Salicassin, an Unprecedented Chalcone–Diterpene Adduct and a Quinone Methide Triterpenoid from *Maytenus salicifolia*

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In continuation of our work on *Maytenus salicifolia*, we report herein the isolation and structural elucidation of two new compounds, salicassin (**1**), a diterpene-chalcone adduct with an unprecedented C-framework, and (16*β*)-16-hydroxypristimerin (**2**), a quinone methide triterpenoid. Their structures were elucidated on the basis of spectroscopic analysis, including 1D- and 2D-NMR techniques (COSY, ROESY, HSQC, and HMBC). In addition, 22 known compounds were isolated and characterized by comparison of their spectra with reported data. Compound **2**, structurally related to the well known cytotoxic quinone methide triterpenoids, exhibited an antiproliferative effect on HeLa, A-549, and HL-60 human cell lines, with IC_{50} values of 2.2, 3.2, and 2.7 μM, respectively.

Introduction. – The *Maytenus* genus, with more than 225 species, is widely distributed in the tropical and subtropical regions of the world, and represents the most diverse and richest genus of the Celastraceae family. In Brazil, 80 species of this genus have so far been documented as being present. Many traditional properties attributed to the *Maytenus* species have been experimentally verified, and these include antimitotic, antioxidant, and DNA polymerase *β*-lyase inhibitory activities [1].

The species of the Celastraceae biosynthesize dimeric [2] and trimeric [3] triterpenes, and octacyclic sesquiterpene–triterpene adducts [4], possibly through hetero-*Diels–Alder* reactions *via* the formation of a C(1)–C(4) dioxane system. Uragogin (triterpene–neolignan ester) and blepharodin (heptacyclic arylpropanoid–nortriterpene phenol) isolated from *Crossopetalum uragoga* and *Maytenus magellanica*, respectively, are examples of hetero-*Diels–Alder* adducts endowed with dioxane bridges [5]. Furthermore, the isolation of *Diels–Alder* decacyclic $C_{20}-C_{30}$ adducts [6] and diterpene dimers [7][8] have also been reported for Celastraceae.

Maytenus salicifolia REISSEK is endemic in the state of Minas Gerais, southeast of Brazil, and is commonly known as 'cafezinho'. Its leaves have been used in traditional

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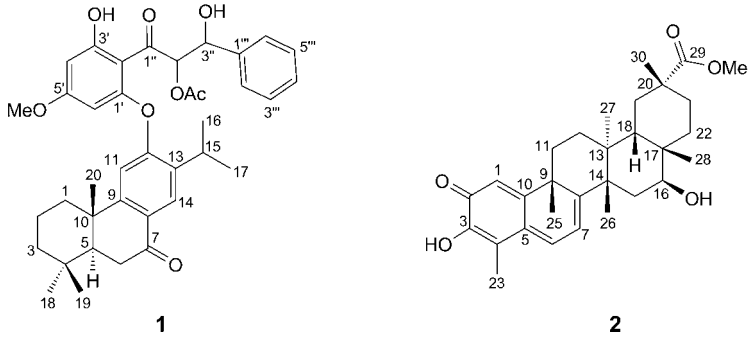
medicine for the treatment of ulcers and other stomach disorders, and the tea (decoction) from fresh leaves is topically applied to alleviate itches and symptoms of skin allergies [9]. As part of our studies on *M. salicifolia*, we have previously reported the isolation of pentacyclic triterpenes, as well as the antioxidant properties of extracts from the aerial part [10].

In continuation of our work on *M. salicifolia*, we report herein the isolation and structural elucidation of two new compounds, salicassin (**1**), an adduct formed by abietane diterpene and chalcone moieties linked *via* an O-bridge, and (16β)-16-hydroxypristimerin (**2**), a quinone methide triterpene (*Fig. 1*), along with 22 known compounds. Their structures were established through ^{1}H- and ^{13}C-NMR spectroscopy, including two-dimensional homonuclear (COSY and ROESY) and heteronuclear (HSQC and HMBC) correlation experiments.



1 **2**

Fig. 1. *Salicassin* (**1**) *and (16β)-16-hydroxypristimerin* (**2**)*, isolated from* M. salicifolia

Based on the cytotoxicity of quinone methide triterpenoids against human tumor cell lines [11 – 14], the related compound **2** was assayed for its effect on HeLa (human cervical adenocarcinoma), A-549 (human lung carcinoma), and HL-60 (promyelocytic leukemia) cell lines.

Results and Discussion. – The hexane and $CHCl_3$ extracts from the root bark of *M. salicifolia* were subjected to repeated chromatography on silica gel and *Sephadex LH-20*, yielding two new terpenes, **1** and **2** (*Fig. 1*). In addition, 22 known compounds were isolated and identified as pinostrobin [15], 4′-hydroxy-3′-methoxy-*trans*-cinnamaldehyde [16], siringaldehyde [17], ferruginol [18], 6,7-dehydroferruginol [19], glochidonol [20], 3-epiglochidiol [20], lupenone [20], lupeol [21], 30-hydroxylup-20(29)-en-3-one [22], rigidenol [23], nepeticin [24], glochidone [25], 11α-hydroxyglochidone [26], friedelin [27], 29-hydroxyfriedelin [28], friedelane-1,3-dione [29], glutinol [30], wilforlide A [31], tingenone, pristimerin [32], and β-sitosterol [33] by comparison of their spectroscopic data with values reported in the literature.

Compound **1** was isolated as a white amorphous solid, and its molecular formula, $C_{38}H_{44}O_8$, was deduced by the HR-ESI-MS (*m/z* 628.2949 (M^+)) and ^{13}C-NMR data. Its IR spectrum exhibited absorption bands at 3401 and 1642 cm^{-1}, characteristics of OH and C=O groups, respectively. The absorption bands at 1574, 1503, and 1454 cm^{-1} were attributed to C=C bonds of aromatic systems, and those at 769 and 762 cm^{-1}

suggested the presence of a monosubstituted aromatic ring [34]. The ^{1}H-NMR (*Table 1*) spectrum of **1** showed resonances for three tertiary Me groups at δ(H) 0.95 (Me(18)), 1.01 (Me(19)) and 1.25 (Me(20)), as well as those for an ^{i}Pr group attached to an unsaturated C-atom at δ(H) 1.27 and 1.29 (each *d*, J = 7.0 Hz, Me(16), Me(17)) and 3.18 (*sept.*, J = 7.0 Hz, H–C(15)). Signals for two aromatic H-atoms at δ(H) 6.72 (*s*, H–C(11)) and 7.94 (*s*, H–C(14)), one CH group at δ(H) 1.85 (*dd*, J = 13.8, 3.8 Hz, H–C(5)), and a CH_2 group at δ(H) 2.68 (*dd*, J = 18.1, 3.8 Hz, H_α–C(6)) and 2.59 (*dd*, J = 18.1, 13.8 Hz, H_β–C(6)), characteristic of geminal H-atoms vicinal to a C=O group, were also observed. The ^{13}C-NMR (*Table 1*) and DEPT NMR spectra displayed diterpene signals for 20 C-atoms: five Me, four CH_2, and four CH groups, and seven quaternary C-atoms, which included one C=O at δ(C) 198.7 and six aromatic C-atoms at δ(C) 109.9, 124.6, 132.6, 135.2, 156.5, and 158.3. The profile of these signals resembled that of sugiol (=(4a*S*,10a*S*)-2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-methylethyl)-phenanthren-9(1*H*)-one) [18], a previously reported abietane diterpenoid. The ^{1}H-NMR spectrum of compound **1** also displayed signals at δ(H) 7.36 (*dd*, J = 2.1, 7.8 Hz, H–C(3‴), H–C(4‴), H–C(5‴)) and 7.40 (*dd*, J = 7.8, 1.8 Hz, H–C(2‴), H–C(6‴)), attributed to a monosubstituted aromatic ring [34], and signals at δ(H) 6.10 (*d*, J = 2.0 Hz, H–C(4′)) and 6.15 (*d*, J = 2.0 Hz, H–C(6′)), characteristic of a 1,2,3,5-tetrasubstituted benzene ring. In addition, two OCH moieties at δ(H) 5.40 (*d*, J = 11.8 Hz, H–C(3″)) and 5.85 (*d*, J = 11.8 Hz, H–C(2″)), and signals for a MeO group at δ(H) 3.76 (*s*) and an AcO group at δ(H) 2.03 (*s*) were observed. The signals at δ(H) 5.78 and 11.49 (OH–C(3″) and OH–C(3′), resp.), interchangeable with D_2O, indicated

Table 1. *^{1}H- and ^{13}C-NMR Data*[a]) (600 and 150 MHz, resp.; $CDCl_3$) *of* **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	Position	δ(H)	δ(C)
CH_2(1)	1.49–1.52, 2.18–2.22 (2*m*)	37.9	Me(20)	1.25[b]) (*s*)	23.2
CH_2(2)	1.78–1.80, 1.63–1.65 (2*m*)	18.5	C(1′)		169.3
CH_2(3)	1.50–1.53, 1.29–1.30 (2*m*)	41.3	C(2′)		101.9
C(4)		33.3	C(3′)		164.1
H–C(5)	1.85 (*dd*, J = 13.8, 3.8)	49.4	H–C(4′)	6.10 (*d*, J = 2.0)	94.7
CH_2(6)	2.59 (*dd*, J = 18.1, 13.8),	36.1	C(5′)		162.2
	2.68 (*dd*, J = 18.1, 3.8)		H–C(6′)	6.15 (*d*, J = 2.0)	95.7
C(7)		198.7	*Me*O–C(5′)	3.76 (*s*)	55.8
C(8)		132.6	C(1″)		191.7
C(9)		156.5	H–C(2″)	5.85 (*d*, J = 11.8)	72.4
C(10)		37.9	H–C(3″)	5.40 (*d*, J = 11.8)	81.3
H–C(11)	6.72 (*s*)	109.9	C(1‴)		153.2
C(12)		158.3	H–C(2‴)	7.40[b]) (*d*, J = 7.8, 1.8)	126.6
C(13)		135.2	H–C(3‴)	7.36[b]) (*dd*, J = 7.8, 2.1)	128.7
H–C(14)	7.94 (*s*)	124.6	H–C(4‴)	7.36[b]) (*dd*, J = 7.8, 2.1)	129.6
H–C(15)	3.18 (*sept.*, J = 7.0)	26.8	H–C(5‴)	7.36[b]) (*dd*, J = 7.8, 2.1)	128.7
Me(16)	1.27[b]) (*d*, J = 7.0)	22.3	H–C(6‴)	7.40[b]) (*d*, J = 7.8, 1.8)	126.6
Me(17)	1.29[b]) (*d*, J = 7.0)	22.5	O*H*–*C*(3′)	11.49 (*s*)	
Me(18)	0.95 (*s*)	32.6	O*H*–*C*(3″)	5.78 (*s*)	
Me(19)	1.01 (*s*)	21.4	*Ac*O–C(2″)	2.03 (*s*)	20.3, 168.5

[a]) Based on DEPT, HSQC, and HMBC experiments. [b]) Overlapping signals.

the presence of two chelated OH groups in the molecule. These data were also supported by the ^{13}C-NMR spectrum which, except for the signals of the diterpene unit, displayed 15 C-atom signals, including those of 12 aromatic C-atoms, three of which were O-bearing (δ(C) 169.3, 164.1, 162.2), two O-bearing C-atoms (δ(C) 72.4 and 81.3), and a C=O group (δ(C) 191.7). In addition, signals for an AcO (δ(C) 20.3 and 168.5) and MeO group (δ(C) 55.8) were observed. These data suggested the presence of a C_6–C_3–C_6 moiety [35] in the molecule.

The above data strongly suggested **1** to be a diterpene–chalcone adduct, which was further supported by the fragmentation peaks observed in the EI-MS at *m*/*z* 300 ($C_{20}H_{28}O_2^+$) and *m*/*z* 328 ($C_{18}H_{16}O_3^+$). This structure was established by analysis of the 2D-NMR data, including ^{1}H,^{1}H-COSY, HSQC, and HMBC experiments. In particular, the structure of the abietane diterpene unit was demonstrated by the correlations of the signals at δ(H) 1.27 (Me(16)) and 1.29 (Me(17)) with that at δ(H) 3.18 (H–C(15)) in the COSY plot, associated with the ^{i}Pr group. The observed HMBCs δ(H) 7.94 (H–C(14))/δ(C) 198.7 (C(7)) and 26.8 (C(15)), and δ(H) 3.18 (H–C(15))/δ(C) 158.3 (C(12)), 135.2 (C(13)), and 124.6 (C(14)) located the ketone at C(7) and confirmed the positions of substitution at the *B* and *C* rings of the diterpene moiety. Moreover, the structure and substitution pattern of the chalcone unit was confirmed by 2D-NMR experiments. Thus, the HMBC showed the following correlations: H–C(4′) and H–C(6′)/C(5′), C(2′), and C(3′), H–C(4′)/C(3′), and H–C(6′)/C(1′). Moreover, the HMBCs δ(H) 5.85 (H–C(2″))/δ(C) 168.5 (Me*C*OO), 191.7 (C(1″)), and 153.2 (C(1‴)), and δ(H) 5.40 (H–C(3″))/C(1″), C(1‴), C(2‴), and C(6‴) determined the substitution pattern in the molecule. The ROESY experiment showed cross-peaks δ(H) 5.40 (H–C(3″))/δ(H) 7.40 (H–C(2‴), H–C(6‴)), and of the MeO group (δ(H) 3.76) with H–C(4′) and H–C(6′) confirming the position of that substituent at C(5′). To define the linkage between the two units in the molecule, complementary observations were made. In the ^{1}H-NMR spectrum, the signal at δ(H) 11.49 suggested the presence of an OH group at C(3′) chelated to a C=O, which was confirmed by the HMBCs OH–C(3′)/C(2′), C(3′), and C(4′). Moreover, the signal at δ(H) 5.78 (OH–C(3″)) showing an HMBC with δ(C) 81.3 (C(3″)) clearly indicated a free OH group at C(3″). Therefore, the linkage between the diterpene and chalcone units in the molecules was deduced to consist of an ether bond between C(12) and C(1′). Accordingly, the structure of salicassin (**1**) was established as 12-*O*-{2-[2-(acetyloxy)-3-hydroxy-1-oxo-3-phenylpropyl]-3-hydroxy-5-methoxyphenyl}sugiol.

Naturally occurring terpene–chalcone adducts are extremely rare, so far only a few examples having been described, which include those formed by a chalcone and a monoterpene [36], a sesquiterpene [37], or a labdane diterpene [38]. However, to the best of our knowledge, compound **1** represents the first example of a metabolite comprised of an abietane diterpene and a chalcone. Furthermore, compound **1** is a rare adduct example in the Celastraceae species, as it is formed through an ether linkage between the units in the molecule, instead of the much more frequent *Diels–Alder* adducts, where biosynthesis has been hypothesized to be effected by *Diels–Alder*ase enzyme systems [5].

Compound **2** was isolated as a red amorphous solid, and its molecular formula, $C_{30}H_{40}O_5$, was established by HR-EI-MS and ^{13}C-NMR data (*Table 2*), indicating eleven degrees of unsaturation. In its IR spectrum, absorption bands at 3375 (OH

group), 1645 (α,β-unsaturated ketone), and 1725 and 1078 cm^{-1} (ester C=O group) were observed. The ^{1}H-NMR spectrum revealed the presence of six Me *s* at δ(H) 2.23, 1.47, 1.31, 1.24, 1.09, and 0.49, attributed to Me(23), Me(25), Me(26), Me(30), Me(28), and Me(27), and one MeO group at δ(H) 3.58 (*Table 2*). The quinone methide portion of the structure was characterized by a *d* at δ(H) 6.35 ($J = 7.1$ Hz, H–C(7)), a *s* at 6.53 (H–C(1)) and a *d* at 7.03 ($J = 7.1$ Hz, H–C(6)). The *s* at δ(H) 2.23 (Me(23)), characteristic of a Me group attached to an sp^2 C-atom, also confirmed the presence of a quinone methide system. On the basis of the ^{13}C-NMR spectrum of **2**, the presence of

Table 2. *^{1}H- and ^{13}C-NMR Data*[a]) (500 and 125 MHz; $CDCl_3$) *of* **2**. δ in ppm, *J* in Hz.

Position	δ(H)	δ(C)	HMBC (H → C)
H–C(1)	6.53 (*s*)	164.7	C(3), C(5)
C(2)		178.3	
C(3)		146.0	
C(4)		118.2	
C(5)		127.5	
H–C(6)	7.03 (*d*, $J = 7.1$)	134.0	CH(7), C(8), C(10)
H–C(7)	6.35 (*d*, $J = 7.1$)	117.2	C(5), C(14)
C(8)		169.7	
C(9)		47.8	
C(10)		164.0	
CH_2(11)	2.20 (*dd*, $J = 13.7, 2.3$), 2.21–2.23 (*m*)	33.7	C(9), CH_2(12), Me(25)
CH_2(12)	1.24–1.26 (*m*)	30.2	
C(13)		42.6	
C(14)		44.6	
CH_2(15)	2.37 (*dd*, $J = 12.8, 3.4$), 2.18 (*d*, $J = 2.5$)	28.4	CH(16)
H–C(16)	4.12 (*dd*, $J = 11.7, 4.6$)	68.2	CH_2(15), CH_2(22), Me(28)
C(17)		30.2	
H–C(18)	1.84 (*dd*, $J = 8.4, 1.2$)	46.9	
CH_2(19)	2.40[b]) (*dd*, $J = 8.2, 3.2, H_\alpha$), 1.73 (*d*, $J = 8.0, H_\beta$)	29.6	CH(18), CH_2(21)
C(20)		39.4	
CH_2(21)	2.31 (*d*, $J = 4.6, H_\alpha$), 1.53 (*d*, J = 5.8, H_β)	28.4	
CH_2(22)	2.40[b]) (*dd*, $J = 8.2, 3.2, H_\alpha$), 1.39 (*dd*, $J = 6.7, 3.4, H_\beta$)	38.3[b])	CH(18)
Me(23)	2.23 (*s*)	10.3	C(3), C(4), C(5)
Me(25)	1.47 (*s*)	38.3[b])	C(8), C(9), C(10), CH_2(11)
Me(26)	1.31 (*s*)	21.7	C(8), C(13), CH_2(15)
Me(27)	0.49 (*s*)	19.7	CH_2(12), C(13), C(14), CH(18)
Me(28)	1.09 (*s*)	24.2	CH(16), C(17), CH(18), CH_2(22)
C(29)		178.2	
Me(30)	1.24 (*s*)	32.4	CH_2(19), C(20), CH_2(21), C(29)
MeO	3.58 (*s*)	51.8	

[a]) Based on DEPT, HSQC, and HMBC experiments. [b]) Overlapping signals.

30 C-atoms was established, further classified by DEPT experiments as seven Me, six CH_2, and five CH groups, and twelve quaternary C-atoms (*Table 2*). The characteristic downfield signals at δ(C) 164.7 (C(1)), 178.3 (C(2)), 146.0 (C(3)), 118.2 (C(4)), 127.5 (C(5)), 134.0 (C(6)), 117.2 (C(7)), 169.7 (C(8)), and 164.0 (C(10)) were due to a quinone methide system, while the signal at δ(C) 178.2 was attributed to an ester C=O group and that at δ(C) 68.2 to a hydroxylated C-atom. These data indicated that compound **2** is a quinone methide triterpene related to pristimerin (= methyl (9β,13α,14β,20α)-3-hydroxy-9,13-dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oate) [32]. A complete set of 2D-NMR experiments allowed the unambiguous assignment of all H- and C-atoms. Thus, the correlations observed in the HSQC spectrum between δ(H) 1.84 (H–C(18)) and δ(C) 46.9 (C(18)), and of δ(H) 2.40 (H_α–C(19)) and 1.39 (H_β–C(22)) with δ(C) 38.3 (C(22)) allowed the assignments of C(18) and C(22), respectively. Furthermore, the HMBCs δ(H) 1.09 (Me(28))/δ(C) 68.2 (C(16), *i.e.*, OH–*C*H) and δ(H) 4.12 (H–C(16))/δ(C) 38.3 (C(22), *i.e.*, CH_2) confirmed the attachment of the OH group at C(16). The ester group at C(29) was also confirmed by the long-range correlation of the Me group at δ(H) 1.24 (Me(30)) with the ester C=O group at δ(C) 178.2. The relative configuration of H–C(16) was deduced by the J values ($J(16_{ax},15_{ax}) = 11.7$ Hz and $J(16_{ax},15_{eq}) = 4.6$ Hz), which indicated an equatorial orientation of the OH group, as confirmed by a ROESY experiment, showing NOEs effect from H_α–C(19) (δ(H) 2.40) and Me(27) (δ(H) 0.49) to H–C(16) (δ(H) 4.12). Accordingly, compound **2** was identified as (16β)-16-hydroxypristimerin.

Pristimerin, a quinone methide frequently found in species of the Celastraceae family [32], has been reported to induce apoptosis in breast-cancer cell lines [12], and also exhibited cytotoxic activity against human promyelocytic leukemia (HL-60) cell line [13]. Due to the structural similarity with pristimerin, compound **2** was evaluated against cancer cells. Thus, the antiproliferative effect of **2** against HeLa, A-549, and HL-60 cell lines was examined by the MTT assay [39]. After 48 and 72 h of exposure to different concentrations of (16β)-16-hydroxypristimerin (**2**) induced a dose-dependent inhibition of cell proliferation (*Fig. 2*). Compound **2** exhibited high cytotoxicity against the human cell lines HeLa ($IC_{50} = 2.6 \pm 0.3$ μM), A-549 ($IC_{50} = 3.4 \pm 0.6$ μM), and HL-60 ($IC_{50} = 2.7 \pm 0.2$ μM) after 48 h and independently of exposure time (data not shown).

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; *Merck*, 70–230 μm) and *Sephadex LH-20* (*Pharmacia*). HP-TLC: *Sil 20 UV254* plates (*Panreac*). Optical rotations: *Perkin-Elmer-241* automatic polarimeter; $[\alpha]_D$ values in 10^{-1} deg cm^2 g^{-1}. UV Spectra: *Jasco-V-560* spectrophotometer; in abs. EtOH; λ_{max} (log ε) in nm. IR Spectra (film): *Bruker-IFS-55* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR Spectra: *Bruker-Avance-DRX-600* or *Bruker-AMX-500* spectrometers; δ in ppm rel. to Me_4Si as internal standard, J in Hz. High- and low-resolution MS: *VG-Autospec* spectrometer; in m/z (rel. %).

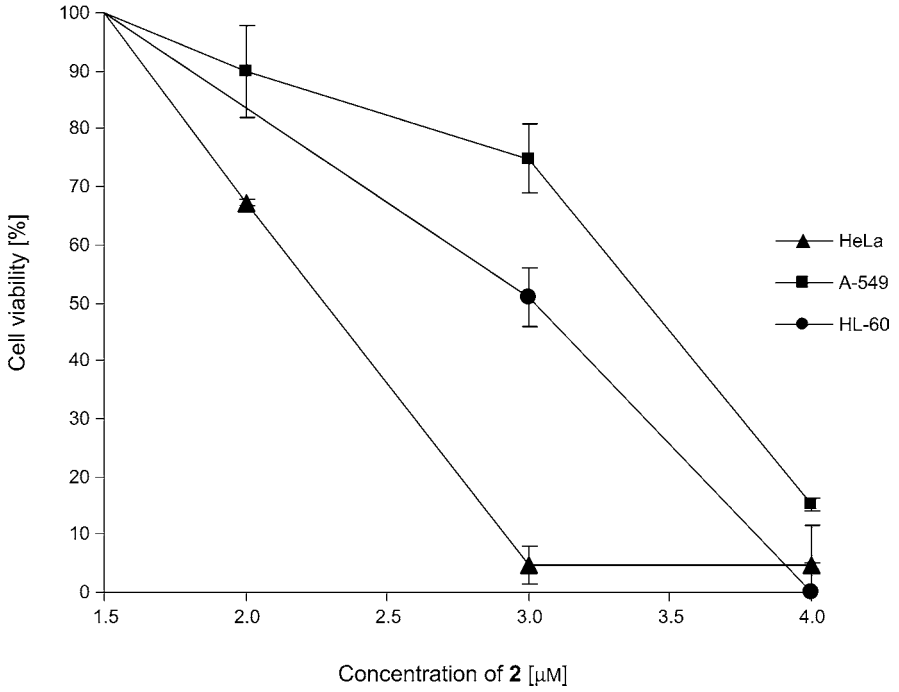

Fig. 2. *Inhibitory effect of (16β)-hydroxypristimerin* (**2**) *on HeLa* (▲), *A-549* (■), *and HL-60* (●) *cell growth.* The cells ($2 \cdot 10^4$ cells/well) were incubated with various concentrations of the compound for 48 h.

Plant Material. Maytenus salicifolia REISSEK (Celastraceae) was collected at Serra de Ouro Branco, a mountain located in the Ouro Branco City region, Minas Gerais, Brazil. A voucher specimen was deposited with the Herbarium José Badini of the Universidade Federal de Ouro Preto, Minas Gerais, Brazil (Collection No. OUPR-18094).

Extraction and Isolation. The root bark of *M. salicifolia* (678.0 g) was extracted with hexane and then with $CHCl_3$ at r.t. The extract obtained with hexane (4.20 g) was submitted to CC (SiO_2, CH_2Cl_2, CH_2Cl_2/acetone of increasing polarity, six fractions of 200 ml): *Fractions 1–6. Fr. 2* gave *ferruginol* (1.0 mg). *Fr. 3* (2.03 g) was subjected to successive CCs (*Sephadex LH-20*, hexane/$CHCl_3$/MeOH 2:1:1; SiO_2, hexane/Et_2O of increasing polarity) and prep. TLC (hexane/AcOEt 75:25): **1** (4.2 mg), *pinostrobin* (4.0 mg), *lupenone* (1.5 mg), *glochidonol* (3.0 mg), *lupeol* (3.7 mg), *glutinol* (3.0 mg), *friedelin* (15.0 mg), *29-hydroxyfriedelin* (12.0 mg), *friedelane-1,3-dione* (2.2 mg), *tingenone* (139.0 mg), and *pristimerin* (208.0 mg). *Fr. 4* gave *30-hydroxylup-20(29)-en-3-one* (2.0 mg) and *Fr. 6* gave *3-epiglochidiol* (2.5 mg). The extract obtained with $CHCl_3$ (25.5 g) was submitted to CC (SiO_2, $CHCl_3$/AcOEt of increasing polarity, 18 fractions of 100 ml): *Frs. 7–12* (by combining fractions with similar TLC profile). *Fr. 8* (782.0 mg) furnished *6,7-dehydroferruginol* (5.0 mg), *nepeticin* (9.0 mg), and **2** (15 mg), by successive CCs (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1; SiO_2, CH_2Cl_2/acetone of increasing polarity) and prep. TLC (hexane/^{i}PrOH 8:2). *Fr. 10* gave an additional amount of *tingenone* (3.3 mg). *Fr. 11* (155.0 mg) was submitted to CC (SiO_2, CH_2Cl_2, CH_2Cl_2/acetone of increasing polarity): *wilforlide A* (3.0 mg). *Fr. 12* (708.4 mg) was submitted to CC (SiO_2, hexane/AcOEt of increasing polarity): *β-sitosterol* (5.0 mg), *glochidone* (1.3 mg), *rigidenol* (20.0 mg), *11α-hydroxyglochidone* (7.0 mg), *4′-hydroxy-3′-methoxy*-trans-*cinnamaldehyde* (9.0 mg), and *siringaldehyde* (1.7 mg).

Salicassin (=*(4a*S*,10a*S*)-6-{2-[2-(Acetyloxy)-3-hydroxy-1-oxo-3-phenylpropyl]-3-hydroxy-5-methoxyphenoxy}-2,3,4,4a,10,10a-hexahydro-1,1,4a-trimethyl-7-(1-methylethyl)phenanthren-9(1*H*)-one*; **1**):

White solid ($CHCl_3$). M.p. 151.6–152.0°. $[\alpha]_D^{20} = +53.08$ ($c = 0.13$, $CHCl_3$). UV (EtOH): 291 (4.49). IR (KBr): 3401, 2926, 1757, 1642, 1574, 1503, 1454, 1216, 1158, 1091, 769, 762, 496. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 628 (34, M^+), 328 (58), 268 (15), 179 (29), 166 (100), 91 (19). HR-ESI-MS: 628.2949 (M^+, $C_{38}H_{44}O_8^+$, calc. 628.2934).

(16β)-16-Hydroxypristimerin (= *Methyl (9β,13α,14β,16β,20α)-3,16-Dihydroxy-9,13-dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oate*; **2**): Red solid ($CHCl_3$). M.p. 99.1–100.0°. $[\alpha]_D^{20} = -80.0$ ($c = 0.10$, $CHCl_3$). UV (EtOH): 421 (3.91). IR (KBr): 3375, 2927, 1725, 1645, 1590, 1140, 1285, 1184, 1078, 950, 754. ^{1}H- and ^{13}C-NMR: *Table 2*. EI-MS: 480 (8, M^+), 241 (22), 201 (100), 145 (4), 95 (4), 55 (5). HR-EI-MS: 480.2869 (M^+, $C_{30}H_{40}O_5^+$, calc. 480.2876).

Cytotoxic Activity. HeLa (human-cervix carcinoma), A-549 (human-lung carcinoma), and HL-60 (leukemia promyelocytic) cell lines were each grown in *Dulbecco*'s modified *Eagle*'s medium (DMEM; *Sigma*), supplemented with 10% fetal bovine serum (FBS; *Gibco*), 1% of penicillin/streptomycin mixture (10.000 UI/ml and 10 mg/ml, resp.), and 200 nM L-glutamine. The cells were maintained at 37° in 5% CO_2 and 98% humidity. Cytotoxicity was assessed by using the colorimetric MTT (=2-(4,5-dimethylthiazol-2-yl)-3,5-diphenyl-2*H*-tetrazolium bromide) reduction assay [39]. HeLa, A-549, and HL-60 cells ($2 \cdot 10^4$ cells/well) were seeded in DMEM, supplemented with 5% fetal bovine serum, in microtiter well plates (96 wells; *Iwaki*, London, UK) together with compound **2**, predissolved in DMSO at different concentrations, for 48 h or 72 h at 37°. At the end of each experimental time period, the optical density was measured with a *microELISA* reader (*Titertek*, *Multiskan Plus II*) at 550 nm after dissolving the formazan (formed from MTT) with DMSO (150 µl). The 50% cell viability value (IC_{50}) was calculated from the curve. Each experiment was performed at least three times in duplicates. Data are given as arithmetic means ± s.d.

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