

Article

A New Depside Isolated from the Bark of *Rauwolfia mattfeldiana*

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Do extrato hexânico da casca do caule de *Rauwolfia mattfeldiana* Markgr. (Apocynaceae) foram isolados um novo depsídeo (**1**, 4'-O-metilartonioato de metila), a 1-hidroxi-3,6-dimetoxi-8-metilxantona (**4**), lupeol (**5**), sitosterol (**6**) e stigmasterol (**7**). As estruturas destas substâncias foram elucidadas pela análise de seus dados espectrais. RMN bidimensional (2D) foi utilizada para definir a estrutura e garantir a atribuição inequívoca dos deslocamentos químicos dos átomos de hidrogênio e carbono do novo depsídeo.

From the hexane extract from stem bark of *Rauwolfia mattfeldiana* Markgr. (Apocynaceae) a new depside (**1**, methyl 4'-O-methylarthonioate), along with 1-hydroxy-3,6-dimethoxy-8-methylxanthone (**4**), lupeol (**5**), sitosterol (**6**) and stigmasterol (**7**) were isolated. The structures of these natural products were elucidated by spectral data analysis. 2D NMR experiments were used to establish the structure and the complete hydrogen and carbon chemical shift assignments of the new depside.

Keywords: *Rauwolfia mattfeldiana*, *Apocynaceae*, *depside*, *xanthone*, *terpenoids*, *spectral data*

Introduction

In the course of our phytochemical investigations on Brazilian plants, we studied the constituents of *Rauwolfia mattfeldiana* Markgr., Apocynaceae. The genus *Rauwolfia* is known for its diversity and abundance of bioactive alkaloids¹, which provided additional interest to undertake the chemical investigation.

The new depside methyl 4'-O-methylarthonioate (**1**), a derivative of arthonioic acid [**2**, isomer of microphyllinic acid (**3**)]^{2,3}, was isolated from *n*-hexane extract of the stem bark, along with the known 1-hydroxy-3,6-dimethoxy-8-methylxanthone (**4**, lichexanthone), lupeol (**5**), sitosterol (**6**) and stigmasterol (**7**).

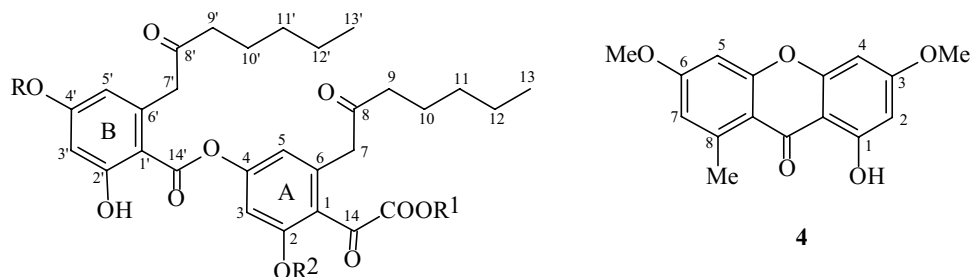
The polyketides **2**, **3** and **4** are classified as lichen (symbiont consisting of a fungus and an algae growing together in a composite structure)⁴ bioproducts. The depsides arthonioic acid (**2**) and microphyllinic acid (**3**) were

isolated from *Arthonia impolita*² and *Centaria collata*³, respectively, and lichexanthone (**4**) from *Parmelia formosana*, *Pertusaria sulphurata* and *Anthocleista djalonenensis*⁵. The structures of the natural products **1**, **4**, **5**, **6** and **7** were deduced on the basis of spectral data, including 2D NMR techniques (HMQC-¹J_{CH} and HMBC-ⁿJ_{CH}, n = 2 and 3).

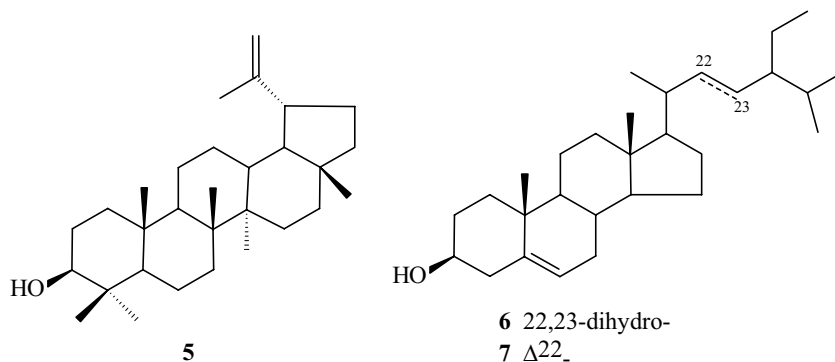
Results and Discussion

The known natural products lichexanthone (**4**), lupeol (**5**), sitosterol (**6**) and stigmasterol (**7**), were identified mainly by their ¹H- and ¹³C-NMR spectral data, including comparison with literature values (**4**⁶, **5**⁷, **6** and **7**⁸).

The IR spectrum of **1** showed absorption bands for carbonyl ketone (ν 1715 cm⁻¹), conjugated carbonyl ester group (ν 1720 cm⁻¹) and aromatic ring (ν 1597 and 1499 cm⁻¹). The molecular formula was inferred to be C₃₁H₄₀O₉ based on the 40 hydrogen and 31 carbon signals



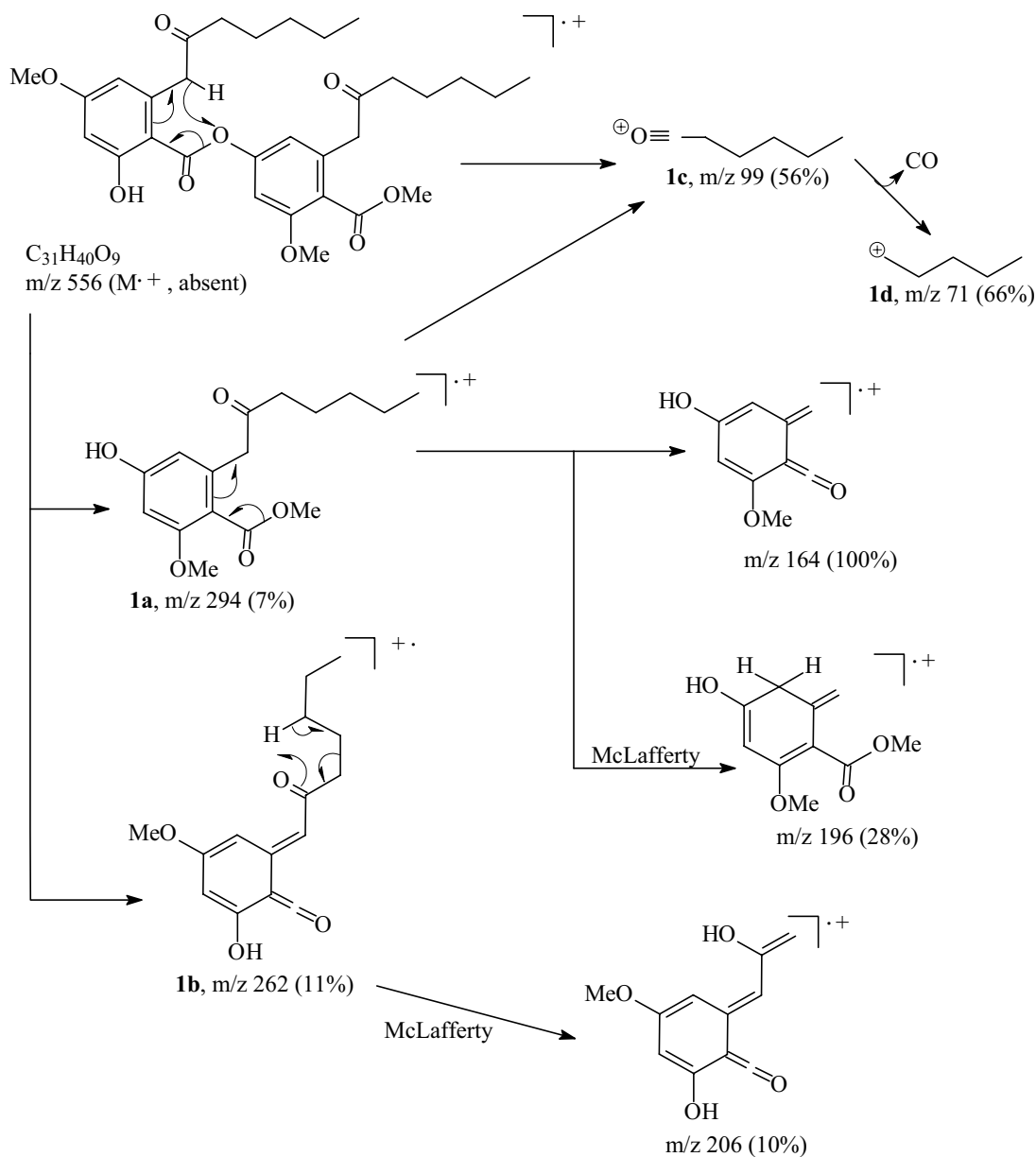
- 1 R = R¹ = R² = Me
 2 R = R¹ = H, R² = Me
 3 R = Me, R¹ = R² = H



- 6 22,23-dihydro-
 7 Δ^{22}

observed in the ¹H- and ¹³C- (PND and DEPT) NMR spectra (Table 1), respectively. This deduction was supported by the presence in the mass spectrum of peaks at *m/z* 294 (C₁₆H₂₂O₅) and 262 (C₁₅H₁₈O₄) which were presumed to be caused by fragments **1a** and **1b** (Scheme 1), generated from the molecular ion [C₃₁H₄₀O₉, *m/z* 556 (M⁺, absent)] after aromatic *ortho*-substituents fragmentation and hydrogen rearrangement (Scheme 1). The hydrogenation pattern for each ¹³C signal was deduced by comparative analysis of the proton noise decoupled (PND) and distortionless enhancement by polarization transfer (DEPT)⁹ spectra (Table 1). The presence of three methoxyl [δ_{H} 3.82 (s), 3.84 (s) and 3.87 (s)] and a chelatogenic hydroxyl [δ_{H} 11.26 (s)] groups was confirmed by ¹H-NMR spectrum. These data, in combination with ¹³C-NMR spectra (Table 1) and the peaks at *m/z* 294 (7%) and 262 (11%) observed in the EI mass spectrum (Scheme 1), were used to deduce the two moieties of the molecule, each sustaining *meta* aromatic hydrogens [δ_{H} 6.59 (*d*, *J* = 1.9 Hz, H-3), 6.56 (*d*, *J* = 1.9 Hz, H-5); 6.49 (*d*, *J* = 2.5 Hz, H-3'), 6.29 (*d*, *J* = 2.5 Hz, H-5'); two AB systems]. The presence of two -CH₂COCH₂CH₂CH₂CH₂CH₃ chains was recognized by the chemical shifts and multiplicities of the following signals clearly observed in the ¹H NMR spectrum: two benzylic and σ -carbonyl methylene hydrogens [δ_{H} 3.69 (*s*, 2H-7) and 4.06 (*s*, 2H-7')], two α -carbonyl methylenes bound to β -CH₂ [δ_{H} 2.43 (*t*, *J* = 7.4 Hz, 2H-9) and 2.41 (*t*, *J* = 7.5 Hz, 2H-9')] and two methyl groups attached to CH₂

[δ_{H} 0.88 (*t*, *J* = 6.8 Hz, 3H-13 or 3H-13' and 0.83 (*t*, *J* = 7.1 Hz, 3H-13' or 3H-13)]. This proposition was corroborated by the ¹³C-NMR spectra (Table 1: ten signals of methylene carbon atoms and two of methyl groups) and by EI mass spectrum, in which the peaks at *m/z* 99 (56%) and 71 (66%) were attributed to fragments **1c** and **1d**, respectively (Scheme 1). The unambiguous location of the substituents at the aromatic rings and the assignment of the ¹H- and ¹³C-NMR chemical shifts were established by heteronuclear ¹H x ¹³C - COSY - ⁿJ_{CH} [n = 1, HMQC (¹J_{CH}); n = 2 and 3, HMBC (²J_{CH} and ³J_{CH})] 2D shift-correlated NMR spectra¹⁰, after assignment of the signals corresponding to quaternary, methine, methylene and methyl carbon atoms by comparative analysis of the PND- and DEPT-¹³C-NMR spectra (Table 1) along with application of the usual shift parameters⁹. The starting point to deduce the substitution pattern for aromatic ring B was the singlet signal of the chelatogenic hydroxyl group (δ_{H} 11.26) at C-2'. This group provides an example where the cross peaks corresponding to OH signal (δ_{H} 11.26) and ¹³C-NMR signals from two quaternary carbons at δ_{C} 104.34 (C-1', ³J_{CH}) and 166.52 (C-2', ²J_{CH}) and one methine carbon at δ_{C} 100.10 (CH-3', ³J_{CH}) were observed in the HMBC spectrum, allowing identification of the signals of C-1', C-2' and C-3'. The signal of C-1' (δ_{C} 104.34) also revealed cross peaks to ¹H frequencies corresponding to H-3' (δ_{H} 6.49, ³J_{CH}), H-5' (δ_{H} 6.29, ³J_{CH}) and 2H-7' (δ_{H} 4.06, ³J_{CH}). The signals of 2H-7' (δ_{H} 4.06) and 2H-9' (δ_{H} 2.41) showed correlations with ¹³C



Scheme 1. Proposed fragmentation mechanisms of **1** (only peaks classified as principals).

signal at δ_C 207.34 (C-8', $^2J_{CH}$). Working along the molecule in this fashion, in combination with analysis of the HMQC ($^1J_{CH}$) spectrum, it was possible to establish the substitution patterns of the aromatic rings and to assign the 1H - and ^{13}C - NMR signals, as summarized in Table 1.

Thus, the structure of the new depside isolated from hexane extract of *Rauwolfia mattfeldiana* was established as methyl 4'-O-methylarthonioate (**1**). This natural product together with lichexanthone (**4**) are polyketide classified due to being produced by the secondary metabolism of lichens⁴. Consequently, it appears probable that the stem bark from the specimen of *Rauwolfia mattfeldiana* used in

this chemical investigation was in association with this symbiont.

Finally, our attention was directed to the mass spectrum of the depside **1**. A proposal for the fragmentation patterns justifying the major peaks is described in Scheme 1.

Experimental

General experimental procedures

M.p. are uncorrected. 1H - (400 MHz) and ^{13}C - (100 MHz) NMR spectra were recorded in $CDCl_3$ using a Bruker ARX-400 spectrometer; chemical shifts are quoted in δ units relative to TMS as the internal standard. Coupling constants (J) are expressed in Hz. The mass spectrum was

measured in a VG Platform II-Fisons instrument using electron impact (EI) at 70 eV. Recycling HPLC was carried out using a Asahipak GS-310P column (21.5 mm x 50.0 cm; 13 μ m), MeOH/CHCl₃ (7:3) as mobile phase, flow rate 3.0 mL/min and UV detection 254 nm.

Plant material

Aerial parts of *Rauwolfia mattfeldiana* Markgr., Apocynaceae family, were collected in September 1996 at Reserva Florestal de Linhares, Espírito Santo State, Brazil,

Table 1. ¹H- (400 MHz) and ¹³C- (100 MHz) NMR spectral data for depside 1 in CDCl₃ and TMS as internal standard. The chemical shifts are in δ (ppm) and coupling constants (*J*, in parenthesis) in Hz.*

		HMQC		HMBC
C	δ_C	δ_H	² <i>J</i> _{CH}	³ <i>J</i> _{CH}
1	121.79	-		H-3, H-5, 2H-7
2	158.37	-	H-3	MeO-2
4	151.26	-	H-3, H-5	
6	135.39	-	2H-7	
8	206.52	-	2H-7	
14	169.07	-		MeO-14
1'	104.34	-		H-3', H-5', 2H-7', HO-2'
2'	166.52	-	HO-2'	
4'	164.90	-		MeO-4'
6'	138.93	-	2H-7'	
8'	207.34	-	2H-7', 2H-9'	
14'	167.44	-		
CH				
3	104.50	6.59 (<i>d</i> , <i>J</i> = 1.9)		H-5
5	116.16	6.56 (<i>d</i> , <i>J</i> = 1.9)		H-3, 2H-7
3'	100.10	6.49 (<i>d</i> , <i>J</i> = 2.5)		HO-2', H-5'
5'	113.34	6.29 (<i>d</i> , <i>J</i> = 2.5)		H-3', 2H-7'
CH ₂				
7	47.50	3.69 (<i>s</i>)		H-5
9	42.47	2.43 (<i>t</i> , <i>J</i> = 7.4)	2H-10	
10	23.30 ^a	1.54 (<i>m</i>)	2H-9	
11	31.26 ^b	1.21 (<i>m</i>)	2H-10, 2H-12	2H-9, 3H-13
12	22.41 ^c	1.18 (<i>m</i>)	3H-13, 2H-11	
7'	51.22	4.06 (<i>s</i>)		H-5'
9'	42.16	2.41 (<i>t</i> , <i>J</i> = 7.5)	2H-10'	
10'	23.38 ^a	1.54 (<i>m</i>)	2H-9'	
11'	31.36 ^b	1.21 (<i>m</i>)	2H-10', 2H-12'	2H-9', 3H-13'
12'	22.41 ^c	1.18 (<i>m</i>)	3H-13', 2H-11'	
CH ₃				
13	13.87 ^d	0.88 (<i>t</i> , <i>J</i> = 6.8)		
13'	13.80 ^d	0.83 (<i>t</i> , <i>J</i> = 7.1)		
MeO-2	55.50	3.82 (<i>s</i>)		
MeO-4'	56.26	3.84 (<i>s</i>)		
MeO-14	52.30	3.87 (<i>s</i>)		
HO-2'		11.26 (<i>s</i>)		

*Multiplicity of signals of carbon atoms deduced by comparative analysis of PND and DEPT-¹³C-NMR spectra. Chemical shifts and coupling constants of hydrogen atoms obtained from 1D ¹H-NMR spectrum. The signals with same letter can be interchanged.

and identified by a botanist from the Companhia Vale do Rio Doce. The voucher specimen (CVRD-368) is deposited at the Herbarium of the Companhia Vale do Rio Doce, Linhares, Espírito Santo State, Brazil.

Extraction and isolation of the constituents

Dried and powdered stem bark (615 g) was extracted at room temperature with *n*-hexane. The solvent was removed under vacuum to yield 2 g of residue. This residue (2 g) was chromatographed on a silica gel column (75 x 5 cm) using *n*-hexane with increasing amounts of ethyl acetate. Eleven fractions of 200 mL each were collected. Fraction 3 (300 mg), eluted with *n*-hexane-ethyl acetate (9:1), was rechromatographed as described above and ten fractions of 100 mL each were collected. Fraction 7 (75 mg) eluted with *n*-hexane-ethyl acetate (80:20), containing the mixture of **4**, **5**, **6** and **7**, was submitted to recycling HPLC to afford **4** (40 mg) and a mixture of **5**, **6** and **7** (20 mg) as revealed by ¹³C-NMR spectrum. Fraction 5 (first column, 286 mg) was rechromatographed using the same procedure and the fractions **5** and **6** furnished the depside **1** (22 mg) after recrystallization from CH₂Cl₂-MeOH (2:1).

Methyl 4'-O-methylarthonioate (1)

M. p. 208-210° C. IR ν (cm⁻¹, KBr): 3424, 1717, 1686, 1646, 1617, 1597, 1256, 1146. UV λ (nm, MeOH): 210 (ε 11943), 270 (ε 4793), 305 (ε 2558). ¹H-NMR (400 MHz, CDCl₃): Table 1. ¹³C-NMR (100 MHz, CDCl₃): Table 1. EIMS *m/z* (rel. int.): Scheme 1.

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