Tetrahedron 68 (2012) 2671–2678

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Cytotoxic and NF-κB inhibitory sesquiterpene lactones from *Piptocoma rufescens*

Yulin Ren [a], Ulyana Muñoz Acuña [b], Francisco Jiménez [c], Ricardo García [c], Melciades Mejía [c], Heebyung Chai [a], Judith C. Gallucci [d], Norman R. Farnsworth [e,†], Djaja D. Soejarto [e,f], Esperanza J. Carcache de Blanco [a,b], A. Douglas Kinghorn [a,*]

[a] *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[b] *Division of Pharmacy Practice and Administration, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*
[c] *Jardín Botánico Nacional 'Dr. Rafael Ma. Moscoso', Santo Domingo, Dominican Republic*
[d] *Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210, USA*
[e] *Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA*
[f] *Botany Department, Field Museum of Natural History, Chicago, IL 60605, USA*

ARTICLE INFO

Article history:
Received 5 December 2011
Received in revised form 18 January 2012
Accepted 19 January 2012
Available online 28 January 2012

Keywords:
Piptocoma rufescens
Goyazensolide-type sesquiterpene lactones
Germacranolide-type sesquiterpene lactones
Cytotoxicity for HT-29 human colon cancer cells
NF-κB (p65) inhibition assay

ABSTRACT

Six new (**1**–**6**) and eight known germacranolide-type sesquiterpene lactones, along with several known phenylpropanol coumarates and methylated flavonoids, were isolated from the leaves of *Piptocoma rufescens*, collected in the Dominican Republic. The new compounds were identified by analysis of their spectroscopic data, with the molecular structure of **3** being established by single-crystal X-ray diffraction. The absolute configurations of the sesquiterpene lactones isolated were determined from their CD and NOESY NMR spectra, together with the analysis of Mosher ester reactions. Bioassay screening results showed the majority of the sesquiterpene lactones isolated (**1**–**13**) to be highly cytotoxic toward the HT-29 human colon cancer cell line, with the most potent compound being 15-deoxygoyazensolide (**10**, IC_{50}, 0.26 μM). In addition, several of the sesquiterpene lactones exhibited NF-κB (p65) inhibitory activity.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Piptocoma is a small genus of the plant family Asteraceae that occurs in tropical and sub-tropical regions of the Western Hemisphere.[1] There are no previous reports on the chemical constituents of any member of this genus, including the type species, *Piptocoma rufescens* Cass. As part of search for new natural product anticancer agents from diverse organisms,[2] a leaf crude methanol extract of *P. rufescens* collected in the Dominican Republic was found to be cytotoxic toward the HT-29 human colon cancer cell line. Using column chromatography, several new (**1**–**6**) and known cytotoxic sesquiterpene lactones, together with other known non-cytotoxic compounds, were isolated from this species. The isolation, structural characterization, and bioactivity evaluation of these isolates are reported herein.

2. Results and discussion

A methanol extract of the ground leaves of *P. rufescens* was partitioned with *n*-hexane and then chloroform, with these extracts subjected to cytotoxicity testing using the HT-29 human colon cancer cell line. The active *n*-hexane-soluble extract was separated by column chromatography over silica gel to afford two new compounds, rufesolides A (**3**) and B (**4**), and two known compounds, ereglomerulide (**7**)[3] and 15-deoxygoyazensolide (**10**).[4] In turn, separation of the chloroform-soluble extract of this plant part yielded four active fractions. Purification of the first fraction gave a new compound, rufesolide C (**5**), and five known compounds, 15-deoxygoyazensolide (**10**),[4] 2′,3′-dihydro-15-deoxygoyazensolide (**11**),[5] lychnopholide (**12**),[6] 4,5-dihydrolychnopholide (**13**),[7] and eremantholide C.[8] Separation of the second fraction produced three new compounds, rufescenolides A and B (**1** and **2**) and rufesolide D (**6**), and a known compound, 5-epiisogoyazensolide (**9**).[9] Work up of the third fraction afforded three known compounds, goyazensolide (**8**),[10] [3-(3,5-dimethoxy-4-hydroxyphenyl)]-1-propanol-*trans*-coumarate,[3] and 4′-methoxy-5,7,3′-trihydroxyflavone.[11] Finally,

* Corresponding author. Tel.: +1 614 247 8094; fax: +1 614 247 8081; e-mail addresses: kinghorn.4@osu.edu, kinghorn@pharmacy.ohio-state.edu (A.D. Kinghorn).
† Deceased September 10, 2011.

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2012.01.061

separation of the fourth fraction yielded a further quantity of goyazensolide (**8**),[10] in addition to 3-(4-hydroxyphenyl)-1-propanol-*trans*-coumarate,[12] 4′-methoxy-3,5,7,3′-tetrahydroxyflavone,[11] 3-methoxy-5,7-4′-trihydroxyflavone,[11] and 3,4′-dimethoxy-5,7-3′-trihydroxyflavone.[11] The structures of the cytotoxic sesquiterpene lactones isolated (**1**–**13**) are shown in Fig. 1, and those of the inactive compounds are presented in Fig. S10 (Supplementary data).

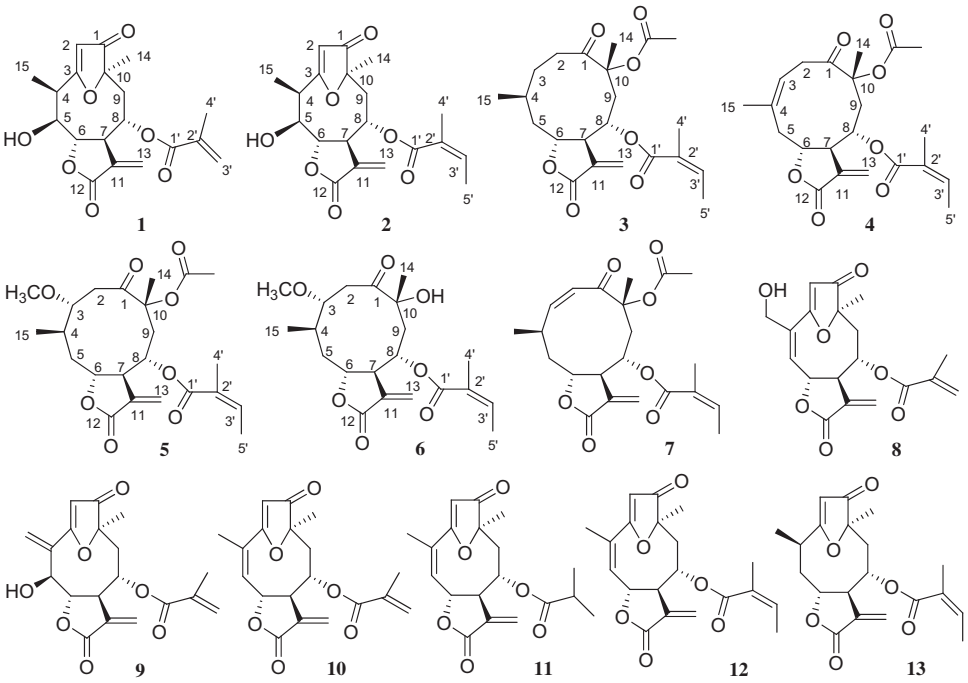


Fig. 1. Structures of the cytotoxic sesquiterpene lactones (**1**–**13**) isolated from *P. rufescens*.

Compound **1** was isolated as an amorphous white powder with a molecular formula of $C_{19}H_{22}O_7$, as determined by HRESIMS (*m*/*z* 385.1253 $[M+Na]^+$, calcd for 385.1263). It showed UV (λ_{max} 212 and 262 nm) and IR [ν_{max} 3466 (hydroxy), 1766 and 1634 (α,β-unsaturated γ-lactone), 1712 and 1634 (α,β-unsaturated ester), 1712 and 1588 (dihydrofuran-3-one ring) cm^{-1}] absorptions typical for a furan ring-containing germacranolide, with such compounds also known as furanoheliangolides or goyazensolides.[13] This preliminary structural assignment was supported in the ^1H NMR spectrum by a three-proton methyl singlet at δ_H 1.47, a three-proton methyl doublet at δ_H 1.36, two doublets at δ_H 5.50 and 6.23 for two terminal olefinic protons (H-13), and a broad singlet at δ_H 5.78 for a vinylic proton H-2 (Table 1). In the ^{13}C NMR spectrum, signals were observed for a carbonyl group at δ_C 204.7, an oxygen-substituted vinylic group at δ_C 106.2 and 192.0, a lactone carbonyl group at δ_C 168.2, and three oxygen-bearing carbons at δ_C 74.6, 80.7, and 71.0 (Table 2).[10]

An oxygen bridge between C-3 and C-10 was deduced from the HMBC correlations from H-2 to C-10, H-4 to C-2, and H-15 to C-3 (Fig. 2). In addition, the ^1H and ^{13}C NMR spectra of **1** revealed the presence of a methacrylate group, characterized by a methyl singlet at δ_H 1.81 and two broad singlets at δ_H 5.53 and 5.99 in the ^1H NMR spectrum, and four signals that appeared at δ_C 166.9 (C-1′), 135.3 (C-2′), 126.6 (C-3′), and 17.9 (C-4′) in the ^{13}C NMR spectrum.[10] This methacrylate group was assigned to C-8, as supported by a HMBC correlation between H-8 and C-1′ (Fig. 2).

Comparison of the ^1H and ^{13}C NMR data of **1** with those of **10**[4] (Tables 1 and 2) indicated both compounds to be methacryloxy-bearing goyazensolide-type sesquiterpene lactones, with differences evident at their C-4 and C-5 positions. Replacement of the observed ^{13}C NMR signals at δ_C 130.4 (C-4) and 135.0 (C-5) for **10**[4] with those at δ_C 37.5 (C-4) and 74.6 (C-5) for **1** (Table 2) indicated the presence of a 5-hydroxy substituent for the latter compound. This was evidenced by the molecular formula of $C_{19}H_{22}O_7$ for **1** ($C_{19}H_{20}O_6$ for **10**) indicated by the mass spectrum, and supported by HMBC correlations between H-2/C-10, H-4/C-2 and C-6, H-15/C-3 and C-5, and H-5/C-7, and the proton-proton spin system from H-4/H-5, H-5/H-6, H-6/H-7, H-7/H-8 to H-8/H_2-9 established from the $^1H-^1H$ COSY NMR spectrum (Fig. 2).

The relative configurations at C-4, C-5, C-6, C-7, C-8, and C-10 of **1** were deduced by analysis of the NOESY 2D NMR spectrum (Fig. 3), and the absolute configuration at C-7 of **1** was determined by analysis of its CD spectrum. Negative Cotton effects at 232 and 264 nm observed in the CD spectrum (Fig. 4) supported a 7*R* absolute configuration, consistent with previous studies demonstrating that sesquiterpene lactones with a trans-fused α-methylene-γ-lactone ring connected between the C-6 and C-12 positions with an α-configuration for H-7 (7*R*) show negative Cotton effects at ca. 220 and 260 nm in their CD spectra, arising from the $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions of the lactone ring, respectively.[14,15]

To determine the absolute configuration at C-5, the modified Mosher ester NMR method was applied to **1**. Following a literature procedure, the (*S*)-MTPA ester of **1** (**1s**) was prepared by reaction of **1** and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, and the (*R*)-MTPA ester of **1** (**1r**) was prepared by reaction of **1** and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride.[16] Analysis of ^1H NMR chemical shift differences ($\Delta\delta_{S-R}$) between **1s** and **1r** revealed negative values for H-2, H-5, H-9, H-13a, H-14, and H-15, and positive values for H-6, H-8, H-13b, H-3′, and H-4′ (Fig. 5), which indicated a *S* configuration for C-5.[16] In turn, the absolute configurations at C-4, C-6, C-8, and C-10 were determined as *R*, *S*, *S*, and *R*, respectively, by analysis of the NOESY correlations between H-5/H-14, H-6/H-8 and H-15, H-7/Hb-9 and H-14, and H-8/H-15 (Fig. 3). Thus,

Table 1
^{1}H NMR data of compounds **1**–**6** in $CDCl_3$[a]

Position	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[c]
2a	5.78 br s	5.78 s	2.74 m	3.07 m	2.79 dd (9.9, 19.8)	2.45 dd (2.9, 16.6)
2b			2.59 m	3.38 m	3.22 dd (4.5, 19.0)	2.66 dd (3.9, 16.6)
3a			2.01 m	5.74 br s	3.67 m	3.70 m
3b			1.58 m			
4	3.39 m	3.36 m	2.01 m		2.14 m	2.13 m
5a	4.34 m	4.32 m	1.73 m	2.60 br s	2.03 m	2.19 dd (4.2, 16.5)
5b			1.67 m		1.45 m	1.12 m
6	4.49 dd (5.0, 9.5)	4.51 dd (5.0, 9.4)	4.30 m	4.19 m	4.45 m	4.68 m
7	3.35 m	3.36 m	2.97 m	3.27 m	2.88 m	2.86 m
8	4.34 m	4.32 m	4.94 m	5.22 m	4.98 m	4.88 dt (3.1, 11.4)
9a	2.34 dd (1.5, 14.2)	2.33 m	2.21 m	2.00 m	2.14 m	2.86 m
9b	2.43 dd (11.9, 12.9)	2.45 t (13.4)	2.59 m	2.43 t (12.0)	2.61 dd (9.0, 17.1)	3.00 m
13a	6.23 d (3.2)	6.23 d (3.2)	6.27 d (2.2)	6.35 br s	6.29 br s	6.24 br s
13b	5.50 d (2.7)	5.47 d (2.7)	5.63 d (1.5)	5.74 br s	5.67 br s	5.64 br s
14	1.47 s	1.47 s	1.77 s	1.79 s	1.88 s	1.34 s
15	1.36 d (7.0)	1.38 d (7.7)	1.02 d (6.4)	1.86 s	1.13 d (7.0)	1.06 d (6.3)
3′a	5.53 br s	6.10 q (8.6)	6.14 q (6.5)	6.13 q (6.0)	6.17 q (7.0)	6.09 dq (1.3, 7.2)
3′b	5.99 br s					
4′	1.81 s	1.75 s	1.84 s	1.77 s	1.82 s	1.86 d (1.3)
5′		1.87 d (8.6)	1.98 d (6.5)	1.98 d (6.0)	1.97 d (7.0)	1.98 dd (1.3, 7.2)
OMe-3					3.34 s	3.37 s
OAc-10			2.03 s	2.08 s	2.01 br s	
OH-10						3.81 s

[a] Assignments based on analysis of 2D NMR spectra.
[b] Data (δ) measured at 400 MHz.
[c] Data (δ) measured at 300 MHz; s=singlet, br s=broad singlet, d=doublet, dd=double doublet, t=triplet, dt=double triplet, q=quartet, dq=double quartet, m=multiplet. *J* values are presented in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from ^{1}H–^{1}H COSY, HSQC, and HMBC spectra without designating multiplicity.

Table 2
^{13}C NMR data of compounds **1**–**6** in $CDCl_3$[a]

Position	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[c]	**6**[b]
1	204.7 C	204.8 C	208.5 C	207.2 C	207.6 C	214.5 C
2	106.2 CH	106.2 CH	36.0 CH_2	38.6 CH_2	41.7 CH_2	38.5 CH_2
3	192.0 C	192.0 C	29.6 CH_2	122.3 CH	79.7 CH	79.4 CH
4	37.5 CH	37.5 CH	29.6 CH	133.6 C	29.5 CH	30.8 CH
5	74.6 CH	74.7 CH	38.6 CH_2	36.1 CH_2	33.0 CH_2	36.6 CH_2
6	80.7 CH	80.7 CH	78.0 CH	78.4 CH	78.4 CH	76.8 CH
7	51.8 CH	51.8 CH	47.1 CH	47.7 CH	46.4 CH	44.3 CH
8	71.0 CH	70.7 CH	67.1 CH	65.5 CH	66.3 CH	70.0 CH
9	45.3 CH_2	45.4 CH_2	43.8 CH_2	43.5 CH_2	43.9 CH_2	38.8 CH_2
10	89.7 C	89.8 C	84.0 C	84.8 C	83.3 C	78.8 C
11	132.8 C	133.1 C	134.5 C	133.9 C	134.2 C	135.0 C
12	168.2 C	168.2 C	169.7 C	169.9 C	169.0 C	168.6 C
13	125.2 CH_2	125.1 CH_2	124.8 CH_2	122.8 CH_2	124.1 CH_2	126.7 CH_2
14	20.9 CH_3	21.0 CH_3	21.1 CH_3	21.3 CH_3	21.9 CH_3	28.6 CH_3
15	9.4 CH_3	9.4 CH_3	24.0 CH_3	25.8 CH_3	20.4 CH_3	20.5 CH_3
1′	166.9 C	167.2 C	166.2 C	166.3 C	166.4 C	166.3 C
2′	135.3 C	126.3 C	126.7 C	126.7 C	126.7 C	126.9 C
3′	126.6 CH_2	141.1 CH	140.7 CH	140.8 CH	140.9 CH	139.9 CH
4′	17.9 CH_3	20.0 CH_3	20.4 CH_3	20.4 CH_3	20.4 CH_3	20.5 CH_3
5′		15.7 CH_3	15.9 CH_3	16.1 CH_3	16.0 CH_3	15.8 CH_3
OAc			169.1 C	169.1 C	169.6 C	
			21.1 CH_3	21.9 CH_3	21.0 CH_3	
OMe-3					58.0 CH_3	58.2 CH_3

[a] Assignments based on analysis of 2D NMR spectra.
[b] Data (δ) measured at 100.6 MHz.
[c] Data (δ) measured at 75.5 MHz; CH_3, CH_2, CH, and C multiplicities determined by DEPT 90, DEPT 135, and HSQC experiments.

the new compound **1** was determined as (4*R*,5*S*,6*S*,7*R*,8*S*,10*R*)-1-oxo-3,10-epoxy-5-hydroxy-8-methacryloyloxygermacra-2,11(13)-dien-6,12-olide,[17] and has been assigned the trivial name, rufescenolide A.

Compound **2** was isolated as an amorphous white powder with a molecular formula of $C_{20}H_{24}O_7$, 14 (CH_2) units greater than that of **1**, as determined by HRESIMS (*m*/*z* 399.1404 $[M+Na]^+$, calcd for 399.1420). The closely related UV and IR spectra of **1** and **2** indicated the latter compound to be also a goyazensolide-type sesquiterpene lactone. Direct comparison of the ^{1}H and ^{13}C NMR spectra of **1** and **2** showed that both compounds contain identical sesquiterpene lactone units but different ester residues at their C-8 positions. Compound **2** was assigned as having an angelate group, which displayed a methyl singlet at δ_H 1.75, a methyl doublet at δ_H 1.87, and a vinylic quartet at δ_H 6.10 in the ^{1}H NMR spectrum (Table 1).[6]

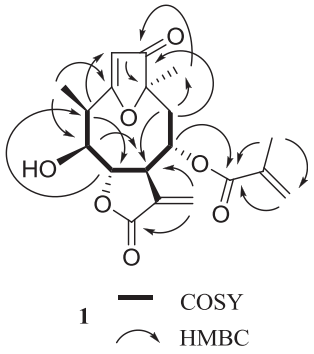


Fig. 2. COSY and the key HMBC correlations of **1**.

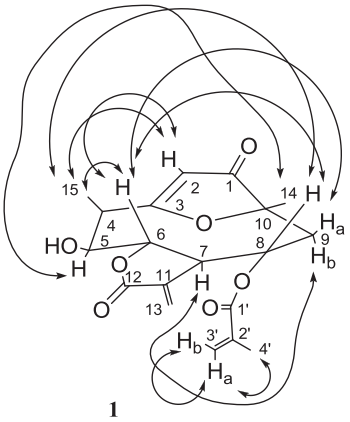


Fig. 3. Selected NOESY correlations of **1**.

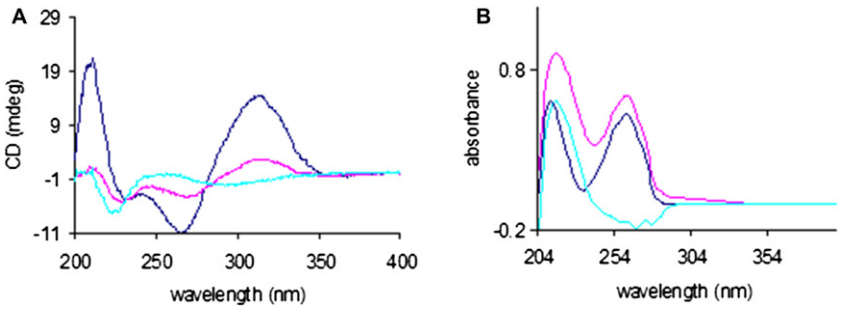


Fig. 4. CD (A) and UV (B) spectra of **1** (dark blue), **2** (pink), **3** (gray blue). The data were obtained in MeOH corrected by subtracting a spectrum of the appropriate solution in the absence of the samples recorded under identical conditions.

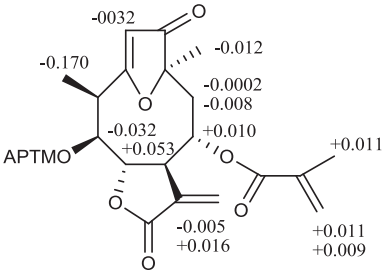


Fig. 5. $\Delta\delta_{S-R}$ values for the Mosher esters of **1**.

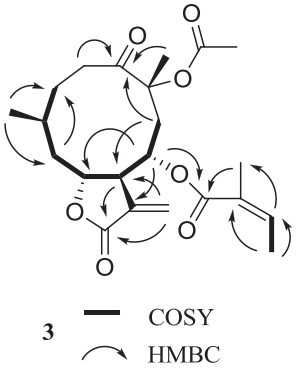


Fig. 6. COSY and the key HMBC correlations of **3**.

In turn, five signals for this ester group appeared at δ_C 167.2 (C-1′), 126.3 (C-2′), 141.1 (C-3′), 20.0 (C-4′) and 15.7 (C-5′) in its ^{13}C NMR spectrum (Table 2).[6] The NOESY correlations between H-4′/H-3′ and H-3′/H-5′ indicated a trans configuration for the C-4′ and C-5′ methyl groups (Fig. S8, Supplementary Data). The closely comparable ^{1}H and ^{13}C NMR data (Tables 1 and 2), specific rotation values, and the CD and NOESY NMR spectra of **1** and **2** (Fig. 4 and Fig. S8, Supplementary data) suggested that both compounds have the same absolute configurations at their C-4, C-5, C-6, C-7, C-8, and C-10 stereogenic centers. Therefore, compound **2** (rufescenolide B) was determined as (4*R*,5*S*,6*S*,7*R*,8*S*,10*R*)-1-oxo-3,10-epoxy-5-hydroxy-8-angeloyloxygermacra-2,11(13)-dien-6,12-olide.[17]

Compound **3**, obtained as colorless needles (mp 190–191 °C), showed UV (λ_{max} 216 nm) and IR [ν_{max} 1769 (α,β-unsaturated γ-lactone), 1738 (ester) 1716 and 1698 (α,β-unsaturated ester) cm^{-1}] absorptions typical for a germacranolide.[3] The positive HRESIMS exhibited a sodiated molecular ion peak at *m*/*z* 429.1890 (calcd 429.1889 for $C_{22}H_{30}O_7Na$), indicating 8° of unsaturation. Among these, four resulted from carbonyl groups, two from carbon-carbon double bonds, and the remaining two from a bicyclic system of a germacranolide.

A ketone group could be located at the C-1 position of **3**, as indicated by the HMBC correlations between H_2-2 and H_2-9/C-1 (Fig. 6). A saturated ten-membered ring was suggested from the long $^{1}H-^{1}H$ COSY sequence represented by H_2-2/H_2-3/H-4 (H-15)/H_2-5/H-6/H-7/H-8/H_2-9. A cyclic lactone containing an exo-methylene group could be proposed at the C-6 and C-7 positions, as supported by HMBC correlations between H-13/C-7 and C-12 (Fig. 6). An angelate group was linked to the C-8 position, as supported by the HMBC correlation between H-8/C-1′ (Fig. 6). In addition, an acetoxy group was proposed at C-10, which was found to be the sole oxygen-substituted quaternary carbon in the molecule.

The complete structure of **3** was established by single-crystal X-ray diffraction analysis (Fig. 7), which confirmed the structure determined by analysis of the NMR spectroscopic data. The crystal structure of **3** showed that this molecule contains two rings, an

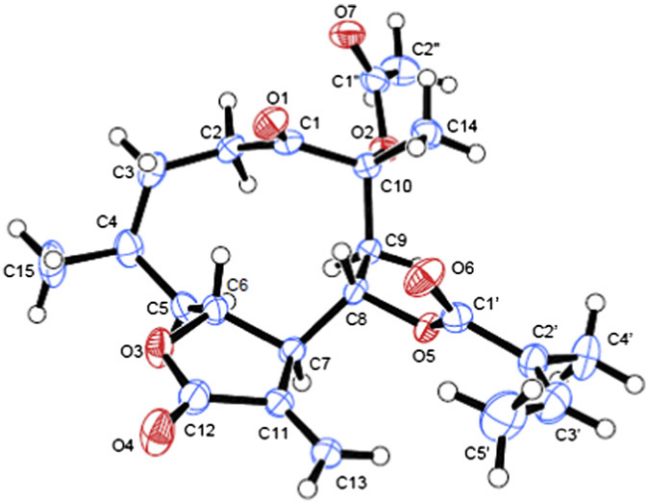


Fig. 7. ORTEP plot for the molecular structure of **3** drawn with 50% probability displacement ellipsoids (oxygen atoms are red, carbon atoms are blue, and the small white circles represent hydrogen atoms, which are drawn with an artificial radius).

almost planar trans-fused α-methylene-γ-lactone ring and a ten-membered ring adopting a half chair-boat-chair conformation, with C-1, C-2, C-3, C-4, C-5, C-9, C-10 forming a half chair conformation, C-1, C-3, C-4, C-5, C-6, C-8, C-9, and C-10 forming a boat conformation, and C-4, C-5, C-6, C-7, C-8, C-9, and C-10 comprising a chair conformation (Fig. 8). The axial proton H-7 is coupled with two trans-axial protons, H-6 and H-8, with Ha-2, H-6, H-8, H-14, and H-15 being β-configured, and Hb-2, H-4 and H-7 being α-configured, as indicated by its NOESY correlations (Fig. 9). An angelate residue is bonded to the C-8 position, and the carbonyl group is oriented cis to H-8 and in a cis relationship with the

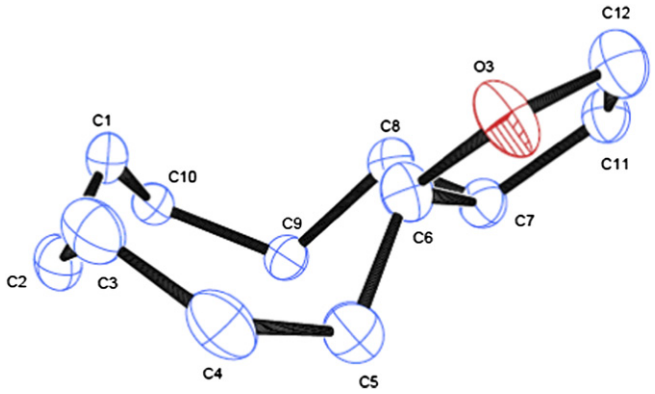
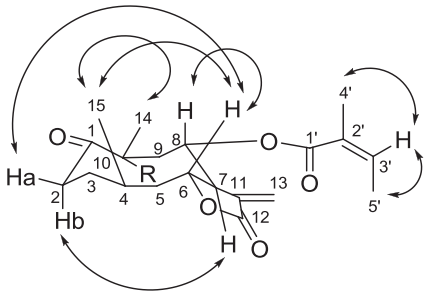


Fig. 8. View of the central portion of compound **3** showing the conformation of the 10-membered ring.



3 R = OAc

Fig. 9. Selected NOESY correlations of **3**.

conjugated carbon-carbon double bond at the C-2′ and C-3′ positions. The two methyl groups of C-4′ and C-5′ are trans (Fig. 7).

Even though the conformation of the ring system and relative configuration of **3** have been established by X-ray analysis (Figs. 7 and 8), it is not possible to determine its absolute configuration in this manner. Refinement of the Flack parameter to determine the absolute configuration was inconclusive, as its value was 0.04(60).[18] The small value of 0.04 indicates that the absolute configuration of the rufesolide A (**3**) molecule is probably correct, but the large error value of 0.60 gives little confidence in this number. As a result, it was not possible to determine the absolute configuration of **3** with the Flack parameter obtained. The Cotton effects displayed in the CD spectrum of **3** were sufficient to make such a determination. Negative Cotton effects at 223 and 272 nm in the CD spectrum (Fig. 4) of **3** implied a α-configuration for H-7 (7*S* configuration),[14,15] and the 4*S*, 6*R*, 8*S*, and 10*R* configurations thus were established relative to 7*S* by X-ray crystallographic analysis and supported by NOESY correlations between Ha-2/H-6, Hb-2/H-7, H-6/H-8 and H-15 (Fig. 9). Thus, the structure of **3** was determined as (4*S*,6*R*,7*S*,8*S*,10*R*)-1-oxo-8-angeloyloxy-10-acetoxygermacra-11(13)-en-6,12-olide.[17] This germacranolide was accorded the trivial name, rufesolide A.

Compound **4** was isolated as an amorphous white powder with a molecular formula of $C_{22}H_{28}O_7$, from the HRESIMS sodiated molecular ion peak at *m*/*z* 427.1715, two hydrogen atoms less than that of **3**. This compound exhibited closely comparable ^{1}H and ^{13}C NMR resonances to those of **3**, except for the signals for an extra carbon-carbon double bond at the C-3 and C-4 positions, as assigned in Tables 1 and 2 by HMBC correlations between H-2/C-1, H-5/C-3 and C-7, and H-15/C-3 and C-5 (Fig. S7, Supplementary data). The closely comparable NOESY NMR and CD spectra of **4** with those of **3** (Figs. S8 and S9, Supplementary data) indicated the same absolute configurations at the C-6, C-7, C-8, and C-10 stereogenic centers for both compounds. Therefore, compound **4** (rufesolide B) was determined as (6*R*,7*S*,8*S*,10*R*)-1-oxo-8-angeloyloxy-10-acetoxygermacra-3(4),11(13)-dien-6,12-olide.[17]

Compound **5** was isolated as an amorphous white powder with a molecular formula of $C_{23}H_{32}O_8$, 30 mass units more than that of **3**, consistent with the presence of a methoxy group at the C-3 position, as indicated by a singlet at δ_H 3.34 displayed in the ^{1}H NMR spectrum and a signal at δ_C 58.0 that appeared in the ^{13}C NMR spectrum of **5**. This was supported by observed HMBC correlations between the protons of the methoxy group and C-3, H-5/C-3, and H-15/C-3 and C-5 (Fig. S7, Supplementary data). The consistent NOESY and CD data of **5** with those of **3** (Figs. S8 and S9, Supplementary data) suggested the same absolute configurations at the C-6, C-7, C-8, and C-10 stereogenic centers for both compounds, and 3*R* and 4*R* absolute configurations were determined by the NOESY correlations between H-3/H_3-15, H-6/Ha-2, H-8/Ha-2 and H-14 (Fig. S8, Supplementary data). Therefore, compound **5** (rufesolide C) was determined as (3*R*,4*R*,6*R*,7*S*,8*S*,10*R*)-1-oxo-3-methoxy-8-angeloyloxy-10-acetoxygermacra-11(13)-en-6,12-olide.[17]

Compound **6** was isolated as an amorphous white powder with a molecular formula of $C_{21}H_{30}O_7$, 41 mass units ($COCH_2$) less than that of **5**, consistent with being a deacetyl derivative of the latter compound. This was supported by two signals at δ_C 21.0 and 169.6 for an acetyl group in the ^{13}C NMR spectrum of **5** being absent in the analogous data for **6**. Comparison of the NMR data of **6** with literature values indicated this compound to be a new isomer of 3-methoxy-8β-angeloyloxyternifolin.[19] The CD spectrum of **6** consistent with those of **3**–**5** (Fig. S9, Supplementary data) indicated a 7*S* absolute configuration, and 3*R*,4*R*,6*R*,8*S*, and 10*R* absolute configurations were supported by NOESY correlations between Ha-2/H-6 and H-8, H-3/H_3-15, H_3-15/Ha-5, and Ha-5/H-6 (Fig. S8, Supplementary data). Therefore, compound **6** (rufesolide D) was determined as (3*R*,4*R*,6*R*,7*S*,8*S*,10*R*)-1-oxo-3-methoxy-8-angeloyloxy-10-hydroxygermacra-11(13)-en-6,12-olide.[17]

The structures of the known compounds (Fig. S10, Supplementary data) isolated from *P. rufescens*, including several sesquiterpene lactones, phenylpropanol coumarates, and methylated flavonoids, were determined by analysis of their spectroscopic data and comparison of these data with reference values,[3–12] and the assignments of their ^{1}H and ^{13}C NMR spectroscopic data are listed in the Tables S1–S5 (Supplementary data). The absolute configurations of the known sesquiterpene lactones were determined by comparison of their NOESY NMR and CD spectra with those of **1**–**6** (Figs. S8 and S9, Supplementary data).

All isolated goyazensolides and germacranolides (**1**–**13**) displayed negative Cotton effects around 220 and 260 nm in their CD spectra and UV absorption maxima around 215 nm (Fig. S9, Supplementary data), indicating the absolute configuration for the C-7 position.[14,15] Additionally, the goyazensolides (**1**–**2** and **8**–**13**) all showed positive Cotton effects around 210 and 310 nm in their CD spectra and UV absorption maxima around 260 nm (Fig. S9, Supplementary data), which disappeared in the CD and UV spectra of the germacranolides (**3**–**7**), indicating that these positive Cotton effects are indicative of exciton coupling arising from the α,β-unsaturated carbonyl chromophore at C-10[20] for an α-configuration for H-14 (a 10*R* configuration for compounds **1**–**2** and **8**–**13**).

All compounds obtained in this study from *P. rufescens* were tested in terms of their cytotoxicity against the HT-29 human colon cancer cell line, using paclitaxel as the positive control. Both the goyazensolides (**1**–**2** and **8**–**13**) and germacranolides (**3**–**7**) showed potent cytotoxicity toward this cell line (Table 3), with 15-deoxygoyazensolide (**10**) being the most active compound with an IC_{50} value of 0.26 μM. However, all other compounds were inactive in this assay system. All the cytotoxic sesquiterpene lactones contain a α-methylene-γ-lactone ring at the C-6 and C-7 positions and

Table 3
Cytotoxicity and NF-κB p65 inhibition of **1**–**13**[a]

Compound	Cytotoxicity[b]	NF-κB p65 inhibition[c]
1	1.9	NT[d]
2	6.6	NT[d]
3	3.0	>10
4	4.0	>10
5	1.4	2.1
6	1.0	>10
7	1.2	>10
8	0.56	3.8
9	0.28	NT[d]
10	0.26	3.2
11	0.58	NT[d]
12	1.4	2.9
13	0.56	>10

[a] All data presented as 95% confidence intervals obtained by non-linear regression in these assays.
[b] Data presented as IC_{50} values (μM) toward HT-29 cells, with paclitaxel used as a positive control (IC_{50} 1 nM).
[c] Data presented as IC_{50} values (μM), with rocaglamide used as positive control (IC_{50} 0.075 μM).
[d] Not tested due to sample limitations.

a α,β-unsaturated ester group at the C-8 position, which have been reported as key structural requirements for sesquiterpene lactones to mediate cytotoxicity.[21] To further investigate of the bioactivities of the isolates, all compounds obtained in sufficient quantity in this study, except for **1**, **2**, **9**, **11**, eremantholide C, and 4′-methoxy-5,7,3′-trihydroxyflavone, were tested in a NF-κB (p65) inhibition assay, using rocaglamide as the positive control. Compounds **5**, **8**, **10**, and **12** were active (Table 3), with all other compounds tested being inactive (IC_{50} value>10 μM).

Several goyazensolides and germacranolides, including goyazensolide (**8**), lychnopholide (**12**), and parthenolide, have been reported previously to exhibit cytotoxicity toward a panel of human cancer cell lines.[22] Recently, goyazensolide (**8**) and 15-deoxygoyazensolide (**10**), together with other analogues, have been investigated for their NF-κB inhibitory activity.[23] The NF-κB inhibitory activity of compounds **5** and **12** has not been reported previously.

In summary, a number of germacranolide-type sesquiterpene lactones, phenylpropanol coumarates, and methylated flavonoids were isolated and characterized from the leaves of *P. rufescens* for the first time in this study. Based on the results obtained, both the goyazensolides and germacranolides can be regarded as characteristic chemical components and the cytotoxic principles of this species.

3. Experimental

3.1. General experimental procedures

Melting points were measured using a Fisher Scientific apparatus and are uncorrected. Specific rotation values were obtained on a Perkin–Elmer model 343 polarimeter. UV spectra were recorded on a Hitachi U2910 UV spectrophotometer. CD measurements were performed using a JASCO J-810 spectropolarimeter. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. ^{1}H and ^{13}C, DEPT, HSQC, HMBC, NOESY, and COSY NMR spectra were recorded at room temperature on Bruker Avance DPX-300, DRX-400, DRX-600, or DRX-800 MHz NMR spectrometers, with TMS as internal standard. ESIMS and HRESIMS were measured on a LCT–TOF mass spectrometer in the positive-ion mode. Column chromatography was conducted using silica gel (65×250 or 230×400 mesh, Sorbent Technologies, Atlanta, GA). Analytical and preparative thin-layer chromatograph (TLC) systems were performed on precoated silica gel 60 F_{254} plates (Sorbent Technologies, Atlanta, GA). Sephadex LH-20 was purchased from Amersham Biosciences, Uppsala, Sweden. For visualization of TLC plates, sulfuric acid reagent was used. All procedures were carried out using anhydrous solvents purchased from commercial sources and employed without further purification.

3.2. Plant material

The leaves of *P. rufescens* Cass. (Asteraceae) were collected from a shrub 1.5 m tall with violet stamens, at Villa Mella (18°39′ N; 69°58′ E; 150 m), on the road to Yamasá, in a forest on serpentine rock, Sierra Prieta, Distrito Nacional, Dominican Republic, in January, 1996. A voucher herbarium specimen (*Jiménez & Mejía 2040*) was identified by F. J., R. G., and D. D. S. and has been deposited both at the Herbarium of the Jardín Botánico Nacional 'Dr. Rafael Ma. Moscoso', Santo Domingo, Dominican Republic, and at the John G. Searle Herbarium of the Field Museum of Natural History, Chicago, IL, under the accession number FM2169750.

3.3. Extraction and isolation

The ground leaves of *P. rufescens* (470 g) were extracted with MeOH (4 L×5) at room temperature. The solvent was evaporated in vacuo. The resultant dried MeOH extract (90 g, 19.2%) was suspended in 10% H_2O in MeOH (700 mL) and partitioned with *n*-hexane (500, 500, and 400 mL) to yield a *n*-hexane-soluble residue (18.0 g, 3.8%). A volume of 100 mL of H_2O was added to the aqueous-MeOH layer and this was then partitioned with $CHCl_3$ (500, 500, and 400 mL) to afford a chloroform-soluble extract (8.4 g, 1.8%), which was washed with 1% aqueous solution of NaCl to partially remove any plant polyphenols present. Both the *n*-hexane- and chloroform-soluble residues showed inhibitory activity toward the HT-29 human colon cancer cell line in a cytotoxicity assay.

The active *n*-hexane-soluble extract (18.0 g, IC_{50}<10 μg/mL) was subjected to passage over a silica gel column (4.5×45 cm), eluted with gradient mixtures of *n*-hexane/acetone (100:1→1:1; 500 mL each). The eluates were pooled by TLC analysis to give thirteen combined fractions. Of these, fraction 9 (IC_{50}<2 μg/mL) was chromatographed further over a silica gel column (2.5×20 cm), eluted with gradient mixtures of *n*-hexane/acetone (20:1→3:1, 200 mL each), and then purified by separation over a Sephadex LH-20 column (2.5×25 cm), by elution with CH_2Cl_2/MeOH (1:1), affording rufesolide B (**4**, 6.0 mg) and 15-deoxygoyazensolide (**10**, 50 mg). Fractions 10–12 (IC_{50}<1 μg/mL) were combined and separated by silica gel chromatography, eluted with *n*-hexane/acetone (5:1), and then purified by passage over a Sephadex LH-20 column, by elution with CH_2Cl_2/MeOH (1:1), to afford rufesolide A (**3**, 11.0 mg) and ereglomerulide (**7**, 45 mg). Compound **3** was recrystallized from a mixture of *n*-hexane and acetone to give fine needles suitable for single-crystal X-ray analysis.

The active chloroform-soluble extract (8.0 g, IC_{50}<2 μg/mL) was subjected to passage over a silica gel column (4.5×45 cm), and eluted with gradient mixtures of *n*-hexane/acetone (100:1→1:1; 500 mL each). Fractions were pooled by TLC analysis to yield nine combined fractions. Of these, fractions 1–4 were deemed cytotoxic toward the HT-29 cell line (IC_{50}<1 μg/mL). Fraction 1 was chromatographed over a silica gel column, and eluted with gradient mixtures of *n*-hexane/acetone (10:1→3:1), to yield two combined sub-fractions. The first sub-fraction was chromatographed over silica gel using *n*-hexane/acetone (5:1) as solvent, and then purified by separation over a Sephadex LH-20 column, eluted with CH_2Cl_2/MeOH (1:1), affording rufesolide C (**5**, 13.0 mg) and 15-deoxygoyazensolide (**10**, 550 mg). The second sub-fraction was separated by silica gel chromatography, eluted with a gradient mixture of *n*-hexane/acetone (5:1→1:1), and then purified by

passage over a Sephadex LH-20 column, eluted with CH_2Cl_2/MeOH (1:1), to afford 2′,3′-dihydro-15-deoxygoyazensolide (**11**, 3.0 mg), lychnopholide (**12**, 5.0 mg), 4,5-dihydrolychnopholide (**13**, 2.0 mg), and eremantholide C (1.0 mg).

Fraction 2 was chromatographed over a silica gel column, and eluted with gradient mixtures of *n*-hexane/acetone (10:1→1:1), to yield two combined sub-fractions. The first sub-fraction was chromatographed over silica gel using *n*-hexane/acetone (3:1) as solvent, and then finally purified by passage over a Sephadex LH-20 column, eluted with CH_2Cl_2/MeOH (1:1), affording rufescenolide A (**1**, 10.0 mg) and rufescenolide B (**2**, 1.0 mg). The second sub-fraction was separated by silica gel chromatography, eluted by *n*-hexane/acetone (3:1), and then purified by passage over a Sephadex LH-20 column, eluted with CH_2Cl_2/MeOH (1:1), to afford rufesolide D (**6**, 8.0 mg) and 5-epiisogoyazensolide (**9**, 2.0 mg).

Fraction 3 was chromatographed over silica gel, eluted by *n*-hexane/acetone (3:1), and then purified over a Sephadex LH-20 column, with CH_2Cl_2/MeOH (1:1) as eluent, affording goyazensolide (**8**, 15 mg), [3-(3,5-dimethoxy-4-hydroxyphenyl)]-1-propanol-*trans*-coumarate (6.0 mg), and 4′-methoxy-5,7,3′-trihydroxyflavone (1.0 mg).

Fraction 4 was chromatographed over silica gel, eluted with a gradient mixture of *n*-hexane/acetone (50:1→1:1), to produce three combined sub-fractions. The precipitate from the first sub-fraction was triturated and purified over a Sephadex LH-20 column, using CH_2Cl_2/MeOH (1:1) for elution, to afford 3,4′-dimethoxy-5,7-3′-trihydroxyflavone (31 mg). The mother liquor was evaporated in vacuo, and the residue was chromatographed over silica gel, eluted with *n*-hexane/acetone (3:1), and then purified over a Sephadex LH-20 column, using CH_2Cl_2/MeOH (1:1) for elution, to furnish goyazensolide (**8**, 90 mg). The second sub-fraction was chromatographed over silica gel, eluted by *n*-hexane/acetone (3:1), and then separated over Sephadex LH-20, using CH_2Cl_2/MeOH (1:1) for elution, to yield 3-(4-hydroxyphenyl)-1-propanol-*trans*-coumarate (15.0 mg). The third sub-fraction was separated by silica gel, eluted by *n*-hexane/acetone (1:1), and then purified finally over a Sephadex LH-20 column, using CH_2Cl_2/MeOH (1:1) for elution, furnishing 4′-methoxy-3,5,7,3′-tetrahydroxyflavone (2.0 mg) and 3-methoxy-5,7-4′-trihydroxyflavone (13.0 mg).

*3.3.1. Rufescenolide A (**1**).* Amorphous colorless powder (*n*-hexane); $[\alpha]_D^{20}$ +95 (*c* 0.12, MeOH); $[\alpha]_D^{20}$ +132.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 212 (3.87), 262 (3.81) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 210 (+7.8), 232 (−1.8), 264 (−4.0), 312 (+5.3); IR (dried film) ν_{max} 3466, 1766, 1712, 1634, 1588, 1453 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z*: 385.1253 $[M+Na]^+$ (calcd for $C_{19}H_{22}O_7Na$, 385.1263).

*3.3.1.1. Preparation of the (S)-MTPA ester (**1s**) of rufescenolide A (**1**).* Compound **1** (1.5 mg) was transferred to a NMR tube and dried under vacuum in an oil pump for 8 h. Deuterated pyridine (0.5 mL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) were transferred into the NMR tube under argon. Then, the NMR tube was shaken and kept at room temperature for 8 h. The ^1H NMR spectrum was measured directly at room temperature, and the data were assigned based on comparison of the ^1H NMR spectrum of the product with that of **1**. ^1H NMR data of **1s** (400 MHz, pyridine-d_5): δ 6.38 (1H, d, *J*=2.78 Hz, H-13a), 6.05 (1H, br s, H-3′), 6.01 (1H, s, H-2), 5.97 (1H, dd, *J*=6.70 Hz, 8.70, H-5), 5.67 (1H, d, *J*=2.33 Hz, H-13b), 5.48 (1H, br s, H-3′), 4.83 (1H, dd, *J*=4.00, 9.03 Hz, H-6), 4.68 (1H, d, *J*=11.51 Hz, H-8), 3.88 (2H, m, H-7 and H-4), 2.73 (1H, t, *J*=13.29 Hz, H-9), 2.49 (1H, d, *J*=12.85 Hz, H-9), 1.76 (3H, s, H-4′), 1.55 (3H, s, H-14), 1.17 (3H, d, *J*=6.92 Hz, H-15).

*3.3.1.2. Preparation of the (R)-MTPA ester (**1r**) of rufescenolide A (**1**).* Using the same procedure as described for **1s**, the (*R*)-MTPA ester derivative of **1** (**1r**) was produced by the reaction of **1** (1.5 mg), (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL), and deuterated pyridine (0.5 mL). ^1H NMR data of **1r** (400 MHz, pyridine-d_5): δ 6.38 (1H, d, *J*=2.81 Hz, H-13a), 6.05 (1H, br s, H-3′), 6.04 (1H, s, H-2), 6.00 (1H, dd, *J*=6.67, 8.86 Hz, H-5), 5.65 (1H, d, *J*=2.35 Hz, H-13b), 5.47 (1H, br s, H-3′), 4.77 (1H, dd, *J*=4.02, 8.82 Hz, H-6), 4.67 (1H, d, *J*=12.45 Hz, H-8), 3.88 (2H, m, H-7 and H-4), 2.74 (1H, t, *J*=13.08 Hz, H-9), 2.50 (1H, d, *J*=12.74 Hz, H-9), 1.75 (3H, s, H-4′), 1.56 (3H, s, H-14), 1.35 (3H, d, *J*=6.94 Hz, H-15).

*3.3.2. Rufescenolide B (**2**).* Amorphous colorless powder (*n*-hexane); $[\alpha]_D^{20}$ +40 (*c* 0.06, MeOH); $[\alpha]_D^{20}$ +33.3 (*c* 0.06, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (3.94), 262 (3.79) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 209 (+1.1), 229 (−4.1), 270 (−3.3), 312 (+2.1); IR (dried film) ν_{max} 3473, 1770, 1710, 1589, 1454 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z*: 399.1404 $[M+Na]^+$ (calcd for $C_{20}H_{24}O_7Na$, 399.1420).

*3.3.3. Rufesolide A (**3**).* Colorless needles (*n*-hexane/acetone); mp 190−191 °C; $[\alpha]_D^{20}$ +7.7 (*c* 0.09, MeOH); $[\alpha]_D^{20}$ +1.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (3.92) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 223 (−4.1), 272 (−0.7), 292 (−1.1); IR (dried film) ν_{max} 1769, 1738, 1716, 1698, 1461 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z*: 429.1890 $[M+Na]^+$ (calcd for $C_{22}H_{30}O_7Na$, 429.1889).

*3.3.4. X-ray crystal data of rufesolide A (**3**).* Data for a small colorless needle of **3** were collected by synchrotron radiation at the Advanced Light Source at Lawrence Berkeley National Laboratory.[24] Crystal data: molecular formula $C_{22}H_{30}O_7$, MW=406.46, orthorhombic, space group $P2_12_12_1$, *a*=5.5228(2) Å, *b*=17.4103(7) Å, *c*=22.1641(10) Å, *V*=2131.2(2) $Å^3$, *Z*=4, D_{calcd}=1.267 gm/cm^3, 150 K, size 0.01×0.02×0.09 mm^3, Bruker APEXII CCD diffractometer with λ=0.8856 Å, 5.42≤2θ≤67.36° (Table S6, Supplementary data). Data were collected using the *APEX2* software and processed with *SAINT* within *APEX2*.[25] Corrections for absorption and beam corrections are based on the multi-scan technique as implemented in *SADABS*.[25] The structure was solved by direct methods in *SHELXS-97*.[26] Full-matrix least-squares refinements based on F^2 were performed in *SHELXL-97*,[26] as incorporated in the *WinGX* package.[26] The final refinement cycle was based on 4356 intensities and 267 variables and resulted in agreement factors of *R1*(*F*)=0.060 and *wR2*(F^2)=0.088. For the subset of data with $I>2\sigma(I)$, the *R1*(*F*) value is 0.040 for 3464 reflections. Refinement of the Flack parameter[18] to determine the absolute configuration was not conclusive, as its value was 0.04 (60). The CIF file of the X-ray data of **3** has been deposited in the Cambridge Crystallographic Data Centre (deposition no.: CCDC 852357).

*3.3.5. Rufesolide B (**4**).* Amorphous colorless powder (*n*-hexane); $[\alpha]_D^{20}$ −46.6 (*c* 0.09, MeOH); $[\alpha]_D^{20}$ −68.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 216 (3.94) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 206 (+10.3), 226 (−7.7), 279 (−3.8), 289 (−4.3); IR (dried film) ν_{max} 1770, 1715, 1669, 1641, 1581, 1455 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z*: 427.1715 $[M+Na]^+$ (calcd for $C_{22}H_{28}O_7Na$, 427.1733).

*3.3.6. Rufesolide C (**5**).* Amorphous colorless powder (*n*-hexane); $[\alpha]_D^{20}$ +29.3 (*c* 0.08, MeOH); $[\alpha]_D^{20}$ +10 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 219 (4.41) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 221 (−4.3), 275 (-2.1), 292 (−2.5); IR (dried film) ν_{max} 1768, 1716, 1461 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z*: 459.2031 $[M+Na]^+$ (calcd for $C_{23}H_{32}O_8Na$, 459.1995).

*3.3.7. Rufesolide D (**6**).* Amorphous colorless powder (*n*-hexane); $[\alpha]_D^{20}$ +66.6 (*c* 0.09, MeOH); $[\alpha]_D^{20}$ +65.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 215 (3.95) nm; CD (MeOH, nm) λ_{max} ($\Delta\varepsilon$) 223 (−3.8); IR

(dried film) ν_{max} 3494, 1770, 1699, 1647, 1456 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m/z*: 417.1875 $[M+Na]^+$ (calcd for $C_{21}H_{30}O_7Na$, 417.1889).

3.4. Cytotoxicity assay

Cytotoxicity of the samples was performed against HT-29 human colon cancer cells by a previously reported procedure.[27] Paclitaxel was used as a positive control, and the IC_{50} values of the test samples in serial dilutions were calculated using non-linear regression analysis (Table Curve2Dv4; AISN Software, Inc., Mapleton, OR). Measurements were performed in triplicate and are representative of two independent experiments in which the values generally agreed within 10%.

3.5. Enzyme-based ELISA NF-κB p65 inhibition assay

A NF-κB p65 inhibition assay was carried out using a published procedure, with an EZ-Detect Transcription Factor Assay System ELISA kit (Pierce Biotechnology, Rockford, IL).[27] Rocaglamide was used as a positive control, and measurements were performed in duplicate and are representative of two independent experiments, with the values generally agreed within 10%. The dose response curve was calculated for IC_{50} determinations using non-linear regression analysis (Table Curve2DV4; AISN Software Inc., Mapleton, OR).

Acknowledgements

This investigation was supported by grants U01 CA52956 and P01 CA125066, funded by the National Cancer Institute, NIH, Bethesda, MD. The leaf sample of *Piptocoma rufescens* was collected under a collaborative agreement between the University of Illinois at Chicago and the Jardín Botánico Nacional 'Dr. Rafael Ma. Moscoso', Santo Domingo, Dominican Republic. We thank Dr. Jeanette Krause of the University of Cincinnati for the X-ray data collection through the SCrALS program. We thank Dr. Kari Green-Church, of the Mass Spectrometry and Proteomics of the Campus Chemical Instrument Center (CCIC), The Ohio State University (OSU), for assistance with the MS measurements, Dr. Chunhua Yuan (CCIC, OSU) for assistance with the HMBC and NOESY measurements on 600 and 800 MHz NMR spectrometers, and Jack Fowble (College of Pharmacy, OSU) for access to the other NMR instrumentations used in this investigation.

Supplementary data

Copies of MS and ^1H and ^{13}C NMR spectra of compounds **1**–**6** and ^1H NMR spectra of (*S*)- and (*R*)-MTPA esters (**1s**/**1r**) of **1**; diagrams of COSY and the key HMBC and NOESY correlations of compounds **2**, **4**–**13**, and eremantholide C; UV and CD spectra of compounds **1**–**13** and eremantholide C; structures of eremantholide C, phenylpropanol coumarates, and methylated flavonoids obtained from *P. rufescens*; crystallographic data and the CIF file of compound **3**; and analytical data of the known compounds. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2012.01.061. These data include MOL files and InChIKeys of the most important compounds described in this article.

References and notes

1. Pruski, J. F. *Novon* **1996**, *6*, 96–102.
2. Kinghorn, A. D.; Carcache-Blanco, E. J.; Chai, H.-B.; Orjala, J.; Farnsworth, N. R.; Soejarto, D. D.; Oberlies, N. H.; Wani, M. C.; Kroll, D. J.; Pearce, C. J.; Swanson, S. M.; Kramer, R. A.; Rose, W. C.; Fairchild, C. R.; Vite, G. D.; Emanuel, S.; Jarjoura, D.; Cope, F. O. *Pure Appl. Chem.* **2009**, *81*, 1051–1063.
3. Bohlmann, F.; Gupta, R. K.; Jakupovic, J.; Robinson, H.; King, R. M. *Phytochemistry* **1981**, *20*, 1609–1612.
4. Heleno, V. C. G.; Crotti, A. E. M.; Constantino, M. G.; Lopes, N. P.; Lopes, J. L. C. *Magn. Reson. Chem.* **2004**, *42*, 364–367.
5. (a) Bohlmann, F.; Mahanta, P. K.; Natu, A. A.; King, R. M.; Robinson, H. *Phytochemistry* **1978**, *17*, 471–474; (b) Schmeda-Hirschmann, G.; Boeker, R.; Jakupovic, J.; Bohlmann, F. *Phytochemistry* **1986**, *25*, 1753–1754; (c) Crotti, A. E. M.; Lopes, J. L. C.; Lopes, N. P. *J. Mass Spectrom.* **2005**, *40*, 1030–1034.
6. (a) Bohlmann, F.; Zdero, C.; Robinson, H.; King, R. M. *Phytochemistry* **1980**, *19*, 2381–2385; (b) Barrero, A. F.; Oltra, J. E.; Raslan, D. S.; Saude, D. A. *J. Nat. Prod.* **1999**, *62*, 726–729.
7. (a) Gao, F.; Wang, H.-P.; Mabry, T. J. *Phytochemistry* **1987**, *26*, 779–781; (b) Gobbo-Neto, L.; Lopes, N. P. *J. Agric. Food Chem.* **2008**, *56*, 1193–1204.
8. Heleno, V. C. G.; Thiago de Oliveira, K.; Lopes, J. L. C.; Lopes, N. P.; Ferreira, A. G. *Magn. Reson. Chem.* **2008**, *46*, 576–581.
9. (a) Lunardello, M. A.; Tomaz, J. C.; Vichnewski, W.; Lopes, J. L. C.; Gutierrez, A. B.; Diaz, J. G.; Herz, W. *J. Braz. Chem. Soc.* **1995**, *6*, 307–311; (b) Valdes, D. A.; Bardon, A.; Catalan, C. A. N.; Gedris, T. E.; Herz, W. *Biochem. Syst. Ecol.* **1998**, *26*, 805–808.
10. Perry, K. S. P.; Miguez, E.; de Amorim, M. B.; Boaventura, M. A. D.; da Silva, A. J. R. *Magn. Reson. Chem.* **2001**, *39*, 219–221.
11. Valesi, A. G.; Rodriguez, E.; Vander Velde, G.; Mabry, T. J. *Phytochemistry* **1972**, *11*, 2821–2826.
12. Bohlmann, F.; Zdero, C. *Phytochemistry* **1977**, *16*, 1773–1776.
13. (a) Herz, W.; Kumar, N. *Phytochemistry* **1980**, *19*, 593–597; (b) Delgado, G.; Romo De Vivar, A.; Herz, W. *Phytochemistry* **1982**, *21*, 1305–1308; (c) Batista Da Costa, F.; Dias, D. A.; Lopes, J. L. C.; Vichnewski, W. *Phytochemistry* **1993**, *34*, 261–263.
14. (a) Waddell, T. G.; Stocklin, W.; Geissman, T. A. *Tetrahedron Lett.* **1969**, *17*, 1313–1316; (b) Stocklin, W.; Waddell, T. G.; Geissman, T. A. *Tetrahedron* **1970**, *26*, 2397–2409.
15. Youssef, D.; Frahm, A. W. *Phytochemistry* **1996**, *41*, 1107–1111.
16. (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519; (b) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *98*, 2143–2147; (c) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096; (d) Rieser, M. J.; Hui, Y.-H.; Rupprecht, J. K.; Kozlowski, J. F.; Wood, K. V.; McLaughlin, J. L.; Hanson, P. R.; Zhuang, Z.; Hoye, T. R. *J. Am. Chem. Soc.* **1992**, *114*, 10203–10213.
17. The semi-systematic names of **1**–**6** are based on naming used in Ref. 9b
18. (a) Flack, H. D.; Bernardinelli, G. *Acta Crystallogr.* **1999**, *A55*, 908–915; (b) Flack, H. D.; Bernardinelli, G. *J. Appl. Crystallogr.* **2000**, *33*, 1143–1148.
19. Gershenzon, J.; Mabry, T. J.; Korp, J. D.; Bernal, I. *Phytochemistry* **1984**, *23*, 2561–2571.
20. Ren, Y.; Lantvit, D. D.; Carcache de Blanco, E. J.; Kardono, L. B. S.; Riswan, S.; Chai, H.; Cottrell, C. E.; Farnsworth, N. R.; Swanson, S. M.; Ding, Y.-Q.; Li, X.-C.; Marais, J. P. J.; Ferreira, D.; Kinghorn, A. D. *Tetrahedron* **2010**, *66*, 5311–5320.
21. (a) Lee, K.-H.; Hall, I. H.; Mar, E.-C.; Starnes, C. O.; ElGebaly, S. A.; Waddell, T. G.; Hadgraft, R. I.; Ruffner, C. G.; Weidner, I. *Science* **1977**, *196*, 533–536; (b) Ren, Y.; Zhou, Y.; Chen, X.; Ye, Y. *Lett. Drug Des. Discovery* **2005**, *2*, 444–450.
22. (a) Canalle, R.; Burim, R. V.; Lopes, J. L. C.; Takahashi, C. S. *Cancer Det. Prev.* **2001**, *25*, 93–101; (b) dos Santos, P. A.; Amarante, M. F. C.; Pereira, A. M. S.; Bertoni, B.; Franca, S. C.; Pessoa, C.; Odorico de Moraes, M.; Costa-Lotufo, L. V.; Pereira, M. R. P.; Lopes, N. P. *Chem. Pharm. Bull.* **2004**, *52*, 1433–1435; (c) Vongvanich, N.; Kittakoop, P.; Charoenchai, P.; Intamas, S.; Sriklung, K.; Thebtaranonth, Y. *Planta Med.* **2006**, *72*, 1427–1430; (d) Zhang, D.-L.; Qiu, L.; Jin, X.-Q.; Guo, Z.-H.; Guo, C.-B. *Mol. Cancer Res.* **2009**, *7*, 1139–1149.
23. Wagmer, S.; Hofmann, A.; Siedle, B.; Terfloth, L.; Merfort, I.; Gasteiger, J. *J. Med. Chem.* **2006**, *49*, 2241–2252.
24. Crystallographic data were collected through the SCrALS (Service Crystallography at Advanced Light Source) program at the Small-Crystal Crystallography Beamline 11.3.1 at the Advanced Light Source (ALS), Lawrence Berkeley National Laboratory. The ALS is supported by the U.S. Department of Energy, Office of Energy Sciences Materials Sciences Division, under contract DE-AC02–05CH11231.
25. APEX2 v2010.3.0 and SAINT v7.60A for data collection and data processing programs, respectively. Bruker Analytical X-ray Instruments, Inc., Madison, WI; SADABS v2008/1 semi-empirical absorption and beam correction program. G. M. Sheldrick, University of Göttingen, Germany.
26. (a) SHELXS-97: Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122; (b) SHELXL-97: Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122; (c) WinGX-Version 1.80.01: Farrugia, L. J. *J. Appl. Crystallogr.* **1999**, *32*, 837–838.
27. Ren, Y.; Matthew, S.; Lantvit, D. D.; Ninh, T. N.; Chai, H.; Fuchs, J. R.; Soejarto, D. D.; Carcache de Blanco, E. J.; Swanson, S. M.; Kinghorn, A. D. *J. Nat. Prod.* **2011**, *74*, 1117–1125.