Acetogenins from the Leaves of *Rollinia laurifolia*

by **Lucia P. S. Pimenta**[a]), **Francisco C. Nascimento**[b]), and **Maria Amélia D. Boaventura***[a])

[a]) Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, 31270–970, Belo Horizonte, MG, Brazil (e-mail: dianadb@netuno.lcc.ufmg.br)

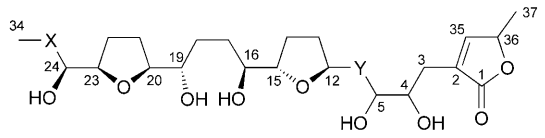
[b]) Departamento de Química, Centro de Ciências e Tecnologia, Universidade Federal de Roraima, Campus do Paricarana, 69310–270, Boa Vista, RO, Brazil

From the EtOH extract of the leaves of *Rollinia laurifolia* SCHL. (Annonaceae), a novel acetogenin, rolifolin (=3-(9-{(2*R**,5*S**)-5-[(1*S**,4*S**)-1,4-dihydroxy-4-{(2*S**,5*R**)-5-[(1*S**)-1-hydroxyundecyl]tetrahydrofuran-2-yl}-butyl]tetrahydrofuran-2-yl}-2,3-dihydroxynonyl)-5-methylfuran-2(5*H*)-one; **1**) was isolated, together with the known acetogenin annonin-I (**2**). Also, from the corresponding hexane extract, a mixture of rolilaurin (**3**, a novel compound), uvariamicin-I (**4**), and uvariamicin-II (**5**) was obtained. The structures of compounds **1**–**5** were elucidated by NMR and MS analysis, and relative configurations were established. Compounds **2** and **5** have never been obtained before from *Rollinia*.

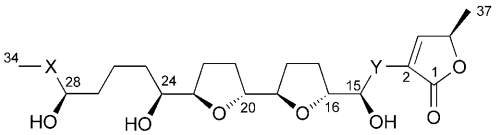
Introduction. – Acetogenins, a relatively new class of secondary metabolites, have been isolated only from the family Annonaceae, and only from ten out of the 130 genera. Acetogenins exhibit a great variety of pharmacological properties such as cytotoxic, antitumoral, insecticidal, and immunosuppressive activities. In continuation of our studies on this family [1–3], we have investigated the acetogenins from the leaves of *Rollinia laurifolia* SCHL. In previous reports, we described the isolation of a new mono-tetrahydrofuran-based acetogenin, laurifolin [4], and of the three known acetogenins [5] uvariamicin-I, solamin, and gonionenin, which were all isolated from the hexane extract of this plant.

The present paper reports the isolation and structural elucidation of two new and three known acetogenins. The novel bis-tetrahydrofuran-based compound rolifolin (**1**; *Fig. 1*) was obtained, together with the known annonin-I (**2**), from the EtOH extract of *R. laurifolia*. In addition, rolilaurin (**3**), another unknown compound, was identified in a mixture with uvariamicin-I (**4**) and -II (**5**) from the corresponding hexane extract. A mixture of **4**/**5** had been isolated before by *Hisham et al.* [6] from the roots of *Uvaria narum*; and annonin-I (**2**; also known as squamocin) has been isolated from *Annona squamosa* [7] and *Rollinia membranaceae* [8]. The acetogenin **5** was isolated for the first time from *Rollinia*.

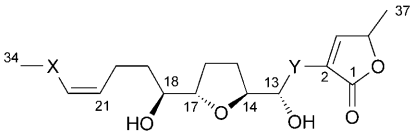
Results and Discussion. – The molecular formula of **1** was determined as $C_{37}H_{66}O_9$ from the $[M+Na]^+$ peak at *m/z* 678 in the liquid secondary-ion mass spectrum (LSI-MS). A positive *Kedde* test [9] and IR absorptions at 3400, 2920, 2850, 1745, 1650 cm^{-1} suggested **1** to be an annonaceous acetogenin bearing an α,β-unsaturated γ-lactone moiety. In the ^{1}H-NMR spectrum of **1** (*Table 1*), the signals of a typical α,β-unsaturated-γ-lactone with an OH group at C(4) [10] appeared at δ(H) 7.22 (H–C(35)),



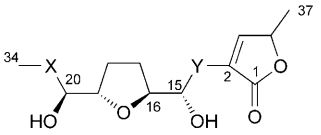
Rolifolin
1 X = $(CH_2)_9$ Y = $(CH_2)_6$



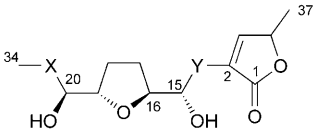
Annonin-I
2 X = $(CH_2)_5$ Y = $(CH_2)_{12}$



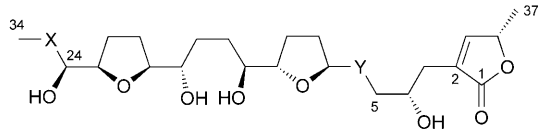
Rolilaurin
3 X = $(CH_2)_{11}$ Y = $(CH_2)_{10}$



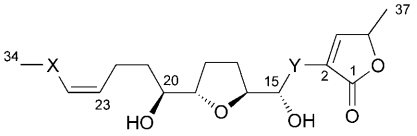
Uvariamicin-I
4 X = $(CH_2)_{14}$ Y = $(CH_2)_{12}$



Uvariamicin-II
5 X = $(CH_2)_{12}$ Y = $(CH_2)_{14}$



Sylvaticin
6 X = $(CH_2)_9$ Y = $(CH_2)_6$



Bulatencin
7 X = $(CH_2)_9$ Y = $(CH_2)_{12}$

Fig 1. *Structures of the acetogenins* **1**–**7**. Relative configurations only.

5.04 (H–C(36)), and 1.41 (H–C(37)). In the ^{13}C-NMR spectrum (*Table 1*), the corresponding signals appeared at δ(C) 174.94 (C(1)), 152.21 (C(35)), 130.87 (C(2)), 78.25

Table 1. *^{1}H- and ^{13}C-NMR Spectroscopic Data of* **1** *and* **6** [13]. At 400 (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$; δ in ppm, J in Hz.

Position	**1**		**6**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	–	174.94	–	174.59
2	–	130.87	–	131.07
3a	2.52–2.57 (*m*)	30.19	2.53 (*ddt*, *J*=15.1, 3.2, 1.6)	33.24
3b	2.52–2.57 (*m*)	30.19	2.40 (*ddt*, *J*=15.1, 8.2, 1.3)	33.24
4	3.63–3.67 (*m*)	72.42	3.84 (*m*)	69.80
5	3.41–3.46 (*m*)	74.40	1.30–1.50 (*m*)	37.31
6–11	1.25–1.52 (*m*)	28.3–30.1	1.30–1.50 (*m*)	22.7–35.47
12	3.81–3.90 (*m*)	79.29	3.89 (*m*)	79.25
13	1.64, 1.92 (*m*)	31.91	1.60, 1.99 (*m*)	22.6–32.4
14	1. 64, 1.95 (*m*)	28.62	1.60, 1.97 (*m*)	22..6–32.4
15	3.74–3.80 (*m*)	81.78	3.80 (*q*, *J*=6.9)	81.80
16	3.41–3.86 (*m*)	73.80	3.44 (*m*)	74.30
17	1.50–1.70 (*m*)	26.0–29.7	1.49 (*m*)	22.6–32.4
18	1.50–1.70 (*m*)	26.0–29.7	1.72 (*m*)	22.6–32.4
19	3.48–3.53 (*m*)[a])	74.23[a])	3.51 (*m*)	74.04
20	3.81–3.90 (*m*)[a])	82.44[a])	3.87 (*m*)	82.34
21	1.84, 1.97 (*m*)[a])	24.0[a])	1.86, 1.98 (*m*)	22.6–32.4
22	1.75, 1.97 (*m*)[a])	28.0[a])	1.77, 1.98 (*m*)	22.6–32.4
23	3.90–3.96 (*m*)[a])	83.01[a])	3.93 (*m*)	82.93
24	3.81–3.90 (*m*)[a])	72.46[a])	3.87 (*m*)	72.29
25–33	1.25–1.52 (*m*)	28.3–30.18	1.30–1.50 (*m*)	22.6–33.04
34	0.88 (*t*, *J*=8.8)	14.1	0.88 (*t*, *J*=7.0)	14.06
35	7.22 (*d*, *J*=1.3)	152.21	7.19 (*q*, *J*=1.4)	151.80
36	5.04 (*qd*, *J*=6.6, 1.3)	78.25	5.06 (*qq*, *J*=6.8, 1.5)	77.93
37	1.41 (*d*, *J*=6.6)	19.03	1.43 (*d*, *J*=6.9)	19.04

[a]) Signals might be interchanged.

(C(36)), and 19.03 (C(37)). According to *Cavé et al.* [11], the lack of a 4-OH group displaces the signals of C(35) and C(2) to δ(C) 148.0 and 134.0, respectively.

The above assignments were confirmed by HMQC and HMBC spectra and by an EI-MS signal at *m/z* 141 (*Fig. 2*). The ^{1}H-NMR signals at δ(H) 3.41–3.96 were correlated, by HMQC, with the signals at δ(C) 74.40, 74.23, 73.80, 72.46, and 72.42, respectively, which suggested a total of five OH groups in **1**. The remaining signals in the same spectral region (δ(C) 83.01, 82.44, 81.78, and 79.29) were associated with four methine groups of two substituted tetrahydrofuran rings. An HMQC experiment showed their correlations with the *multiplet*s at δ(H) 3.90–3.96, 3.81–3.90, 3.74–3.80, and 3.81–3.90, respectively, attributed to H–C(23), H–C(20), H–C(15), and H–C(12). According to the literature [11], a signal at δ(C) 79.29 is consistent with the lack of an OH group adjacent to a substituted tetrahydrofuran ring. The signal at δ(C) 78.25 was associated with C(36) (see *Table 1*). The *multiplet* at δ(H) 3.63–3.67 was assigned to H–C(4), as confirmed by HMBC and ^{1}H,^{1}H-COSY experiments. The chemical shift of C(4) (δ(C) 72.42) was slightly different from those found for acetogenins bearing 4-OH groups, thus pointing to oxygenation at C(5) [12]. Analysis of ^{1}H- and ^{13}C-NMR, and HMQC spectra permitted us to assign the remaining carbinol-type signals (δ(C),

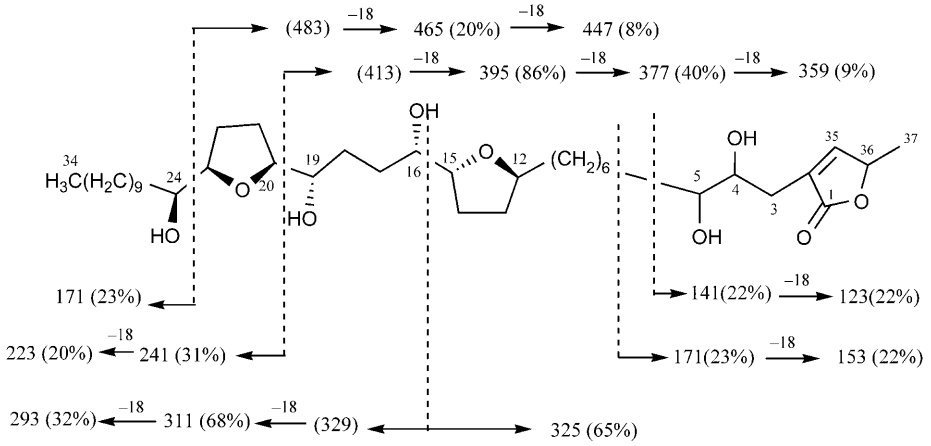


Fig. 2. *Diagnostic EI-MS fragment ions of* **1**

δ(H)): H–C(5) (74.40, 3.41–3.46), H–C(16) (73.80, 3.41–3.86), H–C(19) (74.23, 3.48–3.53), and H–C(24) (72.46, 3.81–3.90). The ^{1}H,^{1}H-COSY spectrum showed connectivities between H–C(4) at δ(H) 3.63–3.67 and H–C(5) at 3.41–3.46, and also allowed a detailed assignment of the tetrahydrofuran CH_2 resonances: δ(H) 1.64 (H_{ax}–C(13,14)), 1.92 (H_{eq}–C(13)), 1.95 (H_{eq}–C(14)), 1.84 (H_{ax}–C(21)), 1.97 (H_{eq}–C(21,22)), and 1.75 (H_{ax}–C(22))[1]).

The location of the two substituted tetrahydrofuran rings and five OH groups along the hydrocarbon chain of **1** was based on EI-MS analysis (*Fig. 2*), and corroborated by comparison with the EI-MS data of the known congener sylvaticin (**6**) isolated from *Rollinia mucosa* [13]. The relative configurations at C(15)/C(16), C(19)/C(20), and C(23)/C(24) were determined by comparing the ^{1}H- and ^{13}C-NMR data of **1** with those of model compounds of known configuration [11]. This comparison showed that the relative configurations at C(15)/C(16), C(19)/C(20), and C(23)/C(24) of **1** are *threo*, *threo*, and *erythro*, respectively. Based on the work of *Cavé et al.* [11], the tetrahydrofuran rings of **1** were found to be *trans*- (C(12)/C(15)) and *cis*-substituted (C(20)/C(24)), just as in sylvaticin (**6**). Actually, the presence of a 5-OH group was the only difference found between rolifolin (**1**) and sylvaticin (**6**). To the best of our knowledge, this type of 'non-adjacent' bis-tetrahydrofuran acetogenin, the most-rare subclass of these compounds, is described here for the first time.

The structure of annonin-I (**2**), a known 'adjacent' bis-tetrahydrofuran acetogenin with two rings flanked by two α-OH groups, was confirmed by comparison of ^{1}H- and ^{13}C-NMR as well as optical-rotation data with those reported in the literature [7].

A mixture of rolilaurin (**3**), uvariamicin-I (**4**), and uvariamicin-II (**5**), isolated in the form of an off-white wax, was isolated from the *hexane* extract of the leaves of *R. laurifolia*. Repeated chromatographic purification (column (CC) and high-pressure liquid chromatography (HPLC)) was required due to very similar retention times in different

[1]) H_{ax} and H_{eq} refer to pseudo-axial and -equatorial H-atoms, resp.

solvent systems and due to the small amount (3.4 mg) of sample. A positive *Kedde* test and IR absorptions at 1675 and 1745 cm^{-1} suggested the presence of acetogenins possessing α,β-unsaturated γ-lactone rings, which was confirmed by the ^{1}H-NMR signals at δ(H) 6.97 (H–C(35)), 4.99 (H–C(36)), 1.41 (H–C(37)), as well as by the ^{13}C-NMR signals at δ(C) 148.8 (C(35)), 134.4 (C(2)), 77.3 (C(36)), and 19.2 (C(37)) in the case of **3** (*Table 2*). The two methine resonances at δ(C) 82.6 and 74.3 suggested the presence of only one tetrahydrofuran ring each, with flanking α-OH groups. The *multiplet*s centered at δ(H) 3.43 (2 H) and 3.82 (2 H) were assigned to the methine-type H-atoms of this system. In a ^{1}H,^{1}H-COSY experiment, these assignments were confirmed, based on correlations of H–C(36) at δ(H) 4.99 with H–C(37) at 1.41, and of H–C(35) at 6.97 with H–C(3) at 2.24. The presence of a tetrahydrofuran ring with α,α'-OH groups was confirmed by the correlation of the signal at δ(H) 3.43 (*m*, H–C(17)) with that at δ(H) 3.82 (*m*, H–C(18)) in the case of **3**, and similarly for **4** and **5** (data not shown).

Table 2 . *^{1}H- and ^{13}C-NMR Spectroscopic Data of* **3** *and* **7**. At 400 (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$; δ in ppm, *J* in Hz.

Position	**3**		**7**[a])	
	^{1}H	^{13}C	^{1}H	^{13}C
1	–	174.8	–	173.82
2	–	134.4	–	134.22
3	2.24 (*m*)	34.1	–	–
4–12	1.26–1.54 (*m*)	25.2–25.7	–	–
13	3.40 (*m*)	74.3	–	–
14	3.82 (*m*)	82.6	–	–
15	1.75–1.93 (*m*)	25.2–29.6	3.42	74.05
16	1.75–1.93 (*m*)	25.2–29.6	3.81	82.68
17	3.82 (*m*)	82.6	–	–
18	3.43 (*m*)	74.3	–	–
19	1.45 (*m*)	22.7	3.81	82.68
20	2.04 (*m*)	22.7	3.42	74.05
21	5.37	128.9	–	–
22	5.39	129.3	–	–
23	2.19	27.4	5.37 (*m*)	130.73
24	1.59	25.2–29.6	5.37 (*m*)	128.89
25–33	1.26–1.54 (*m*)	25.2–29.6	–	–
34	0.88 (*t*, *J* = 6.7)	14.11	0.88 (*t*)	14.17
35	6.97 (*d*, *J* = 2.2)	148.8	6.99 (*q*)	148.77
36	4.99 (*m*)	77.3	5.00 (*qq*)	77.41
37	1.41 (*d*, *J* = 6.0)	19.2	1.41 (*d*)	19.24

[a]) Data recorded at 500 (^{1}H) and 125 MHz (^{13}C) [14].

The presence of a C=C bond in **3** was apparent from the signals at δ(H) (δ(C)) 5.37 (128.9) and 5.39 (129.3) (*Table 2*), as confirmed by 2D connectivities. A ^{1}H,^{1}H-COSY experiment showed correlations of δ(H) 5.37 (H–C(21)) and 5.39 (H–C(22)) with δ(H) 2.04 (CH_2(20)) and 2.19 (CH_2(23)), respectively. The signal at δ(H) 2.04

(CH_2(20)) was further correlated with that at δ(H) 1.45 (CH_2(19)), which, in turn, was correlated with δ(H) 3.43 (H–C(18)). Hence, the C=C bond was two CH_2 groups away from the tetrahydrofuran ring.

The EI-MS data of the mixture **3**/**4**/**5** revealed the presence of three acetogenins with molecular formulae of $C_{37}H_{68}O_5$ and $C_{37}H_{66}O_5$. Based on analysis of fragment ions (*Fig. 3*), the compounds only differ by the position of the tetrahydrofuran ring and, in the case of **3**, by a C=C bond. Generally, the major fragments ions in acetogenins arise from cleavage in α-position to the rings, the charge being retained on the fragment containing the α,β-unsaturated γ-lactone moiety. In *Fig. 3*, the major fragment ions observed for **3** are shown. The ion at *m*/*z* 209 further confirmed the location of the C(21)=C(22) bond.

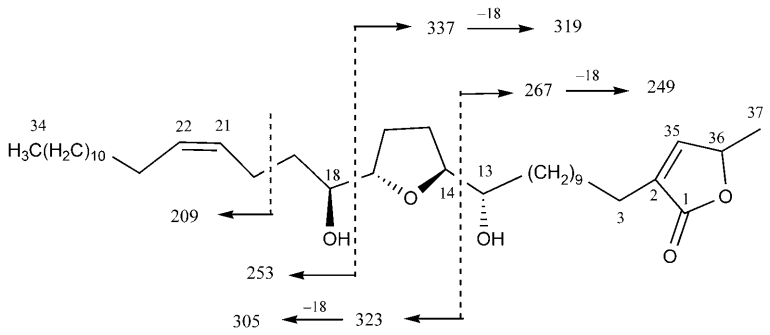


Fig. 3. *Diagnostic EI-MS fragment ions of* **3**

The relative configurations of the HO–CH–ring–CH–OH moieties in **3**–**5** were found to be *threo–trans–threo*, as determined by comparing the ^{1}H- and ^{13}C-NMR data with those of model compounds of known relative configuration [11].

The structure of uvariamicin-I (**4**) and uvariamicin-II (**5**) were determined by comparison of the spectroscopic data with those described by *Hisham et al.* [6]. Compound **4** has already been isolated from *Rollinia laurifolia* [5], but **5** has not been found before in *Rollinia*. Rolilaurin (**3**) and bulatencin (**7**) [11][14] differ by the position of their C=C bond in the hydrocarbon chain, and **3** is a novel acetogenin described herein for the first time.

Experimental Part

General. HPLC: *Waters 501* apparatus, with a *486* UV-detector and a *746* integrator. M.p.: *Mettler FP-80 HT*; uncorrected. Optical rotation: *Perkin-Elmer 341* polarimeter. IR Spectra: *Shimadzu IR-408* spectrophotometer, KBr samples; in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker DRX-400 AVANCE* instrument, 400 or 100 MHz, resp.; *ca.* 1–2 mg of substance in $CDCl_3$ (0.5 ml); δ in ppm rel. to Me_4Si, *J* in Hz. EI-MS and LSI-MS: *VG-Autospec Q* spectrometer; in *m*/*z* (rel. %).

Plant Material. *Rollinia laurifolia* SCHL. (Annonaceae) was collected on the campus of Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Minas Gerais, Brazil, and identified by Dr. *Júlio Antônio Lombardi*. A voucher specimen (No. 22749) was deposited at the Herbarium of the Natural History Museum of UFMG, Belo Horizonte, Minas Gerais, Brazil.

Extraction and Isolation. The leaves of *R. laurifolia* were cleaned, dried at r.t., ground to a powder (3.0 kg), and submitted to successive cold extraction with hexane and EtOH, which afforded, after solvent evaporation *in vacuo*, 68.0 and 180.0 g of extract, resp. A part of the EtOH extract (63.0 g) was submitted to column chromatography (CC) (191 g of polyamide gel, <0.07 mm; *Macherey Nagel*), eluting with AcOEt and MeOH. The MeOH fraction (43.0 g) was submitted to CC (430 g of silica gel *60*, 70–230 mesh; *Merck*), eluting with hexane, CH_2Cl_2, AcOEt, and MeOH, either neat or in mixtures of increasing polarity: 446 fractions. *Fr. 369–377* (1.37 g) were combined and subjected to CC (*Sephadex LH-20*; *Sigma*), and a resulting subfraction (subgroup *4*; 107.6 mg) was purified by reverse-phase (RP) HPLC (*C18*, 5.0 μm; MeCN/H_2O 50:50 (isocratic) at 3 ml/min; detection at 220 nm) to afford **1** (3.5 mg) as a white waxy material. *Fr. 232–241* (199 mg) were combined and submitted to successive RP-HPLC (*C18*; MeCN/H_2O 50:50 → 1:99 in 35 min) to afford **2** (14.0 mg) as a white wax.

The original hexane extract (68.0 g) was submitted to CC (700 g of SiO_2, 70–230 mesh), eluting with hexane, CH_2Cl_2, AcOEt, and MeOH, either neat or in mixtures of increasing polarity: 320 fractions. *Fr. 305–317* (4.12 g) were combined and partitioned between equal volumes of hexane and 10% aq. MeOH. The aq. MeOH fraction (1.03 g) was subjected to CC (*Sephadex LH-20*), and a resulting subfraction (subgroup *5*; 72.6 mg) was purified by RP-HPLC (*C18*; MeOH/H_2O/THF 90.0:9.5:0.5 (isocratic)) to afford a mixture of **3**/**4**/**5** (3.4 mg) as a white waxy material.

Rolifolin (= *3-(9-{(2*R*,5*S*)-5-[(1*S*,4*S*)-1,4-dihydroxy-4-{(2*S*,5*R*)-5-[(1*S*)-1-hydroxyundecyl]tetrahydrofuran-2-yl}butyl]tetrahydrofuran-2-yl}-2,3-dihydroxynonyl)-5-methylfuran-2(5*H*)-one*; **1**). White wax. M.p. 79.9–81.6°. $[\alpha]_D^{25} = -3.6$ ($c = 0.2$, MeOH). IR (KBr): 3400, 1745, 1650, 1460, 1390, 1070. ^{1}H- and ^{13}C-NMR: see *Table 1*. EI-MS: 678 ($[M+Na]^+$, $C_{37}H_{66}NaO_9^+$). LSI-MS (70 eV): 465 (223), 395 (100), 377 (13), 325 (63), 297 (14), 267 (6).

Mixture of Compounds **3**, **4**, *and* **5**. White wax. M.p. 81.4–83.0°. ^{1}H- and ^{13}C-NMR: see *Table 2*. EI-MS: 615.7 ($[M+Na]^+$). LSI-MS (70 eV): 267 (100), 295 (17), 323 (28).

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REFERENCES

[1] L. A. R. Santos, M. A. D. Boaventura, L. P. S. Pimenta, *Biochem. Syst. Ecol*. **2005**, in press.
[2] L. P. S. Pimenta, G. V. Pinto, J. A. Takahashi, L. G. F. Silva, M. A. D. Boaventura, *Phytomedicine* **2003**, *10*, 209.
[3] L. P. S. Pimenta, M. A. D. Boaventura, N.-J. Sun, J. M. Cassady, A. B. Oliveira, *Phytochemistry* **1996**, *42*, 705.
[4] L. P. S. Pimenta, F. C. Nascimento, A. C. Assunção, M. A. D. Boaventura, *Tetrahedron Lett.* **2001**, *42*, 8433.
[5] F. C. Nascimento, A. C. Assunção, L. P. S. Pimenta, M. A. D. Boaventura, *Quim. Nova* **2003**, *26*, 319.
[6] A. Hisham, L. A. Pieters, M. Claeys, E. Esmans, R. Dommisse, A. J. Vlietinck, *Tetrahedron Lett.* **1990**, *31*, 4649.
[7] Y. Fujimoto, T. Eguchi, K. Kakinuma, *Chem. Pharm. Bull*. **1988**, *36*, 4802.
[8] J. Saez, S. Sahpaz, L. Villaescusa, R. Hocquemiller, A. Cavé, *J. Nat. Prod*. **1993**, *56*, 351.
[9] F. Q. Alali, X.-X. Liu, J. L. McLaughlin, *J. Nat. Prod*. **1999**, *62*, 504.
[10] D. H. Kim, E. S. Ma, K. D. Suk, J. K. Son, J. S. Lee, M. H. Woo, *J. Nat. Prod.* **2001**, *64*, 502.
[11] A. Cavé, D. Cortes, A. Figadère, A. Laurens, G. R. Pettit, in 'Progress in the Chemistry of Organic Natural Products', Eds. W. Herz, G. W. Kirby, R. E. Moore, W. Steglich, C. Tamm, Springer-Verlag, Wien, 1997, p. 81.
[12] D. Alfonso, T. Colman-Saizarbitoria, G.-X.Zhao, G. Shi, Q. Ye, J. T. Schwedler, J. L. McLaughlin, *Tetrahedron* **1996**, *52*, 4215.
[13] G. Shi, L. Zeng, Z.-M. Gu, J. M. McDougal, J. L. McLaughlin, *Heterocycles* **1995**, *41*, 1785.
[14] X.-P. Fang, M. J. Rieser, Z.-M. Gu, G.-X. Zhao, J. L. McLaughlin, *Phytochem. Anal.* **1993**, *4*, 27.

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