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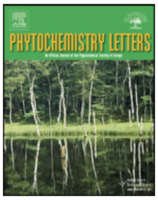

The first record of neolignans from the marine phanerogam *Posidonia oceanica*

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ABSTRACT

Chemical analysis of the secondary metabolites of *Posidonia oceanica* rhizomes led to the identification of several compounds. In particular, two neolignans, co-occurring with related metabolites previously described from the plant kingdom, have been isolated and characterised by spectroscopic methods. To the best of our knowledge, this is the first report of neolignans from a marine phanerogam.

1. Introduction

Posidonia oceanica is a seagrass species only present in the Mediterranean basin whose importance in the marine ecosystem is well ascertained (Dawes, 1998; Gobert et al., 2006; Gillanders, 2006; Ruiz et al., 2009). *P. oceanica* is an important primary producer in the coastal waters, providing shelter and food to marine fauna, and habitat for epiphytes. Most papers dealing with this phanerogam are focused on the impact of human activities on *Posidonia* population whereas chemical studies investigating the secondary metabolism are in limited number. Very recently, Zidorn has published a comprehensive review on secondary metabolites of *P. oceanica* describing lipids (as sterols and fatty acids), phenols as simple derivatives, phenyl-methane, -ethane and -propane compounds as well as phenylpropanoic acid esters, flavonols and chalcones (Heglmeier and Zidorn, 2010). He also stated the importance of further studies on the secondary metabolites from *P. oceanica* and other seagrasses with special regard to classes of compounds never detected.

As part of our ongoing investigation on marine phanerogams (Bitam et al., 2010) and since the chemical data reported in the literature so far involve the aerial part of *P. oceanica* (Agostini et al., 1998; Cuny et al., 1995), we have examined the lipophilic extract of rhizomes of this marine plant collected off the Bay of Naples. Previous studies (Viso et al., 1993) on the rhizomes revealed lipid components as fatty acids and alkanes in few amount with respect to the aerial part and in diverse distribution depending on the geographical sampling. Among phenolic compounds, *p*-hydroxybenzoic acid and vanillic acid are the only molecules reported from the rhizomes (Cariello et al., 1979).

Our chemical investigation has resulted in the identification of two new neolignans (compounds **1** and **2**) isolated along with known related neolignans, quiquesetinerviusin A (**3**) (Chang et al., 2010) and 4,9-dihydroxy-9-(*p*-hydroxybenzoyloxy)-3,5′-dimethoxy-4,7′-epoxy-8,5′-neolignan-7-en-9′-al (**4**) (Hashimoto et al., 1994), a polar sterol fraction (Kontiza et al., 2006) and 3-indole-aldehyde (Evidente and Surico, 1986). All known compounds were identified by comparing the spectroscopic data with those reported in the literature. The new neolignans **1** and **2**, structurally related to co-occurring quiquesetinerviusin A (**3**) and to quiquesetinerviusin C (**5**) (Chang et al., 2010), respectively, were characterised by an extensive NMR analysis (see Fig. 1).

2. Results and discussion

The $CHCl_3$ soluble portion of the Me_2CO extract of *P. oceanica* rhizomes was analysed by TLC chromatography ($CHCl_3$/MeOH in different ratio) revealing the presence mainly of triglycerides and sterols along with a series of UV absorbing spots at R*f* 0.4 ($CHCl_3$/MeOH, 9:1). The extract was then purified on a Sephadex LH-20 column ($CHCl_3$/MeOH, 1:1 as eluent) to obtain three main fractions I–III containing compounds evidenced by TLC analysis. The ^1H

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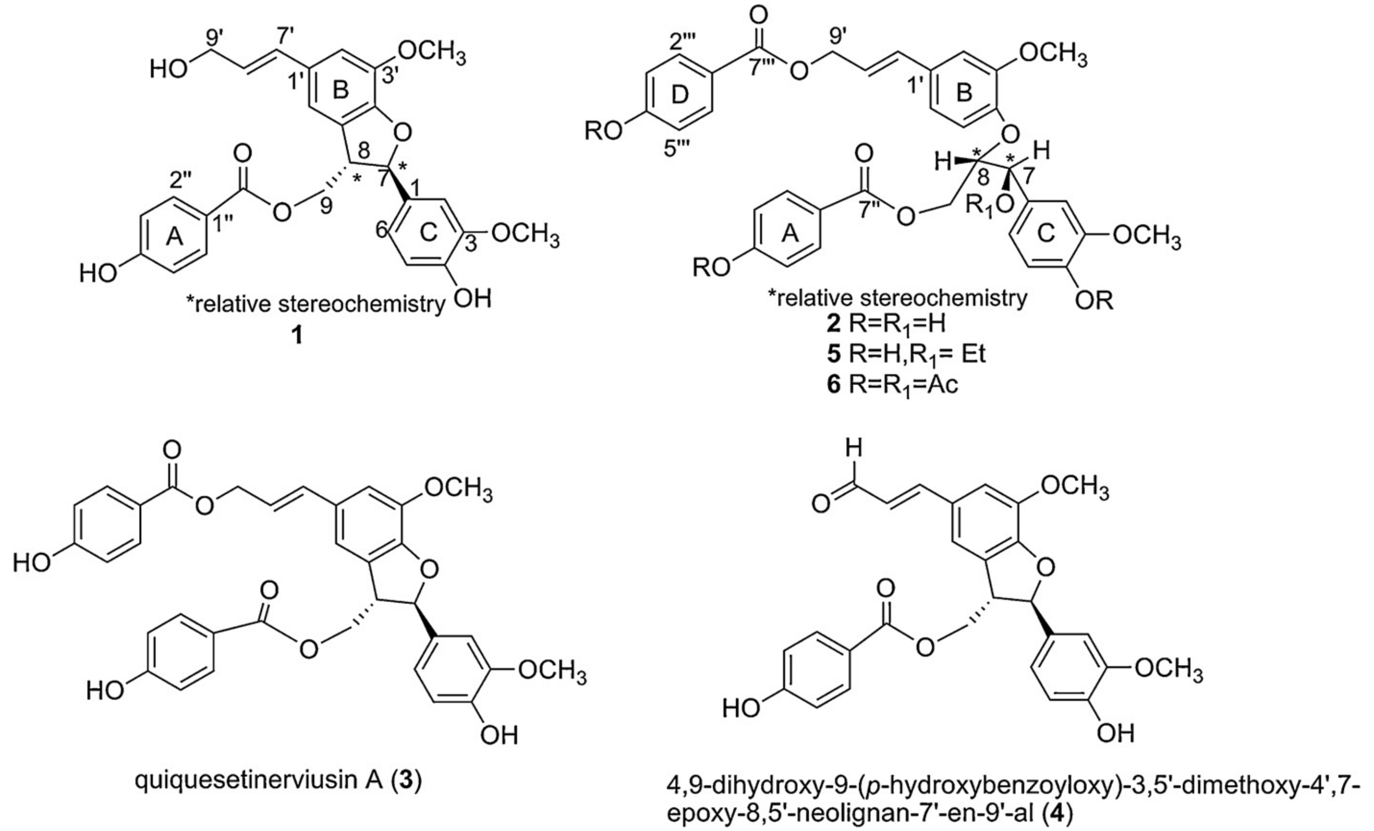

Fig. 1. Structures of new (**1**–**2**) and known neolignans (**3**–**4**) isolated from *Posidonia oceanica*.

NMR spectrum of fraction II contained a series of signals attributable to sterols and aromatic compounds. Thus, the fraction was subjected to reverse-phase HPLC (MeOH/H_2O gradient) purification to afford four neolignans, compounds **1**–**4**, and a mixture of polar sterols. This mixture were constituted by 3-keto-6-hydroxysterols identified as those previously reported from *Cymodocea nodosa* (Kontiza et al., 2006). Compounds **3** and **4** were identified as quiquesetinerviusin A (Chang et al., 2010) and 4,9-dihydroxy-9-(*p*-hydroxybenzoyloxy)-3,5′-dimethoxy-4,7′-epoxy-8,5′-neolignan-7-en-9′-al (Hashimoto et al., 1994), respectively, by comparison of their spectroscopic data with the literature.

Compound **1** showed a molecular formula $C_{27}H_{26}O_8$ as deduced from the sodiated ion peak in the HRESIMS at *m*/*z* 501.1521 implying 15 unsaturation degrees. ^1H NMR spectrum of **1** (recorded in MeOD) was almost reminiscent with that of known quiquesetinerviusin A (**3**) suggesting close structural analogies. However, due to the best resolution observed in the spectra recorded in $CDCl_3$ with 10% of MeOD, the NMR characterisation of compound **1** was conducted in this solvent. The ^1H NMR spectrum showed three sets of signals attributable to aromatic, olefinic and aliphatic protons. The multiplets at δ_H 6.66, (2H, d, J = 8.8 Hz, H-3″ and H-5″) and δ_H 7.64 (2H, d, J = 8.8 Hz, H-2″ and H-6″) were consistent with the presence of a *p*-hydroxybenzoyloxy unit (ring A) whereas two broad 1H singlets at δ_H 6.76 (H-2′) and δ_H 6.81 (H-6′) were assigned to the protons of a 1,3,4,5-tetrasubstituted benzene ring (ring B). The presence of an additional 1,3,4-trisubstituted benzene moiety (ring C) was suggested by the signals at δ 6.75 (1H, br s, H-2), δ_H 6.74 (1H, br d, J = 8.2, H-6) and δ_H 6.68 (1H, d, J = 8.2, H-5). Two olefinic signals observed at δ_H 6.06 (1H, dt, J = 15.7, 5.5 Hz, H-8′) and δ_H 6.40 (1H, d, J = 15.7 Hz, H-7′) were attributed to the protons of an *E*-double bond. In addition, the spectrum contained resonances due to oxygenated protons: a methine [δ_H 5.42 (d, J = 7.3 Hz, H-7)], two methylenes [δ_H 4.52 (1H, dd, J = 11.0, 5.2 Hz, H-9a), δ_H 4.34 (1H, dd, J = 11.0, 8.1 Hz, H-9b), and δ_H 4.10 (2H, br d, J = 5.5 Hz, H_2-9′)], and two –OMe groups [δ_H 3.78 (s, MeO-3′) and 3.64 (s, MeO-3)]. An overlapped signal at δ_H 3.78, which was attributed to the angular methine H-8, was also present in the spectrum (Table 1). Analysis of the ^1H–^1H COSY experiment showed mutual couplings among protons H-7, H-8, and H_2-9 as well as H-7′, H-8′, and H_2-9′ thus defining two C_3 sequences in the molecule. By comparing these data with those of co-occurring **3**, the structure of compound **1** was proposed to be tetracyclic, with two phenylpropanoid moieties forming a dihydrobenzo[*b*]furan neolignan further esterified with a *p*-hydroxybenzoic acid unit. Diagnostic correlations observed in the HMBC spectrum secured the connection of two C_6–C_3 moieties in the molecule and the location of the *p*-hydroxybenzoic acid residue at 9-OH. In fact, C-1′ (δ_C 131.8) showed cross-peaks with H-8′, whereas C-7′ showed correlations with H-2′ and H-6′ connecting the ring B to the propenyl portion C7′–C9′; on the other hand, both carbons C-4 and C-6 were correlated to the oxygenated methine H-7 linking the ring C to the propyl chain C7–C9. Diagnostic cross-peaks between C-5′ (δ_C 127.5) with both H-8 and H_2-9 aided us to link ring B to ring C through the C_3 chain. Finally, the carboxyl C-7″ (δ 166.7) showed HMBC correlations with H_2-9 along with the expected correlations with H-2″/H-6″. According to the literature, the relative configuration of the chiral centres C-7 and C-8 in compound **1** was suggested to be *trans* by coupling constant analysis (J_{H7-H8} = 7.3 Hz), the same as compound **3**. Thus, **1** was 9′-*O-de-p*-hydroxybenzoyl derivative of **3**. The small amount of pure isolated compound **1** prevented any further chemical study focused on the determination of the absolute configuration.

Compound **2** was isolated as an oil and showed the molecular formula $C_{34}H_{32}O_{11}Na$ as deduced by the sodiated ion peak at *m*/*z* 639.1853 in the HRESIMS spectrum. The ^1H NMR