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Neolignans from *Callistemon lanceolatus*

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ABSTRACT

Two neolignans, named callislignan A and B together with known *C*-methyl-flavonoids, a lignan and pentacyclic triterpenoid esters were isolated from the leaves of *Callistemon lanceolatus*. Their structures were characterized by spectroscopic methods. Callislignan A and B had antibacterial activity against *Staphylococcus aureus* ATCC25923 and MRSA SK1 with callislignan B having an MIC of 8 μg/mL.

1. Introduction

Callistemon lanceolatus DC., also known as Crimson Bottlebrush, is a shrub in the family Myrtaceae. Preparations from it have been used in folk medicine for the treatment of coughs and bronchitis (Marzouk, 2008). Previous chemical investigations of compounds from this family have revealed the presence of various types of secondary metabolites, including acylphloroglucinols (Lounasmaa et al., 1977), *C*-methyl flavonoids (Huq and Misra, 1997), tannins (Hanaa and Mohamed, 2002) and triterpenoids (Varma and Parthasarathy, 1975). The flowers of this plant have been reported to contain tannins with antioxidant and hepatoprotective activities (Hanaa and Mohamed, 2002). The essential oil from the leaves has also exhibited significant antimicrobial activities (Silva et al., 2010). The search for bioactive metabolites and chemical constituents from natural sources has been an ongoing project in our laboratory. We therefore tested fractions obtained from a crude extract of the leaves of *C. lanceolatus* for antibacterial activity. Positive results prompted us to further evaluate the chemical constituents of the leaves of *C. lanceolatus*.

2. Result and discussion

Separation of a CH_2Cl_2 extract of the leaves of *C. lanceolatus* has resulted in the isolation of two new compounds, callislignan A and B (**1**, **2**) together with nine known compounds (Fig. 1). The known compounds were identified as 5,4′-dihydroxy-7-methoxy-6,8-dimethylflavanone (Wollenweber et al., 2000), nectandrin B (Le et al., 1980), 8-demethylsideroxylin (Cardona and Seoan, 1982), 5,4′-dihydroxy-7-methoxy-6-methylflavanone (Wollenweber et al., 2000), 5,4′-dihydroxy-7-methoxy-6,8-dimethylflavone (Hillis and Isoi, 1965), 5-hydroxy-7,4′-dimethoxy-6-methylflavone (Huq and Misra, 1997), 5-hydroxy-7,4′-dimethoxy-6,8-dimethylflavone (Huq and Misra, 1997), 3β-trans-feruloyloxy-2α-hydroxyurs-12-en-28-oic acid (Ito et al., 2001) and olean-12-en-28-oic acid (Siddiqui et al., 1997). This is the first time that all of the flavones except 5-hydroxy-7,4′-dimethoxy-6,8-dimethylflavone have been isolated from *C. lanceolatus*. All structures were determined from analyses of ^1H, ^{13}C NMR, COSY, HMQC and HMBC spectra.

Callislignan A (**1**) was isolated as a yellowish gum. Its molecular formula of $C_{19}H_{20}O_4$ was established from analysis of high resolution EI mass spectrometry (EI-MS) ($[M]^+$ m/z 312.1362). The IR spectrum showed an absorption band for a hydroxyl group at 3392 cm^{-1}. Inspection of ^1H NMR spectroscopic data in combination with COSY correlations indicated that **1** contained resonances assigned to two meta coupled aromatic protons (δ_H 6.67, H-4; δ_H 6.65, H-6), methine (δ_H 3.29, H-3) and oxy-methine protons (δ_H 4.97, *d*, H-2) that were *trans* coupled (9.6 Hz) (Li et al.,

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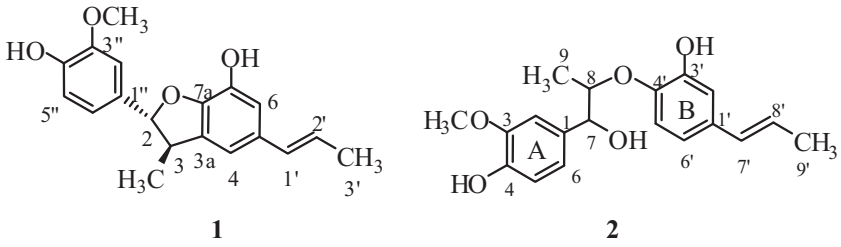

Fig. 1. Structure formula of callislignan A (**1**) and callislignan B (**2**).

1997), an aliphatic methyl doublet (δ_H 1.26, *d*, *J* = 6.6 Hz) that was vicinal to H-3, a (*E*)-propenyl group (δ_H 6.23, *dq*, H-1′; δ_H 5.94, *dq*, H-2′; δ_H 1.72, *dd*, H-3′), a 1,2,4-trisubstituted phenyl group (δ_H 6.76 *d*, *J* = 8.4, H-5″; δ_H 6.84, *dd*, *J* = 8.4, 1.8, H-6″ and δ_H 7.03, *d*, *J* = 1.8, H-2″), an aromatic methoxyl group (δ_H 3.77, 3″-OCH_3) and two phenolic protons (δ_H 7.85, 7-OH; δ_H 7.63, 4″-OH). The ^{13}C NMR spectrum contained seven sp^2 hybridised quaternary carbons, seven protonated sp^2 hybridised carbons and five additional protonated carbons that resonated upfield of 100 ppm. This data suggested that **1** contained a 2,3-dihydrobenzofuran skeleton (Nascimento and Lopes, 1999; Achenbach et al., 1987; Li et al., 1997). HMBC correlations from 7-OH to C-6 (δ_C 113.3), C-7 (δ_C 140.9) and C-7a (δ_C 146.8), from H-4 to C-3 (δ_C 45.5), C-6 (δ_C 113.3), C-7a (δ_C 146.8) and C-1′ (δ_C 131.2) and from H-6 to C-4 (δ_C 112.2), C-7 (δ_C 140.9), C-7a (δ_C 146.8) and C-1′ (δ_C 131.2) indicated that the hydroxyl group and the propenyl group were ortho and para to the heteroatom of the furan ring, respectively. The substituents on the furan ring were assigned to a methyl group at C-3 and a 3-methoxy-4-hydroxyphenyl group at C-2 since HMBC correlations were observed from 3-CH_3 to C-2 (δ_C 93.1) and C-3a (δ_C 133.5), while H-2 correlated to C-2″ (δ_C 110.0) and C-6″ (δ_C 119.5). The placement of the methoxyl group at C-3″ was indicated by the NOE enhancement of the H-2″ resonance upon irradiation of the methoxyl resonance (δ_H 3.77). This analysis in total suggested that **1** was 2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-7-hydroxy-3-methyl-5-(*E*)-propenylbenzofuran. The absolute configuration of the stereogenic centers, C-2 and C-3, in **1** were assigned as 2*R*, 3*R* from comparison of its optical rotation (+56.7) with that of the known compound (+)-licarin A, (2*R*, 3*R*)-2,3-dihydro-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methyl-5-(*E*)-propenylbenzofuran ($[\alpha]_D^{21}$ + 52; MeOH) (Achenbach et al., 1987). Callislignan A (**1**) was therefore the 7-demethyl derivative of (+)-licarin A.

Callislignan B (**2**) was isolated as a yellowish gum, $[\alpha]_D^{29}$ − 15.8 (c 4.55, MeOH). The molecular formula was determined as $C_{19}H_{22}O_5$ from analysis of high resolution EI-MS ($[M]^+$ *m*/*z* 330.1466). The IR spectrum exhibited a stretching band for a hydroxyl group at 3393 cm^{-1}. The ^1H NMR spectrum (Table 2) contained resonances that were assigned to two 1,2,4-trisubstituted phenyl groups (ring A: δ_H 6.81 (*d*, H-5), δ_H 6.86 (*dd*, H-6), δ_H 6.93 (*d*, H-2), and ring B: δ_H 6.90 (*d*, H-5′), δ_H 6.74 (*dd*, H-6′), δ_H 6.96 (*d*, H-2′). A doublet at δ_H 4.65, a quartet of doublets at δ_H 4.05 and a doublet at δ_H 1.09 were assigned to a benzylic oxymethine proton (H-7), an oxymethine proton (H-8) and a methyl proton (8-CH_3), respectively. Analysis of COSY correlations and coupling constants indicated that H-7 was vicinal to H-8 and their mutual coupling constant of 8.4 Hz suggested that these two protons possessed a *threo*-relative configuration (*lit.* *J* = 8.0 Hz, *threo*; *J* = 3.0, *erythro*) (Conserva et al., 1990). The presence of a (*E*)-propenyl group was assigned from signals at δ_H 6.30 (*dq*, H-7′), δ_H 6.86 (*dq*, H-8′) and δ_H 1.85 (*dd*, H-9′). Two $^3J_{CH}$ correlations from both H-2′ and H-6′ to C-4′ (δ_C 144.4) and C-7′ (δ_C 130.4) revealed that the propenyl side chain was attached to ring B and was para to C-4′. An HMBC correlation between H-8 and C-4′ (δ_C 144.4) and C-7 (δ_C 78.3) and between H-7 and C-2 (δ_C 113.2) and C-6 (δ_C 118.9) suggested that an ether bond between C-8 and C-4′ linked the two benzylpropanoid groups. A methoxyl group which resonated at δ_H 3.88 was attached at C-3 of ring A since HMBC correlations were observed between 3-OCH_3, H-2 and H-5 and C-3 (δ_C 146.8). Accordingly, **2** was identified as *threo*-1-(4-hydroxy-3-methoxyphenyl)-2-(2-hydroxy-4-(*E*)-propenylphenoxy)-1-propanol.

All of the pure compounds were evaluated for their antibacterial activity against two strains of *Staphylococcus aureus*. *S. aureus* ATTCC25923 is sensitive to many commercially available antibiotics whereas MRSA SK1 is resistant to methicillin and other beta-lactam antibiotics as well as other classes of antibiotics. Only compounds **1** (callislignan A) and **2** (callislignan B) showed antibacterial activity against both strains of *S. aureus* with MIC values of 200 (**1**, ATTCC25923), 64 (**1**, MRSA SK1) and 8 (**2**, ATTCC25923), 8 (**2**, MRSA SK1) μg/mL. Compound **2** exhibited the best anti-*S. aureus* activity (MIC 8μg/mL) as compared to vancomycin, a standard antibiotic (MIC 1 μg/mL). It is worth noting that callislignan B inhibited both *S. aureus* strains with the same MIC. This result indicated that callislignan B may act on different bacterial targets and this compound may be useful for treating MRSA infections.

3. Experimental

3.1. General experimental procedures

Melting points were recorded with a Fisher–Johns melting point apparatus and are uncorrected. The IR spectra were measured on a FTS 165 FT-IR Perkin-Elmer spectrophotometer. UV spectra were recorded on a SPECORD S100 spectrophotometer. ^1H and ^{13}C nuclear magnetic resonance spectra were recorded in $CDCl_3$ or acetone-d_6 using either a FT-NMR Bruker Avance 300 MHz or 500 MHz spectrometers. Optical rotations were recorded in $CHCl_3$ or MeOH solutions at the sodium D line (589 nm) on a JASCO P-1020 polarimeter. The EI-MS and HREIMS mass spectra were obtained using a MAT 95 XL mass spectrometer (Thermofinigan). Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60H (Merck) and silica gel 100 (Merck), respectively. Precoated plates of silica gel 60 GF254 were used for TLC analysis.

3.2. Plant material

The leaves of *C. lanceolatus* (Myrtaceae) were collected from Amphur Mueang Nakhon Si Thammarat, Nakhon Si Thammarat Province in October 2007. The plant was identified by Mr. Ponlawat Pattarakulpisutti and a herbarium specimen (S. Rattanaburi 1) has been deposited at the Herbarium within the Department of Biology, Faculty of Science, Prince of Songkla University, Thailand.

3.3. Extraction and isolation

Ground, dried leaves (4.5 kg) of *C. lanceolatus* were extracted with CH_2Cl_2 at room temperature for 3 days. The viscous extract (349.5 g), after removal of solvent, was sequentially dissolved in