosterol and stigmasterol, as well as hexacosanol and **1** as a pure compound. The EI mass spectrum of the γ -lactone **1** showed a molecular ion at m/z 310, compatible with the molecular formula $C_{19}H_{34}O_3$. The peaks in the mass spectrum at m/z 282 and m/z 266 represent loss of CO and CO₂, respectively, and are characteristic of spectra of ω -ethyl- γ -lactones (Valderama, 1979). The IR spectrum of **1** exhibited a broad absorption band at 3390 cm⁻¹ (hydroxyl group) and intense absorptions at 1729 and 1685 cm⁻¹, suggesting the presence of an α,β -unsaturated γ -lactone (Tanaka et al., 1990). Analysis of the ¹H NMR spectrum of **1** showed two signals at δ 2.4 ppm (*dq*) and δ 1.5 ppm (*m*) which were assigned to methylenic protons α and β to a double bond (H-2', H-3' of **1**, Table 1). Characteristic signals of aliphatic chains at δ 1.2–1.4 ppm, [(CH₂)_{*n*}] and at δ 0.9 ppm (*t*, terminal methyl group) were also evident. The signal at δ 6.9 ppm (*t*, 1H) was ascribed to an olefinic β -proton (H-1') of the α,β -unsaturated lactone system on the basis of its chemical shift. Comparison of data from ¹³C-¹H NMR and APT spectra pointed out

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the nature of the exocyclic double bond (δ 130.2 and δ 147.6 ppm). The geometry of the trisubstituted double bond conjugated to the lactone carbonyl group in **1** was confirmed to be in the *E*-form from the chemical shift value (δ 6.9 ppm) of the olefinic β -proton in the ^1H NMR spectrum (Tanaka et al., 1990). Comparison of these chemical shifts for H-1' with those in the literature for the γ -lactones C, D and E, isolated from Lauraceae (Takeda et al., 1972), shows that marliolide is a derivative of the same type. Data from ^1H - ^1H COSY spectra (Table 1) show that H-1' (δ 6.9 ppm) couples with H-2' (δ 2.4 ppm), $J=7.8$ Hz, confirming that the aliphatic chain is bonded to C-1' of the double bond. In the same spectrum it can be seen that the signal at δ 4.5 ppm (dq , $J=5.1$ and 6.6 Hz, H-4) correlates with a methyl group (d , δ 1.4 ppm, $J=6.6$ Hz, H-5) and to the

carbinolic proton H-3 (δ 4.8 ppm, *br d*). The ^1H - ^{13}C COSY spectrum (Table 1) shows that these protons are attached to C-4 (δ 78.7 ppm) and C-3 (δ 67.5 ppm), respectively. Analysis of coupling constants for H-3 and H-4 (obtained by double irradiation experiments) suggests the *erythro* (*cis*) relative configuration for **1**. We can observe from model compounds A–E (Table 2) that coupling constants on the order of 2 Hz are characteristic for γ -lactones with *threo* relative configuration in relation to C-3 and C-4. The coupling constant between H-4 and H-3 in marliolide is 5.1 Hz, in accordance with those for the *erythro* configuration (ca. 5.0–6.0 Hz) as shown in model compound C. Moreover, DIFFNOE experiments on **1** confirmed these findings; irradiation of H-4, at δ 4.5 ppm showed the enhancement of the signals of H-5 (δ 1.4 ppm) and H-3 (δ 4.8 ppm), whereas irradiation of the latter (H-3), showed no NOE effect on H-5 but did show an enhancement on the signal of H-4.

In an attempt to establish the absolute configuration at C-3/C-4 stereocenters we prepared the Mosher's ester of **1** (Rieser et al., 1992). Esterification of **1** with MTPA chloride (Niwa et al., 1992), even in mild conditions, gave the elimination product, which suffered further conjugate addition of water at the enone system, affording the heteroaromatic derivative **1a** (93% yield).

A circular dichroism spectrum of **1** was run in an attempt to determine the absolute configuration at C-3 and C-4. The CD spectrum of **1** displays an intense negative band at 228 nm, attributed to a $\pi \rightarrow \pi^*$ transition, and an additional weak positive band at ca. 270 nm ($n \rightarrow \pi^*$). The existing sector rules cannot be used to correlate observed Cotton effects and structure for these butenolides. Uchida and Kuriyama (1974) suggested that the chirality at the γ -carbon of α,β -unsaturated endocyclic butenolides is the sign determining factor for the $\pi \rightarrow \pi^*$ Cotton effect. Upon application of their rule to the observed CE at 228 nm the absolute configuration

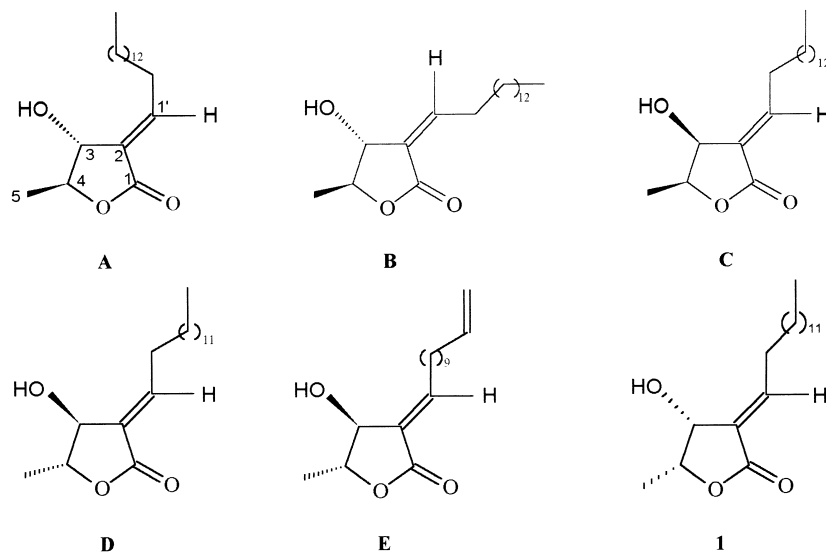
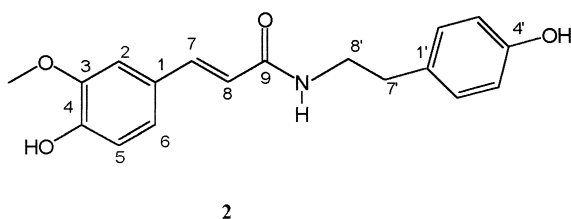
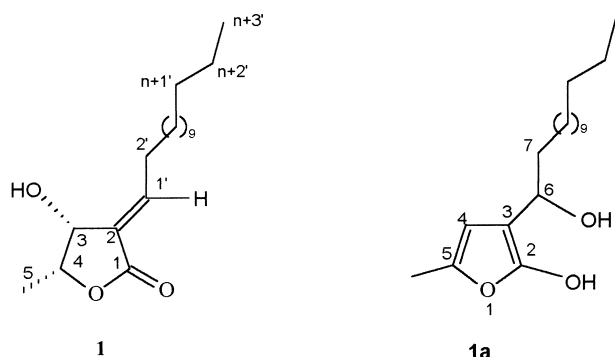


Table 1
¹H and ¹³C NMR spectral data and ¹H–¹H correlations for compound **1**

C/H	δ _C	δ _H	¹ H– ¹ H COSY
1	170.0	—	
2	130.2	—	
3	67.5	4.8 (<i>br d</i>)	H-4
4	78.7	4.5 (<i>dq</i> , <i>J</i> = 5.1, 6.6 Hz)	H-3, H-5
5	13.7	1.4 (<i>d</i> , <i>J</i> = 6.6 Hz)	H-4
1'	147.6	6.9 (<i>dt</i> , <i>J</i> = 7.8 and 1.4 Hz)	H-2'
2'	29.6	2.4 (<i>dq</i> , <i>J</i> = 7.8, 2.0 Hz)	H-1', H-3'
3'	28.2	1.5 <i>m</i>	H-2'
<i>n</i>	29.6–28.2	1.4–1.2 <i>m</i>	
(<i>n</i> + 1)'	31.7	1.4–1.2 <i>m</i>	
(<i>n</i> + 2)'	22.5	1.4–1.2 <i>m</i>	
(<i>n</i> + 3)'	13.9	0.9 <i>t</i>	
OH	—	2.1 <i>br s</i>	

at the γ-carbon (C-4) is assigned as *R*. The same configuration is assigned after application of the rules set by Gawronski and co-workers for 2 (*5H*)-furanones (Gawronski et al., 1997). Thus, considering the evidence from NOE measurements, *J*-coupling and CD, we assigned the configurations at C-3 and C-4 as *R*, *R*.

The CHCl₃ fraction of *M. marliae* and the EtOAc fraction of *M. gilgiana* afforded phytol, in its free form, after purification on column chromatography. Its structure was identified by comparison of its spectral data (mainly ¹³C NMR) with those in the literature for phytol and its fatty acid ester derivatives (Zulueta et al., 1995; Gramatica et al., 1987).

The extracts from *M. gilgiana* displayed a chemical composition based mainly on cinnamic acid derivatives. The EtOAc extract from leaves of this species afforded an alkyl coumarate along with other isomeric compounds. The major constituent of the stems of *M. gilgiana* was feruloyl tyramine **2**, isolated from both EtOAc and

CH₃OH extracts. It occurs together with a minor dimethoxylated isomer.

No alkaloids have been found so far in leaves of either *M. gilgiana* or *M. marliae*. Instead, in *M. gilgiana* we found an abundance of cinnamic acid derivatives; alkyl coumarates in the leaves and cinnamoyl-derived amides in the stems. The γ-lactone, marliolide, **1**, is reported for the first time in the family Monimiaceae as are the cinnamic acid derivatives. Marliolide (**1**) occurs as a major component in the leaves of *M. marliae* (0.1% of the hexane extract). This class of compounds — butanolides — was isolated from plant species of the family Lauraceae (Valderama et al., 1979), which belongs to the same order (Laurales) as the Monimiaceae.

3. Experimental

3.1. Plant material

M. gilgiana and *M. marliae* were collected in Reserva Ecológica de Macaé de Cima (State of Rio de Janeiro, Brasil) in December 1994 and identified by Luis Carlos Giordano and Bruno Kurtz from Jardim Botânico do Rio de Janeiro. Voucher specimens are deposited at the Herbarium of that institution.

3.2. Extraction and isolation

Leaves of *M. gilgiana* and *M. marliae* (770 g and 300 g, respectively) were exhaustively extracted with hexane and methanol consecutively. Evapn. in vacuo gave 17 g and 10 g of hexane extracts for *M. gilgiana* and *M. marliae*, respectively. MeOH extracts of the two species were partitioned between H₂O and EtOAc (*M. gilgiana*) and H₂O and CHCl₃ (*M. marliae*) to give, on evapn., 14 g of EtOAc-soluble and 7.4 g of CHCl₃-soluble

Table 2
¹H NMR spectral data for compound **1** and reference structures^a (Takeda et al., 1972)

δ, ppm, CDCl ₃				
H	A	B	C	
3	4.56 (<i>m</i>)	4.38 (<i>m</i>)	4.63 (<i>m</i>)	
4	4.53 (<i>dq</i> , <i>J</i> = 2.5 and 6.5 Hz)	4.58 (<i>dq</i> , <i>J</i> = 4 and 6 Hz)	4.35 (<i>dq</i> , <i>J</i> = 5 and 6.5 Hz)	
5	1.33 (<i>d</i> , <i>J</i> = 6.5 Hz)	1.38 (<i>d</i> , <i>J</i> = 6.5 Hz)	1.45 (<i>d</i> , <i>J</i> = 6.5 Hz)	
1'	6.69 (<i>dt</i> , <i>J</i> = 1.5 and 7.8 Hz)	6.58 (<i>dt</i> , <i>J</i> = 1.5 and 7.8 Hz)	6.93 (<i>dt</i> , <i>J</i> = 1.5 and 7.8 Hz)	
2'	2.42 (<i>~q</i> , <i>J</i> = 7.8 Hz)	2.76 (<i>~q</i> , <i>J</i> = 7.8 Hz)	2.43 (<i>~q</i> , <i>J</i> = 7.8 Hz)	
H	D	E	1	
3	4.52 (<i>m</i>)	4.52 (<i>m</i>)	4.8 (<i>br d</i>)	
4	4.49 (<i>dq</i> , <i>J</i> = 2.1 and 6.4 Hz)	4.48 (<i>dq</i> , <i>J</i> = 2 and 6.8 Hz)	4.5 (<i>dq</i> , <i>J</i> = 5.1 and 6.6 Hz)	
5	1.34 (<i>d</i> , <i>J</i> = 6.7 Hz)	1.33 (<i>d</i> , <i>J</i> = 6.8 Hz)	1.4 (<i>d</i> , <i>J</i> = 6.6 Hz)	
1'	6.97 (<i>dt</i> , <i>J</i> = 1.7 and 7.8)	6.95 (<i>dt</i> , <i>J</i> = 1.5 and 7.8 Hz)	6.9 (<i>dt</i> , <i>J</i> = 7.8 and 1.4 Hz)	
2'	2.39 (<i>m</i>)	2.4 (<i>lq</i>)	2.4 (<i>dq</i> , <i>J</i> = 7.8 and 2 Hz)	

^a 60 MHz.

residues. The hexane extract of *M. marliae* was fractionated by CC over silica gel, eluted with a solvent gradient from hexane to EtOAc, and EtOAc to MeOH with fractions of 150 ml each. Frs. 2–4 (eluted with hexane–EtOAc 15%) were purified by CC over silica gel, eluted with hexane and EtOAc. Final purification of frs. 7–8 with acetone resulted in the precipitation of the γ -lactone (**1**) (100 mg). Purification of frs. 18–21 gave a mixture of sitosterol and stigmasterol (data not shown). The EtOAc extract of *M. gilgiana* yielded the same steroids (sitos-terol and stigmasterol) and phytol (data not shown).

The stems (small branches including the bark) of *M. gilgiana* were dried and ground to a powder and thoroughly extracted with hexane, CHCl_3 , EtOAc and MeOH. Evapn. in vacuo of the EtOAc extract gave a residue, which was chromatographed on silica gel using hexane, EtOAc and MeOH as eluents. Fr. 18 was purified by CC over silica gel (eluted with hexane, EtOAc and MeOH) and yielded the ferulic acid derivative, *trans*-*N*-feruloyltyramine (**2**), eluted from frs. 31–32 with EtOAc. The MeOH extract from the stems of *M. gilgiana* was further extracted with EtOAc, which contained the same amide. This fraction was purified by counter-current chromatography on a HSCCC apparatus, with solvent system $\text{H}_2\text{O}:\text{EtOH}:\text{hexane}:\text{EtOAc}$ (10:4:7:7), using the upper organic layer as mobile phase (2 ml/min) to give **2** in a pure form (ca. 0.005%).

Marliolide (4-methyl-3-Hydroxy- $\Delta^{2,1'}$ -tetradecene- γ -lactone) (**1**), white solid from hexane, mp 72°C; $[\alpha]_{\text{D}}^{20} -5.29^\circ$ (CHCl_3 ; c 0.10); CD (hexane, 25°C, c 0.11 mg/ml, 0.1 cm) 228 nm ($\pi \rightarrow \pi^*$) $\Delta\epsilon = -11$, 270 nm ($n \rightarrow \pi^*$) $\Delta\epsilon = +1.2$; IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3390, 2924, 2848, 1729, 1685, 1461; EIMS 70 eV, m/z (rel. int.): 310, 292, 266, 140, 129, 70; ^1H and ^{13}C NMR spectral data: see Table 1.

2-Hydroxy-5-methyl-3-[1-tridecanol]-furan (**1a**): ^1H NMR spectral data (400 MHz, CDCl_3/TMS) δ : 6.8 (1H, *s*, H-4), 2.6 (1H, *t*, H-6), 2.3 (3H, *s*, $\alpha\text{-CH}_3$), 1.2 $[(\text{CH}_2)_n]$ and 0.9 (3H, *t*, $\omega\text{-CH}_3$); ^{13}C NMR spectral data (125 MHz, CDCl_3/TMS) δ : 136.2 (C-H, C-4), 135.8 (furan ring), 98.6 (furan ring), 96.5 (furan ring), 76.1 (CH_2 , C-6), 40.4 (CH_2 , C-7), 30.9 (CH_2 , C-8), 28.6, 28.4, 28.3 and 28.1 $[(\text{CH}_2)_n]$, 25.7 (CH_3 , $\alpha\text{-CH}_3$), 22.8 and 21.6 $[(\text{CH}_2)_n]$, 13.0 (CH_3 , $\omega\text{-CH}_3$); UV (hexane, λ_{max} , nm): 205.8.

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