Graphical Abstract

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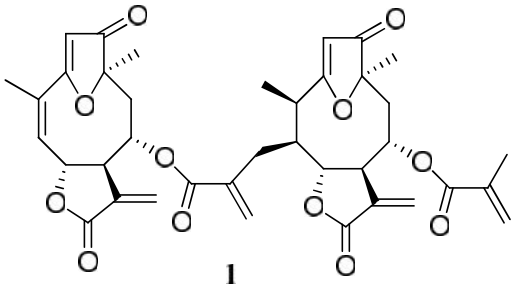

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A cytotoxic dimeric furanoheliangolide from *Piptocoma rufescens*

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ABSTRACT

A new sesquiterpene lactone, rufescenolide C (**1**), the first furanoheliangolide dimer, was isolated from the leaves of *Piptocoma rufescens*, collected in the Dominican Republic. Its structure was determined by analysis of its spectroscopic data, with the absolute configuration being established by analysis of the CD spectrum. A plausible biogenesis of this dimer is proposed. This compound showed potent cytotoxicity with an IC_{50} value of 150 nM, when tested against HT-29 human colon cancer cells.

Keywords:

Piptocoma rufescens

Furanoheliangolide dimer

Cytotoxicity

Piptocoma is a small genus of the plant family Asteraceae, occurring in the Western Hemisphere. A previous investigation of the plant *Piptocoma rufescens* Cass., collected in the Dominican Republic, resulted in the isolation and characterization of several sesquiterpene lactones.[1] Further isolation work (Supplementary data) on this species has led to purification and structure elucidation of a new dimeric goyazensolide-type sesquiterpene lactone, rufescenolide C (**1**, Figure 1). The structure of this new compound was determined by analysis of its spectroscopic data, and the absolute configuration was established using its CD spectrum. The cytotoxicity toward the HT-29 human colon cancer cell line was determined.

The ground leaves of *Piptocoma rufescens* were extracted with MeOH. The MeOH extract was partitioned with *n*-hexane and then $CHCl_3$. The chloroform-soluble extract was found to be active when evaluated by a cytotoxicity assay.[1] Repeated chromatography of the active chloroform-soluble extract over silica gel monitored by cytotoxicity toward HT-29 cells afforded rufescenolide C (**1**).[2]

Compound **1** was isolated as an amorphous white powder. It showed UV (λ_{max} 214 and 263 nm) and IR [ν_{max} 1770 and 1654 (α,β-unsaturated γ-lactone), 1712 and 1629 (α,β-unsaturated ester), 1712 and 1587 (dihydrofuran-3-one ring) cm^{-1}] absorptions typical for a furan ring-containing germacranolide.[1] This compound could be proposed as being a dimeric furanoheliangolide, as indicated by its similar UV and IR spectra to those of 15-deoxygoyazensolide[1] and its molecular formula of $C_{38}H_{40}O_{12}$ (positive HRESIMS *m*/*z* 711.2407, calcd for $C_{38}H_{40}O_{12}Na$, 711.2417), as supported by the ^{13}C NMR spectroscopic data (Table 1), which were comparable to those of 15-deoxygoyazensolide (subunit a),[1] and 4,5-dihydro-15-deoxygoyazensolide (subunit b).[3]

A ten-membered ring fused with a furan ring at the C-3 and C-10 positions was suggested for subunits a and b of **1**, respectively, from the $^{1}H-^{1}H$ COSY sequences of H-5a/H-6a (δ_H 5.24 m)/H-7a/H-8a/H_2-9a and H-15b/H-4b/H-5b/H-6b [δ_H 4.28 dd (3.6, 7.2)]/H-7b/H-8b/H_2-9b (Table 1).[4] A cyclic lactone ring containing an exomethylene group was proposed at the C-6 and C-7 positions for both subunits a and b, as supported by HMBC correlations between H-13a/C-7a and C-12a and H-13b/C-7b and C-12b (Table 1). In addition, the ^{1}H and ^{13}C NMR spectra of **1** revealed the presence of a methacrylate group for both subunits a and b, as characterized by two methylene multiplets at δ_H 2.22 (H-4′a-a) and δ_H 2.73 (H-4′a-b) and two broad singlets at δ_H 5.76 (H-3′a-a) and 6.12 (H-3′a-b) for subunit a, and a methyl singlet at δ_H 1.81 (H-4′b) and broad singlets at δ_H 5.51 (H-3′b-a) and 5.98 (H-3′b-b) for subunit b in the ^{1}H NMR spectrum. In addition, eight signals appeared at δ_C 166.3 (C-1'a), 137.9 (C-2'a), 128.5 (C-3'a), and 30.8 (C-4'a) for subunit a and at δ_C 166.9 (C-1'b), 135.7 (C-2'b), 126.4 (C-3'b), and 18.1 (C-4'b) for subunit b in the ^{13}C NMR spectrum of **1**.[1] These methacrylate groups were assigned to the C-8a and C-8b positions, respectively, as supported by HMBC correlations between H-8a/C-1'a and H-8b/C-1'b (Table 1 and Figure S8, Supplementary data).

Subunit a of **1** contained a structural unit of 15-deoxygoyazensolide, as indicated by comparison of the ^{1}H and ^{13}C NMR data of this part of the molecule (Table 1) with those of 15-deoxygoyazensolide.[1] This subunit showed closely comparable signals for the sesquiterpene lactone core to those of 15-deoxygoyazensolide but different signals for the ester residue at the C-8 position, which appeared at δ 166.7 (C-1'), 135.5 (C-2'), 126.4 (C-3'), and 18.0 (C-4') for 15-deoxygoyazensolide.[1] Also, a signal at δ 18.0 for a methyl group of C-4' of 15-deoxygoyazensolide appeared as a signal at δ 30.7 for the C-4'a methylene group of subunit a of **1**, indicating this subunit to be linked to subunit b at its C-4'a position. This elucidation was

confirmed by HMBC correlations between H-2a/C-1a, -3a, and -10a, H-8a/C-6a, -10a, and 1'a, H-9a/C-1a, -8a, -10a and -14a, H-14a/C-1a, -9a, and -10a, and H-4'a/C-1'a, 2'a, 3'a, -4b, -5b, and 6b, respectively (Table 1).

Subunit b of **1** was proposed as being based on 4,5-dihydro-15-deoxygoyazensolide.[3] Comparison of the NMR data of this subunit with literature data indicated that it exhibited identical signals for the ester residue at the C-8 position to those of 4,5-dihydro-15-deoxygoyazensolide but different signals for the sesquiterpene lactone core, especially at the C-3, -4, -5, -6, -7, -8, and -15 positions, which appeared at δ 192.6 (C-3), 33.6 (C-4), 42.4 (C-5), 82.1 (C-6), 54.4 (C-7), 71.9 (C-8), and 18.5 (C-15) for 4,5-dihydro-15-deoxygoyazensolide,[3] and at δ 193.8 (C-3b), 36.3 (C-4b), 46.3 (C-5b), 81.0 (C-6b), 55.9 (C-7b), 72.4 (C-8b), and 9.9 (C-15b) for subunit b of **1**. A signal at δ 9.9 was assigned to a C-15 methyl group connected to the C-4 position of a goyazensolide core containing a substituent at the C-5 position.[1] The signal at δ 42.4 for the C-5 methylene group of 4,5-dihydro-15-deoxygoyazensolide[3] was observed at δ 46.3 for a methine group at C-5b of subunit b of **1**, indicating that this subunit is linked to subunit a at the C-5 position. This was confirmed by HMBC correlations, in turn, between H-2b/C-1b, -3b, and -10b, H-5b/C-6b, -7b, -15b, and -2'a, H-8b/C-6b, -10b, and 1'b, H-9b/C-1b, -8b, -10b and -14b (Table 1). Based on this spectroscopic evidence, compound **1** was determined as 15-deoxygoyazensolide-(4'→5)-4,5-dihydro-15-deoxygoyazensolide.

The relative configuration of **1** was established by NOESY correlations in combination with comparison of its NMR data with those of both 15-deoxygoyazensolide and 4,5-dihydro-15-deoxygoyazensolide.[1,3] In turn, the absolute configuration of **1** was determined by analysis of the CD spectrum. According to the determination of absolute configuration of goyazensolide-type sesquiterpene lactones,[1] the negative Cotton effects at 235 and 270 nm exhibited in the CD

spectrum (Figure 2) of **1** indicated 7a*R* and 7b*R* configurations, and the positive Cotton effects at 212 and 317.5 nm supported 10a*R* and 10b*R* configurations. The NOESY correlations between H-2a/H-8a and H-6a/H-8a indicated 6a*R* and 8a*S* configurations, as supported by the similar NMR data of this part with those of 15-deoxygoyazensolide.[1] The NOESY correlations between H-2b/H-6b, H-8b, and H-15b indicated 6b*R* and 8b*S* configurations, as supported by the similar coupling constants for H-6b to those of H-6 of rufescenolides A and B[1] and the similar coupling constant for H-8b to that of H-8a, together with the consistent CD spectra with those of rufescenolide A (Figure 2). The NOESY correlations between H-5b/H-7b and H-14b suggested a 5b*S* configuration, and the NOESY correlation between H-15b/H-6b suggested 6b*R* and 4b*R* configurations in **1**. Determination of the absolute configurations at C-4b, -5b, -6b, -7b, -8b, and -10b were supported by the consistent NMR data of **1** with those of 4,5-dihydro-15-deoxygoyazensolide,[3] but not with those of zexbrevin.[5] Therefore, the structure and absolute configuration of compound **1** was proposed as (6a*R*,7a*R*,8a*S*,10a*R*)-1-oxo-3,10-epoxy-8-methacryloyloxygermacra-2,4,11(13)-trien-6,12-olide-(4′→5)-(4b*R*,5b*S*,6b*R*,7b*R*,8b*S*,10b*R*)-1-oxo-3,10-epoxy-8-methacryloyloxygermacra-2,11(13)-dien-6,12-olide,[1] which has been accorded the trivial name, rufescenolide C.

As shown in Scheme 1, it is proposed that rufescenolide C (**1**) may be formed from either an enzyme- or an acid-catalyzed ene-type reaction of 15-deoxygoyazensolide, which was isolated previously from *Piptocoma rufescens* in a high yield,[1] with the C-2′, 3′ and 4′ positions of this molecule as an ene and the C-4 and C-5 positions of the same molecule as an enophile.[6]

Rufescenolide C (**1**) was tested in terms of its cytotoxicity against the HT-29 human colon cancer cell line by a previous procedure,[1] using paclitaxel as positive control (IC_{50}, 0.10 nM). It showed high cytotoxicity toward the HT-29 cell line, with an IC_{50} value of 150 nM.

Dimeric sesquiterpene lactones are rare natural products discovered mainly from the family Asteraceae and exhibit a number of structural types. The most common members of this compound family are symmetrical dimers. Double-linked guaianolide dimers containing either a non-spiro or a spiro linkage are more prevalent than their single-linked variants,[7–10] with dimers having a single ether oxygen bridge being unusual.[11] The connectivity of the monomers of dimeric eudesmanolides may occur either as a C-11-spiro-double linkage or as a single linkage,[12,13] while dimeric eremophilanolides tend to occur in non-spiro-double-linked or single-linked forms.[14,15] These compounds, together with the small member of known germacranolide dimers,[16,17] as well dimeric xanthanolides and elemanolides,[18,19] and the several unsymmetrical sesquiterpene lactone dimers,[20–23] exhibit considerable chemical diversity. It is proposed that doubly-linked dimeric sesquiterpene lactones are formed by Diels-Alder additions,[9,20,22] which has been supported by the subsequent synthesis of several representatives of this compound type using such a synthetic strategy.[24] Also, this same hypothesis was proposed for the biosynthesis of singly-linked sesquiterpene dimers,[10] but supportive evidence for such a proposal is limited.

Dimeric sesquiterpene lactones are known to exhibit many types of biological activities, including cytotoxicity,[9,21,25] anti-HIV potency,[7] antidiabetic activity,[11] antiprotozoal effects,[10,18] anti-inflammatory efficacy,[26] and inhibition of LPS-induced NO production.[8,23] In addition, several guaianolide dimers showed more potent cytotoxicity toward a panel of human cancer cell lines than their monomer.[25] The dimeric guaianolide, microlenin, was found to suppress Walker 256 carcinosarcoma growth *in vivo*,[9] and the antitumor potency of artemisinin, a sesquiterpene lactone endoperoxide, has been improved considerably by dimerization of this molecule.[27,28] Consistent with these previous studies, the present study showed that rufescenolide C (**1**) exhibits more potent cytotoxicity against HT-29 human colon cancer cells than its monomeric

analogues,[1] indicating that this compound might be an enhanced antitumor lead for further investigation.

Acknowledgments

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Supplementary data

Supplementary data about experimental procedures, MS and NMR spectra of rufescenolide C (**1**) associated with this article can be found in the online version at http://dx.doi.org/tetlet.

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2. *Selected data for **1**:* Amorphous colorless powder (*n*-hexane) showing a dark color under UV light at 254 nm; $[\alpha]^{20}_{D}$ +58.9 (*c* 0.09, MeOH); $[\alpha]^{20}_{D}$ +44.0 (*c* 0.1, CH_2Cl_2); UV (MeOH) λ_{max} (log ε) 214 (4.71), 263 (4.66) nm; CD (MeOH, nm) λ_{max} (Δε) 212 (+25.97), 235 (-1.74), 270 (-5.08), 317.5 (+8.24); IR (dried film) ν_{max} 1770, 1712, 1654, 1629, 1587, 814 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; positive HRESIMS *m*/*z* 711.2407, calcd for $C_{38}H_{40}O_{12}Na$, 711.2417.

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Table 1
NMR spectroscopic data for rufescenolide C (**1**) in $CDCl_3$

Position[a]	δ_C(mult.)[b]	δ_H(mult., J, Hz)[c]	COSY (H→H)[d]	HMBC (H→C)[e]	NOESY (H→H)[f]
1a	205.0 C				
2a	104.9 CH	5.70 s		1a, 3a, 10a	8a
3a	187.2 C				
4a	130.6 C				
5a	135.0 CH	5.98 br s	6a	15a	15a
6a	81.9 CH	5.25 m	7a	8a	8a
7a	51.3 CH	3.70 m	6a, 8a		6a, 9a-b, 13a-a
8a	74.2 CH	4.47 dt (2.0, 12.0)	7a, 9a	6a, 10a, 1'a	6a, 9a-a, 9a-b
9a-a	44.2 CH_2	2.26 m	8a, 9a-b	1a, 8a, 10a, 14a	8a
9a-b		2.50 t (12.0)	8a, 9a-a	1a, 8a, 10a, 14a	7a, 8a, 14a
10a	89.8 C				
11a	133.8 C				
12a	168.8 C				
13a-a	124.5 CH_2	5.42 d (2.4)		7a, 12a	7a
13a-b		6.23 d (3.2)		7a, 12a	
14a	20.9 CH_3	1.52 s		1a, 9a, 10a	
15a	20.5 CH_3	2.07 s		3a, 4a, 5a	5a
1'a	166.3 C				
2'a	137.9 C				
3'a-a	128.5 CH_2	5.76 br s		1'a, 2'a, 4'a	4'a-a, 4'a-b
3'a-b		6.12 br s		1'a, 2'a, 4'a	
4'a-a	30.8 CH_2	2.22 m	5b	1'a, 2'a, 3'a, 4b, 5b, 6b	3'a-a, 15b
4'a-b		2.73 m	5b	1'a, 2'a, 3a', 4b, 5b, 6b	3'a-a
1b	205.7 C				
2b	105.9 CH	5.70 s		1b, 3b, 10b	6b, 8b, 15b
3b	193.8 C				
4b	36.3 CH	2.85 m	5b, 15b	3b, 5b, 6b, 15b	
5b	46.3 CH	2.63 m	4'a, 6b	6b, 7b, 15b, 2'a	7b, 14b
6b	81.0 CH	4.28 dd (3.6, 7.2)	5b, 7b	8b, 11b, 12b, 13b	2b, 7b, 15b
7b	55.9 CH	3.40 m	6b, 8b		5b, 6b, 8b, 13b-a
8b	72.4 CH	4.36 dt (2.0, 11.2)	7b, 9b	6b, 10b, 1'b	7b, 9b-b
9b-a	45.8 CH_2	2.35 m	8b, 9b-b	1b, 8b, 10b, 14b	
9b-b		2.63 m	8b, 9b-a	1b, 8b, 10b, 14b	8b
10b	90.1 C				
11b	133.3 C				
12b	169.2 C				
13b-a	125.2 CH_2	5.47 d (2.4)		7b, 12b	7b
13b-b		6.17 d (2.8)		7b, 12b	
14b	21.2 CH_3	1.51 s		1b, 9b	5b
15b	9.9 CH_3	1.25 (overlap)	4b	3b, 4b, 5b	2b, 6b, 4′a-a
1'b	166.9 C				
2'b	135.7 C				
3'b-a	126.4 CH_2	5.51 br s		1'b, 2'b, 4'b	4'b
3'b-b		5.98 brs		1'b, 2'b, 4'b	
4'b	18.1 CH_3	1.81 s		1'b, 2'b, 3'b	3'b-a

[a]Assigned by analysis of ^1H, ^{13}C, DEPT 90, DEPT 135, COSY, HSQC, and HMBC NMR spectra.

[b]Recorded at 100.6 MHz and referenced to the solvent residual peak at δ 77.16.[4] CH_3, CH_2, CH, and C determined by DEPT 90 and DEPT 135 and HSQC experiments.

[c]Recorded at 400.1 MHz and referenced to the solvent residual peak at δ 7.26.[4] The overlapped signals were assigned by $^1H-^1H$ COSY, HSQC, and HMBC spectra are presented without designating multiplicity.

[d]Recorded at 400.1 MHz and referenced to the solvent residual peak at δ 7.26 with proton showing COSY correlation to indicated proton.

[e]Recorded at 800.1 MHz with proton showing HMBC correlation to indicated carbon.

[f]Recorded at 800.1 MHz and referenced to the solvent residual peak at δ 7.26 with proton showing NOESY correlation to indicated proton.

Legends of Figures and Scheme

Figure 1. Structure of rufescenolide C (**1**).

Figure 2. CD spectra of rufescenolide C (**1**) and rufescenolide A.

Scheme 1. Proposed biogenesis of rufescenolide C (**1**) from an ene-type reaction of 15-deoxygoyazensolide.

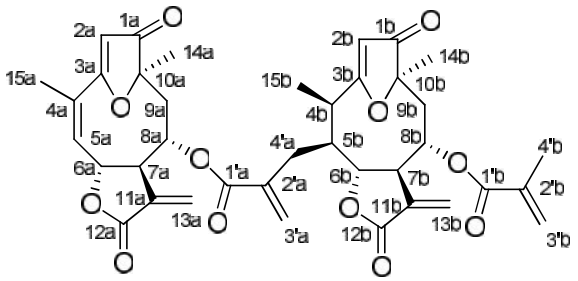


Figure 1. Structure of rufescenolide C (**1**).

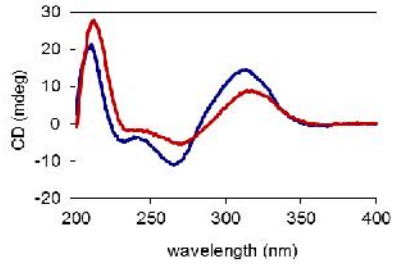
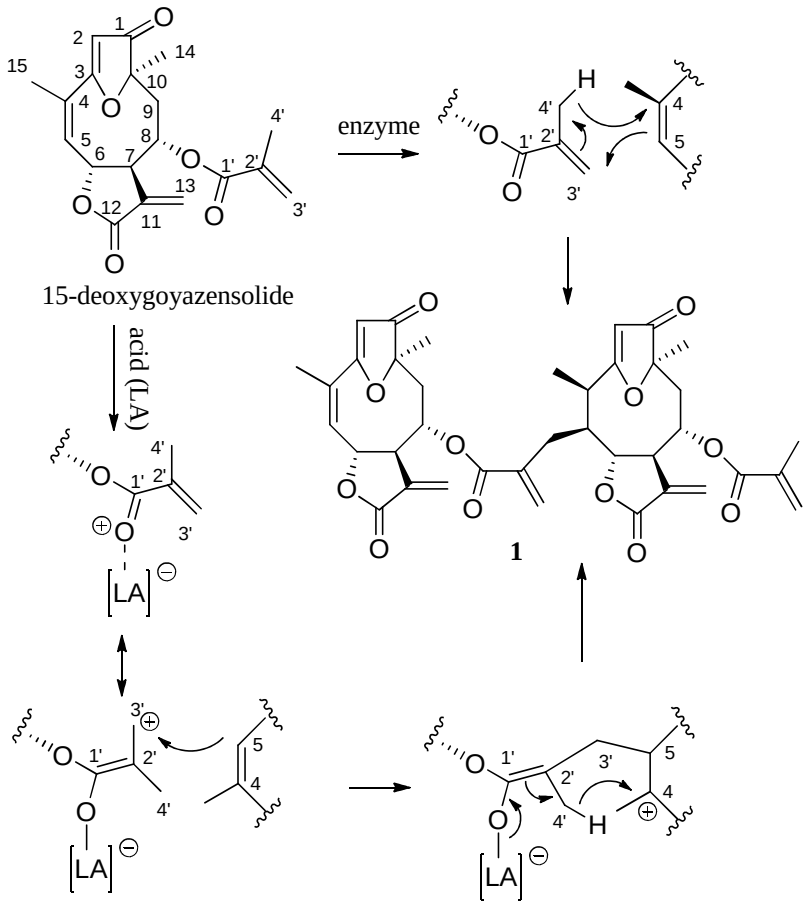


Figure 2. CD spectra of rufescenolide C (**1**, red) and rufescenolide A (blue). The CD data were obtained in MeOH corrected by subtracting a spectrum of the appropriate solution in the absence of the samples recorded under identical conditions.



Scheme 1. Proposed biogenesis of rufescenolide C (**1**) from an ene-type reaction of 15-deoxygoyazensolide.