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Antiproliferative and Antimalarial Sesquiterpene Lactones from *Piptocoma antillana* from Puerto Rico [1]

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Abstract

Bioassay-directed fractionation of an antiproliferative ethanol extract of the leaves and twigs of *Piptocoma antillana* (Asteraceae) afforded two new goyazensolide-type sesquiterpene lactones named 5-*O*-methyl-5-epiisogoyazensolide (**1**) and 15-*O*-methylgoyazensolide (**2**), together with the known compounds 1-oxo-3,10-epoxy-8-(2-methylacryloxy)-15-acetoxygermacra-2,4,11(13)-trien-6(12)-olide (**3**) and 5-epiisogoyazensolide (**4**). The structure elucidation of all compounds was carried out based on NMR and mass spectroscopic data analyses. The relative and absolute configurations of all the isolated compounds were determined from their CD and NOESY NMR spectra. Compounds **1–4** showed moderately potent antiproliferative activities against A2780 ovarian cancer cells, with IC_{50} values of 1.5±0.5, 0.6±0.3, 1.62±0.05, and 1.56±0.04 μM, respectively. They also displayed antimalarial activity against *Plasmodium falciparum*, with IC_{50} values of 6.2 ± 0.5, 2.2 ± 0.5, 8.0 ±0.4, and 9.0±0.6 μM, respectively.

Keywords

Antiproliferative activity; Antimalarial activity; *Piptocoma antillana*; Sesquiterpene lactone; Goyazensolide-type; Dereplication

The genus *Piptocoma*, a member of the Asteraceae family, was first discovered in the West Indies, and consisted initially of only three species. The genus was expanded in 2000, when Pruski described the Central and South American plant species from the genus *Pollalesta* as cogeneric with *Piptocoma*, and this raised the number of *Piptocoma* species to eighteen[2]. A literature search demonstrated that the only reports on the chemical constituents of the

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Supplementary data: ^{1}H and ^{13}C NMR spectra of compounds **1** and **2** and ^{1}H NMR spectra of **3** and **4** are available in electronic form on the publisher's website.

genus *Piptocoma* were in the last two years, describing the isolation of cytotoxic sesquiterpene lactones, phenylpropanol coumarates, and flavonoids from *P. rufescens*[3]. Goyazensolide (**5**), one of the isolated sesquiterpene lactones, was later shown to induce apoptosis in cancer cells in vitro and in vivo[4].

Our ongoing screening of extracts from the Natural Products Discovery Institute collection as part of a collaborative research program to explore the potential of the former Merck collection of extracts[5] indicated that a CH_2Cl_2 extract of the leaves and twigs of *P. antillana* exhibited significant antiproliferative activity against the A2780 human ovarian cancer cell line. We report herein the bioassay-guided isolation and structure identification of the components responsible for the antiproliferative activity, as well as the antiplasmodial activity of the same compounds.

An active CH_2Cl_2-soluble fraction obtained from liquid-liquid partition of the extract (100 mg) was subjected to dereplication studies using size-exclusion chromatography, reverse phase HPLC coupled with bioassay, high-resolution ESIMS (HRESIMS), ^{1}HNMR, and a database search using the online Dictionary of Natural Product (DNP). The results indicated that the extract contained at least one new bioactive compound, and so a 2.0 g sample was investigated. Fractionation of this extract yielded an antiproliferative CH_2Cl_2 fraction which was further subjected to size-exclusion column chromatography on Sephadex LH-20 followed by normal phase silica gel column chromatography. The most active fractions from the silica gel column were subjected to C-18 HPLC to yield two new (**1**, **2**) and two known (**3**, **4**) bioactive sesquiterpene lactones.

Compound **4** had the molecular formula $C_{19}H_{20}O_7$ as determined by high resolution electrospray ionization mass spectroscopy (HRESIMS) analysis. It was determined to be 5-epiisogoyazensolide (**4**) by searching its molecular formula and key ^{1}H NMR spectroscopic features in the DNP database and comparison of its full ^{1}H NMR spectrum with reported literature data[6]. Compound **3** was identified as 1-oxo-3,10-epoxy-8-(2-methylacryloxy)-15-acetoxy-germacra-2,4,11(13)-trien-6(12)-olide (15-acetylgoyazensolide) by comparison of its spectroscopic data with values reported in the literature[7].

Compound **1** was obtained as an amorphous white powder and had the molecular formula $C_{20}H_{22}O_7$ as indicated by HRESIMS analysis (*m/z*: 375.1461 $[M+H]^+$, calcd. for $C_{20}H_{23}O_7^+$, 375.1438). Its IR spectrum showed absorptions characteristic of an α,β-unsaturated γ-lactone (1765 and 1640cm^{-1}) and an α,β-unsaturated ester (1710 and 1645 cm^{-1}) functions, as well as a dihydrofuran-3-one ring (1709 and 1582 cm^{-1})[3a, 6c, 7a, 8]. The similarity of the IR spectroscopic data of **1** with those of **4**, in conjunction with the UV absorption characteristics at λ_{max} 208 and 270 nm, suggested that **1** was also a furan ring-containing germacranolide similar to goyazensolide or furanoheliangolide[6c, 7a, 8]. Its ^{1}H NMR spectrum (Table 1) displayed signals for a methyl group at δ_H1.53 (s, 3H, CH_3-14), two exocyclic methylene groups as two doublets at δ_H 6.21 (*J*= 3.3 Hz, 1H) and 5.46 (*J*=2.9 Hz, 1H) and two singlets at δ_H6.00 and 5.78 (each 1H), representing (H_2-13 and H_2-15), and an olefinic proton at δ_H5.82 (s, 1H, H-2). These signals are all very similar to those in the goyazensolide skeleton of **4**. In the ^{13}C NMR spectrum, the presence of signals attributable

to a conjugated ketone carbonyl at δ_C204.4 ppm (C-1), a lactone carbonyl at δ_C169.1(C-12), the carbons of an oxygen bearing alkene at δ_C 106.8 (C-2) and 185.9 (C-3) and of two exocyclic alkenes at δ_C 133.9 (C-11), 123.8 (C-13), 134.5 (C-4), and 127.1 (C-15), and three oxygen bearing methines at δ_C 84.5 (C-5), 85.1 (C-6), and 72.3 (C-8), supported the preliminary structural assignment[7c]. In the HMBC experiment, protons at δ_H6.00and 5.78 correlated with both C-3 (δ_C 185.9) and C-5 (δ_C 84.5), which allowed us to assign the two olefinic protons to be those of H_2-15 (Fig. 2). In the same manner, the other pair of the exocylic methylene protons at δ_H 6.21 and 5.46 were determined to be those of H_2-13 due to their correlations with δ_C169.1(C-12) and δ_C44.8(C-7). The ^{1}H and ^{13}C NMR spectra of **1** also revealed the expected signals from the goyazensolide methacrylate side chain, namely for a methyl group at δ_H1.82 (s, 3H, H-4'), two olefinic proton signals at δ_H 5.95 and 5.53 (m, 1H for each, H-3'), and four carbon resonances at δ_C 166.9 (C-1'), 135.4 (C-2'), 126.4 (C-3'), and 18.0 (C-4')[7c]. This assignment was confirmed by the HMBC correlations (Fig. 2) between the proton signals of H-4' with both C-1' and C-3'. The acyl group was located at C-8 by the long range HMBC cross peak observed between the signals at δ_H4.31 (ddd, *J*=11.8, 1.4, 1.3 Hz, 1H, H-8) and δ_C 166.9 (C-1'). The^{1}H spectrum of **1** contained an additional proton signal at δ_H3.73 (s, 3H) as compared with the spectrum of **4**. A carbon signal at δ_C57.2 and a deshielded proton signal at δ_H 4.23 (H-5) confirmed the presence of a methoxyl group at C-5 in **1** as compared with the hydroxyl group of **4**. The HMBC correlation between δ_H3.73 (s, 3H) and δ_C 84.5 (C-5) substantiated the placement of the methoxyl group at the C-5 position in **1**. The complete assignments of all protons and carbons of **1** (Table 1) were accomplished by further interpretation of the HMBC and NOESY spectra. The relative configurations at C-5, C-6, C-7, C-8, and C-10 of **1** were suggested by the analysis of a NOESY experiment (Fig. 2). The absolute configuration at C-7 was determined to be *R* by the negative Cotton effects at 225 and 268 nm observed in its CD spectrum. This is consistent with previous studies demonstrating that sesquiterpene lactones with a C-7, C-6 trans-fused α-methylene-γ-lactone moiety display negative Cotton effects in the range of 216–225 and 252–271 nm in their CD spectra, arising from the $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ transitions of the lactone ring, respectively[9]. Compound **1** was thus assigned as (5*S*,6*S*,7*R*,8*S*,10*R*)-1-oxo-3,10-epoxy-5-methoxy-8-(2-methylacryloxy)germacra-2,4(15), 11(13)-trien-6,12-olide[10], or 5-*O*-methyl-5-epiisogoyazensolide.

Compound **2** was isolated as an amorphous white powder with a molecular formula of $C_{20}H_{22}O_7$ based on its HRESIMS spectrum. The close similarity of the UV, IR,^{1}H and ^{13}C NMR spectroscopic data of **2** with those of **1** and **3** demonstrated that all three compounds contain the same goyazensolide skeleton with a methacrylate side chain at the C-8 position. Comparison of the ^{1}H NMR spectroscopic data of **2** with those of **3** indicated that the major differences between them were the lack of signals for the 15-acetyl group in **3** and the addition of signals at δ_H 3.40 (s, 3H) and δ_C 58.5 (15-OMe) for an *O*-methyl group. HMBC correlations from the H-15 with the methoxy carbon at δ_C 58.5 and from the methoxy protons δ_H 3.40 (s, 3H) with the deshielded oxygen bearing methylene carbon at δ_C 72.7 (C-15) confirmed that the methoxy group was at C-15. As in the case of **1**, all proton and carbon signals of **2** were assigned by interpretation of the data obtained from HMBC and NOESY experiments (Table 1). In addition, comparison of the CD and NOESY spectrum of **2** with **1** indicated that both compounds have the same absolute configurations at their C6,

C7, C8, and C10 stereogenic centers. Therefore, compound **2** was determined to be (6*S*,7*R*, 8*S*,10*R*)-1-oxo-3,10-epoxy-15-methoxy-8-(2-methylacryloxy)germacra-2,4(5),11(13)-trien-6,12-olide[7a], or 15-*O*-methylgoyazensolide.

Compounds **1**–**4** were evaluated for their antiproliferative activity against the A2780 human ovarian cancer cell line. They showed micromolar activities with half-maximum inhibitory concentration (IC_{50}) values of 1.5±0.5,0.6 ±0.3, 1.62 ±0.05, and1.56 ±0.04 μM, respectively. All the compounds were further evaluated for their antimalarial activity against *P. falciparum* Dd2 (a chloroquine/mefloquine-resistant strain), and showed IC_{50} values of 6.2 ± 0.5, 2.2 ± 0.5, 8.0±0.4, and 9.0 ± 0.6 μM, respectively.

Experimental section

General experimental procedures

IR and UV spectra were measured on MIDAC M-series FTIR and Shimadzu UV-1201 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 spectrometer in $CDCl_3$, with $CDCl_3$ as reference. Mass spectra were obtained on an Agilent 6220 mass spectrometer. Open column chromatography was performed using Sephadex LH-20 and silica gel (230-400 mesh, Silicycle Co. USA). Semi-preparative HPLC was performed using Shimadzu LC-10AT pumps coupled with a semipreparative Phenomenex C-18 column (5 μm, 250 × 10 mm), a Shimadzu SPD M10A diode array detector, and a SCL-10A system controller. All isolated compounds were purified to 95% purity or better, as judged by HPLC (both UV and ELSD detection) before determining bioactivity.

Plant material

Leaves and twigs of *P. antillana* Urb were collected by Hannah Stevens of the New York Botanical Garden near Manatí, Puerto Rico (18°4.385 N, 66°54.382 W). A voucher specimen is on deposit at the NYBG under the accession number HS 00246a.

Antiproliferative bioassay

The A2780 ovarian cancer cell line antiproliferative bioassay was performed at Virginia Tech as previously reported[11]. The A2780 cell line is a drug-sensitive ovarian cancer cell line[12].Paclitaxel was used as the positive control; it had an IC_{50} value of 0.024 μM.

Antimalarial bioassay

The effect of each compound on parasite growth of the Dd2 strain of *P. falciparum* was measured in a 72 h growth assay in the presence of compound as described previously with minor modifications[13]. Briefly, ring stage parasite cultures (1% hematocrit and 1% parasitemia) were grown for 72 h in the presence of increasing concentrations of the drug in a 5.05% CO_2, 4.93% O_2, and 90.2% N_2 gas mixture at 37 °C. After 72 h in culture, parasite growth was determined by DNA quantitation using SYBR Green I.[31] The half-maximal inhibitory concentration (IC_{50}) calculation was performed with GraFit software using a nonlinear regression curve fitting. IC_{50} values are the average of three independent

determinations with each determination in duplicate and are expressed ± SD. Artemisinin was used as the positive control; it had an IC_{50} value of 7 nM.

Extraction and Isolation

The dried and powdered leaves and twigs of *P. antillana* were exhaustively extracted with ethanol in two 24-hour percolation steps; successive partition of the concentrated extract with hexane and methylene chloride gave an active methylene chloride extract. For purposes of fractionation and purification, 2.0 g of the original ethanol extract designated 1000892-7G (IC_{50} 12 μg/mL) was suspended in 90% aq. MeOH (300 mL), and extracted with hexanes (3 × 200 mL). Evaporation of the hexane-soluble fraction afforded 176 mg of residue. The 90% aq. MeOH layer was then diluted to 60% and extracted with CH_2Cl_2 (3 × 200 mL) to yield 479 mg of CH_2Cl_2-soluble fraction. The aqueous MeOH layer was concentrated to give 1.3 g of brown residue. The CH_2Cl_2 fraction was found to have antiproliferative activity with an IC_{50} value of 0.62 μg/mL, and was subjected to Sephadex LH-20 open column chromatography (CH_2Cl_2:MeOH, 1:1) to give 5 fractions. The most active fraction Fr 3 (IC_{50}0.5 μg/mL) was then divided into 6 sub-fractions by silica gel column chromatography (hexanes:EtOAc, 1:1). Further purification of the most active sub-fraction Fr 3-4 (IC_{50}0.3 μg/mL) was done by using HPLC on a C-18 column with a solvent gradient from H_2O:CH_3CN, 70:30 to 68:32 from 0 to 30 min, to 58:42 from 30 to 60 min, and ending with 100% CH_3CN from 60 to 80 min. This process yielded compounds **4** (2.5 mg, t_R31 min), **2** (1.5 mg, t_R60 min), **1** (1.1 mg, t_R64.5 min), and **3** (1.2 mg, t_R66 min).

5-*O*-methyl-5-epiisogoyazensolide (1)

Amorphous powder, $[\alpha]_D$ -95.6 (c 0.27, MeOH)

CD (c 0.031, MeOH, nm) λ_{max} ($\Delta\varepsilon$) 206 (+10), 225 (−4.0), 268 (−4.2), 320 (+2.5)

UV (MeOH) λ_{max} nm (log ε): 270 (2.20), 208 (3.89)

IR (film) ν_{max} cm^{-1}: 1765, 1710, 1709, 1645, 1640, 1582, 1454 ^{1}H NMR (500 MHz, $CDCl_3$): Table 1.

^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

HRESIMS: *m/z* $[M+H]^+$, calcd. for $C_{20}H_{23}O_7^+$: 375.1438; found: 375.1461.

5-*O*-methylgoyazensolide (2)

Amorphous powder, $[\alpha]_D$ −121.8 (c 0.27, MeOH)

CD (c 0.031, MeOH, nm) λ_{max} ($\Delta\varepsilon$) 203 (+5.7), 216 (−1.8), 276 (−2.3), 318 (+2.9)

UV (MeOH) λ_{max} nm (log ε) : 267 (2.67), 207 (4.01)

IR (film) ν_{max} cm^{-1}: 1769, 1710, 1709, 1653, 1587, 1456;

^{1}H NMR (500 MHz, $CDCl_3$): Table 1.

^{13}C NMR (125 MHz, $CDCl_3$): Table 1.

HRESIMS: *m/z* $[M+H]^+$, calcd. for $C_{20}H_{23}O_7^+$: 375.1438; found: 374.1459.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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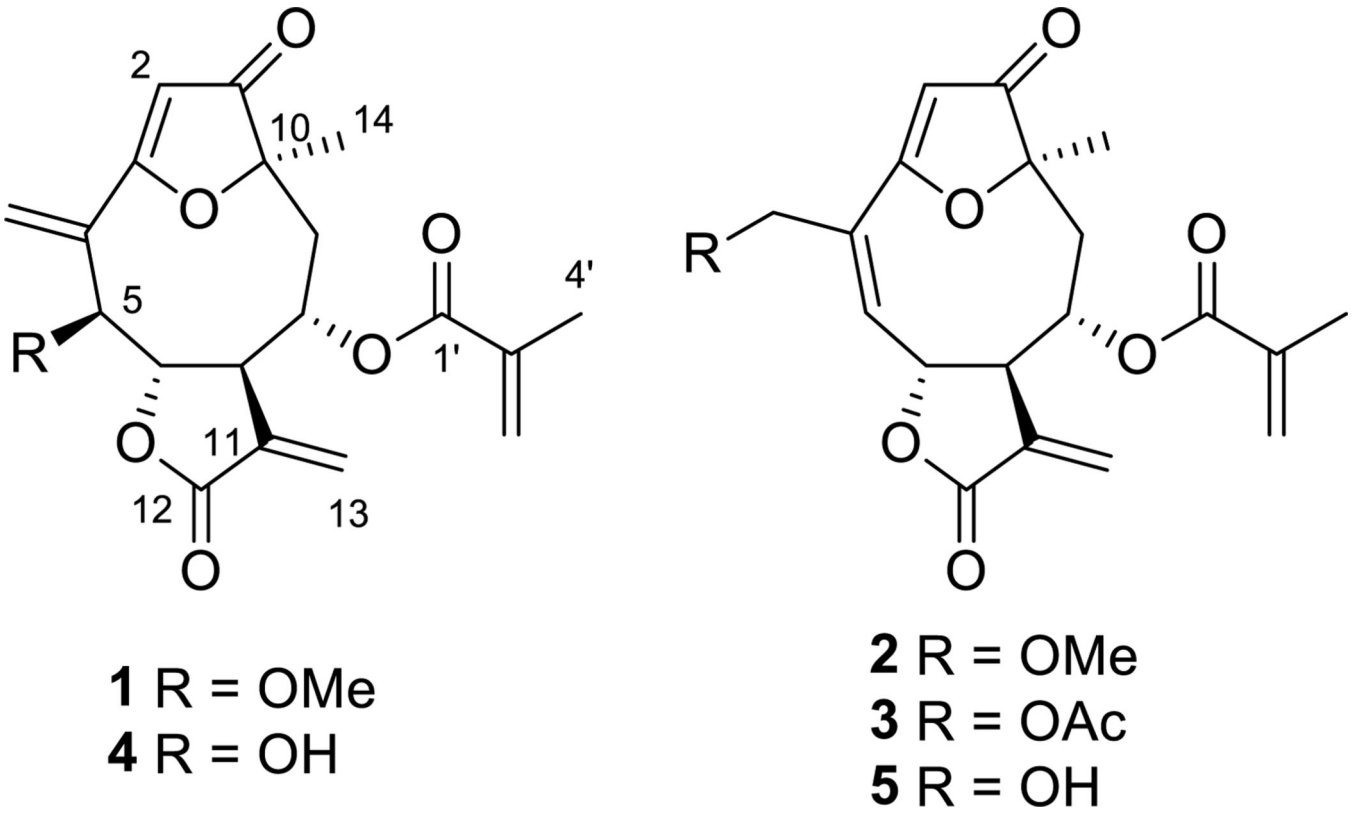

Figure 1.
Structures of compounds **1–5**.

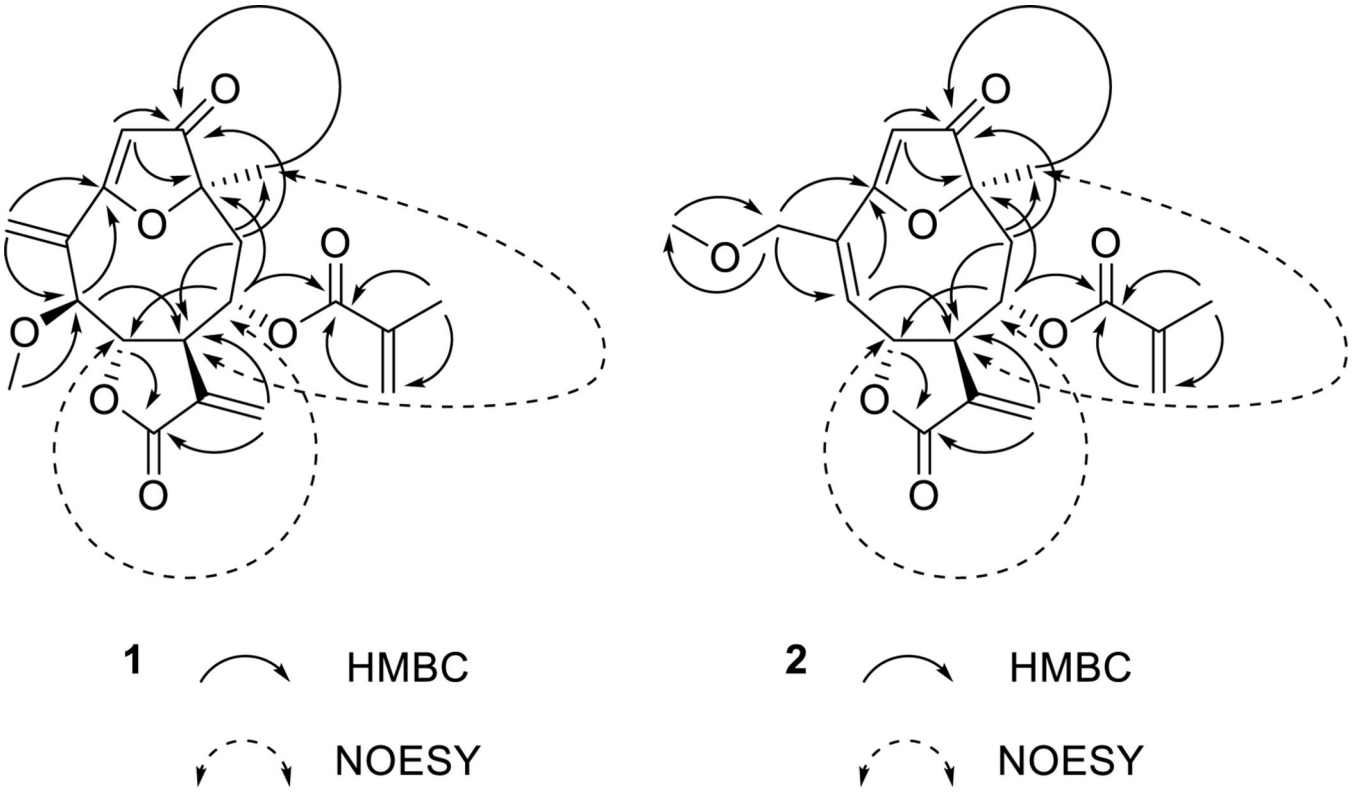

Figure 2.
Key HMBC and NOESY correlations of **1** and **2**.

Table 1

$_1$H and $_{13}$C NMR data for compounds **1** and **2**.

	1		**2**	
Posn	**δH**[b]	**δC**[c]	**δH**[b]	**δC**[c]
1		204.4 C		204.6 C
2	5.82 s	106.8 CH	5.77 brs	106.4 CH
3		185.9 C		184.4 C
4		134.5 C		131.8 C
5	4.23 s	84.5 CH	6.27 dt (2.9, 1.4)	137.3 CH
6	4.74 d (5.2)	85.1 CH	5.33 ddt (5.5, 2.9, 1.4)	81.3 CH
7	4.21 m	44.8 CH	3.80 ddd (5.5, 3.2, 2.7)	50.9 CH
8	4.31 ddd (11.8, 1.4, 1.3)	72.3 CH	4.54 dt (11.8, 2.2)	73.2 CH
9	2.52 dd (13.7, 11.8)	44.6 CH_2	2.49 dd (13.9, 11.8)	43.9 CH_2
	2.33 dd (13.7, 1.4)		2.32 dd(13.9, 2.2)	
10		90.4 C		89.8 C
11		133.9 C		133.1 C
12		169.1 C		168.6 C
13	6.21 d (3.3)	123.8 CH_2	6.23 d (3.2)	124.6 CH_2
	5.46 d (2.9)		5.47 d (2.7)	
14	1.53 s	21.2 CH_3	1.54 s	20.7 CH_3
15	6.00 s	127.1 CH_2	4.16 ddd (12.8, 1.4, 1.3)	72.7 CH_2
	5.78 s		4.12 ddd (12.8,1.4, 1.3)	
1'		166.9 C		166.8 C
2'		135.4 C		135.3 C
3'	5.95 m	126.4 CH_2	6.01 brs	126.6 CH_2
	5.53 m		5.55 m	
4'	1.82 s	18.0 CH_3	1.83 s	18.0 CH_3
5-OMe	3.73 s	57.2 CH_3		
15-OMe			3.40 s	58.5 CH_3

[a] Assignments based on analysis of 2D NMR spectra.

[b] Data (δ) measured at 500 MHz; s= singlet, br s=broad singlet, d=doublet, dd=doublet of doublets, ddd=doublet of doublets of doublets, dt=doublet of triplets, m=multiplet. *J* values are in Hz and are omitted if the signals overlapped as multiplets. The overlapped signals were assigned from HSQC and HMBC spectra without designating multiplicity.

[c] Data (δ) measured at 125 MHz; CH_3, CH_2, CH, and C multiplicities were determined by HSQC experiment.