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Polyhydroxylated macrolide isolated from the endophytic fungus *Pestalotiopsis mangiferae*

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ABSTRACT

A new polyhydroxylated macrolide, named mangiferaelactone (**1**) was isolated from a solid culture of the endophytic fungus *Pestalotiopsis manguiferae*, together with ten known compounds [(6*S*,1′*S*)-LL-P880α; (6*S*,1′*S*,2′*R*)-LL-P880β; (1′*S*,2′*R*)-LL-880γ; (1′*R*)-dehydropestalotin; (−)-5-carboxylmellein; (−)-5-methylmellein; (−)-5-hydroxylmethylmellein; arabenoic acid; 5,6-dihydro-4-methoxy-2*H*-pyran-2-one; and the (−)-2-hexylidene-3-methylsuccinic acid]. *P. manguiferae* was isolated from *Hyptis dilatata*, a small shrub common in the central region of Panama. The structure of compound **1** was elucidated by a combination of spectroscopic methods (IR, MS, optical rotation, 1D and 2D NMR spectroscopy). The absolute configuration of **1** was established as 4*R*,7*R*,8*R*,9*S* by application of vibrational circular dichroism (VCD). Compound **1** showed a minimum inhibitory concentration (MIC) of 1.6863 mg/mL against *Listeria monocytogenes*, and 0.5529 mg/mL against *Bacillus cereus*. No activity was observed for compound **1** against *Plasmodium falciparum* or *Trypanosoma cruzi*; likewise, no cytotoxic activity was observed against A2058 and H522-T1 cells.

Introduction

Endophytic fungi have garnered significant interest in the last decade as a source of unique biologically active and structurally diverse natural products. Previous research has revealed that the majority of natural products isolated from endophytic microorganisms have antimicrobial activities, which have been implicated in protecting the host plant against phytopathogenic microorganisms.[1–3] Medicinal plants have been recognized as a repository of fungal endophytes that produce interesting molecules with potential applications as pharmaceutical agents.[4] *Hyptis dilatata* is a perennial subshrub that is distributed in the central provinces of Panama whose leaves are used as topical anti-parasitic in animals. This plant was selected for the isolation of endophytic fungi as part of our ongoing investigation of natural products from endophytic fungi. From its mature leaves 83 endophytic fungi were isolated. Based on molecular taxonomic identification, database searches (AntiMarin and SciFinder®) and the observed activity of the extracts in different bioassays, 8 fungi were selected for further investigation, including *Pestalotiopsis mangiferae*.

The ethyl acetate extract, from a small-scale culture of *P. mangiferae*, showed activity against *Listeria monocytogenes* (an inhibition of 29 mm by the agar diffusion method). Therefore, a large-scale cultivation, on malt extract agar (MEA; BD Difco) was conducted. From the new extract were isolated ten known compounds and a new polyhydroxylated macrolide, named mangiferaelactone (**1**).[5] The structure of the ten known compounds [(6*S*,1′*S*)-LL-P880α,[6] (6*S*,1′*S*,2′*R*)-LL-P880β,[7] (1′*S*,2′*R*)-LL-880γ,[7] (1′*R*)-dehydropestalotin,[8] (−)-5-carboxylmellein,[9] (−)-5-methylmellein,[9] (−)-5-hydroxylmethylmellein,[9] arabenoic acid,[10] 5,6-dihydro-4-methoxy-2*H*-pyran-2-one,[10] and the (−)-2-hexylidene-3-methylsuccinic acid[11]] was determined by comparison of their ^{1}H NMR, ^{13}C NMR, optical rotation, and MS data with the literature.

Mangiferaelactone is a new stereoisomer of the fungal compound Xyolide, isolated from *Xylaria feejeensis*. This compound was reported with biological activity against *Pythium ultimun*.[12] Its total synthesis was described in Reddy et al. 2013.[13] The *Pythium* spp. are considered as fungus-like organisms or pseudofungi.[14]

Polyhydroxylated macrolides have been isolated from several fungal sources, and have received special attention in natural product research owing to their interesting biological effects, including inhibition of cholesterol biosynthesis and microfilament

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formation; they also have showed phytotoxicity, and antibacterial activity.[15]

Compound **1** showed considerable activity against the bacteria *Listeria monocytogenes* and *Bacillus cereus*, and mild activity against *Enterococcus cloacae*, *Enterococcus faecalis* and *Proteus mirabilis*. The *L. monocytogenes* causes the infection listeriosis,[16] while *B. cereus* is responsible for a minority of foodborne illnesses (2–5%), causing severe nausea, vomiting, and diarrhea.[17] We describe herein the isolation, structural characterization, and biological evaluation of mangiferaelactone (**1**). No significant activity was observed for the ten known compounds against the panel of bacteria and parasites used.

Results and discussion

Compound **1** was obtained as a yellowish-cream solid.[18] The molecular formula $C_{20}H_{32}O_8$ was determined by HRESIMS (*m*/*z* 399.2018 $[M-H]^-$). The IR spectrum showed absorption bands at 1639, 1722, and 3420 cm^{-1}, indicating the presence of a carboxylic acid carbonyl, an ester or lactone carbonyl group, and one or more hydroxyl groups, respectively. The ^{13}C NMR and HSQC spectra, in CD_3OD, showed 17 carbon signals (see Table 1): two carboxylic groups; one from a lactone and a carboxylic acid with the same resonance at δ_C 175.9 (C-1 and C-20) and one from carboxylic ester at δ_C 173.6 (C-17). Furthermore, the spectrum showed two olefinic carbons at δ_C 123.9 (C-5) and δ_C 136.1 (C-6); four oxymethine carbons at δ_C 78.3 (C-4), 73.6 (C-7), 74.7 (C-8), and 72.3 (C-9); 10 methylene carbons at δ_C 32.3 (C-2), 30.9 (C-3), 32.9 (C-10), 25.8 (C-11), 30.8 (C-12 and C-13), 33.2 (C-14), 23.9 (C-15), and 30.5 (C-18 and C-19); and one methyl carbon at δ_C 14.6 (C-16). The ^{13}C NMR spectrum of **1**, in $CDCl_3$, showed 20 carbon signals, allowing us to establish the corresponding signals for the lactone carbonyl (C-1) and the carboxylic acid carbonyl (C-20) at δ_C 174.6 and 176.6, respectively. The ^1H NMR spectrum of **1**, in CD_3OD, possessed signals for two olefinic protons (δ_H 5.92 and 5.49); four oxymethine protons at δ_H 5.17, 5.13, 4.40, and 3.53; four methylene groups at δ_H 2.32, 2.09, 1.98, 1.92, and 2.50–2.60; one methyl group at δ_H 0.89; and signals for a long aliphatic chain (δ_H 1.25–1.84), suggesting the presence of a seven carbon unit, which was confirmed by the ^{13}C NMR, ^1H NMR, and mass spectrum. The gross structure of **1** was further established by 2D NMR studies, particularly ^1H–^1H COSY and HMBC (Fig. 1). In the COSY spectrum, the olefinic protons at δ_H 5.49 (H-5) and 5.92 (H-6) were coupled with the oxymethine protons at δ_H 5.17 (H-4) and 4.40 (H-7), respectively. Furthermore, the following correlations were observed H-2 (a,b)/H-3 (a,b), H-3a/H-4, H-3b/H-4, H-4/H-5, H-5/H-6, H-7/H-8, and H-8/H-9; this contributed to the elucidation of the skeleton of **1** from C-2 to C-9. In addition, the HMBC correlations of δ_H 5.13 (H-9), 2.32 (H-2a), and 2.09 (H-2b) with the lactone carbonyl at δ_C 175.9 (C-1) established this compound as a 10-membered macrolactone, with one double bond (C-5/C-6) and four oxymethine groups (C-4, C-7, C-8, and C-9). The connection of the aliphatic chain was determined through the ^1H–^1H COSY signals between H-9/H-10b and the HMBC correlations of δ_H 5.13 (H-9) with the methylene carbon at δ_C 25.8 (C-11). The HMBC experiment showed a correlation between δ_H 2.50–2.60 (H-18 and H-19) with the ester carbonyl carbon at δ_C 173.6 (C-17) and the acid carbonyl carbon at δ_C 175.9 (C-20), suggesting the presence of a succinic acid moiety; its connection with the macrolactone was determined by the HMBC correlation of δ_H 5.17 (H-4) with the ester carbonyl carbon at δ_C 173.6 (C-17). The existence of the succinic acid moiety was confirmed by a high peak at *m*/*z* 283.23 $[M+H-C_4H_6O_4]^+$ present in the ESIMS (positive mode). Thus the elucidation of the planar structure of **1** was achieved.

The relative configuration of **1** (Fig. 2) was determined by an analysis of coupling constants in conjunction with the NOESY spectrum. The *trans* configuration of the double bond was established by the coupling constant (15.6 Hz) between the two olefinic protons, H-5 and H-6. Moreover, the large coupling constant $J_{H\text{-}4/H\text{-}5}$ (10.3 Hz) and $J_{H\text{-}8/H\text{-}9}$ (9.7 Hz) indicated anti orientation for H-4/H-5 and H-8/H-9, respectively. The short coupling constant $J_{H\text{-}6/H\text{-}7}$ (2.2 Hz) and $J_{H\text{-}7/H\text{-}8}$ (2.4 Hz) indicated the same orientation for H-6/H-7 and H-7/H-8, respectively. Finally, the NOSEY spectrum showed correlation peaks for H-4/H-6, H-6/H-7, H-6/H-8, and H-7/H-8; and a weak correlation peak for H-4/H-5. No-correlation peak for H-7/H-9 was observed; which confirmed the relative stereochemistry of the C-4–C-9 moiety.

Table 1
NMR spectroscopic data of mangiferaelactone (CD_3OD, ^1H, 500 MHz, ^{13}C, 125 MHz)

Position	Mangiferaelactone	
	δ_C	δ_H (mult, *J* in Hz)
1	175.9, qC	
2	32.3, CH_2	2.32 (ddd, 2.3, 6.1, 13.9)
		2.09 (dt, 2.0, 13.9)
3	30.9, CH_2	1.98 (m)
		1.92 (m)
4	78.3, CH	5.17 (dt, 4.9, 10.3)
5	123.9, CH	5.49 (ddd, 2.2, 10.3, 15.6)
6	136.1, CH	5.92 (dd, 2.2, 15.6)
7	73.6, CH	4.40 (br d)
8	74.7, CH	3.53 (dd, 2.4, 9.7)
9	72.3, CH	5.13 (dt, 2.6, 9.7)
10	32.9, CH_2	1.84 (m)
		1.48 (m)
11	25.8, CH_2	1.25–1.36 (m)
12	30.8, CH_2	1.25–1.36 (m)
13	30.8, CH_2	1.25–1.36 (m)
14	33.2, CH_2	1.25–1.36 (m)
15	23.9, CH_2	1.25–1.36 (m)
16	14.6, CH_3	0.89 (t, 7.0)
17	173.6, qC	
18	30.5, CH_2	2.50–2.60 (br s)
19	30.5, CH_2	2.50–2.60 (br s)
20	175.9, qC	

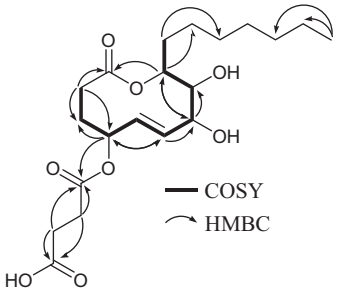

Figure 1. Selected ^1H–^1H COSY and HMBC correlations for mangiferaelactone (**1**).

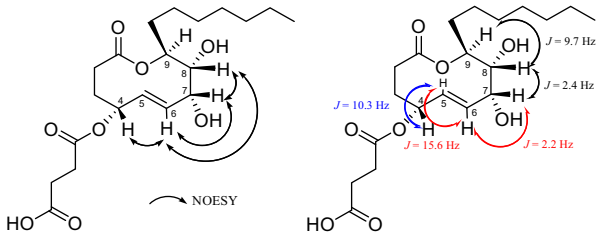

Figure 2. Selected NOESY correlations and key coupling constants between chiral centers for mangiferaelactone (**1**).