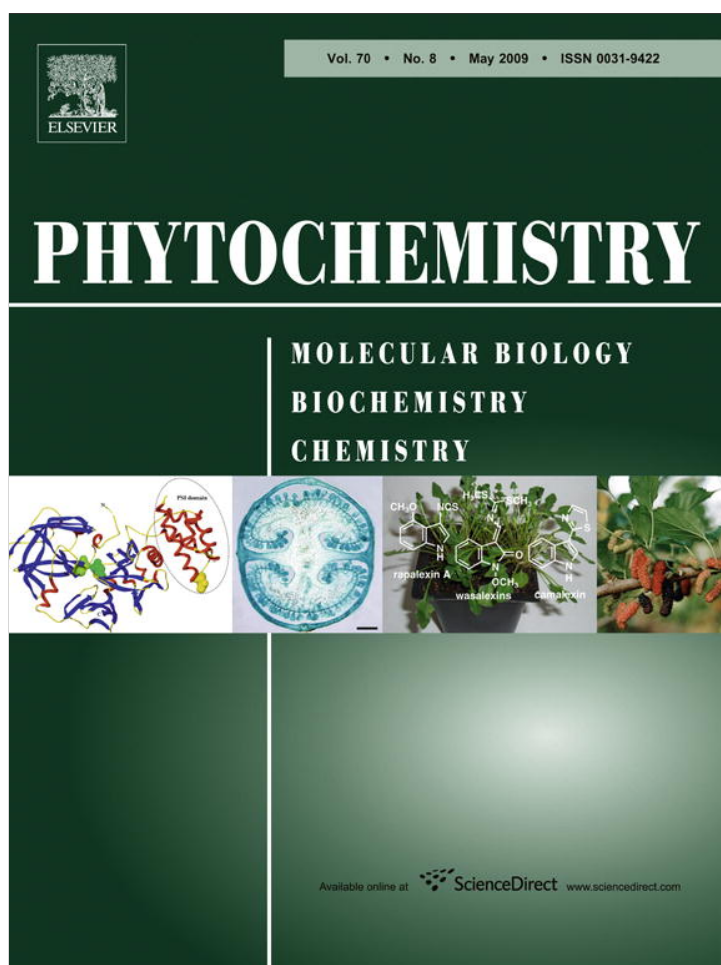




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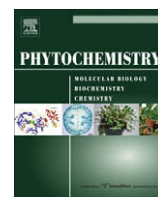
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Phytochemistry

journal homepage: www.elsevier.com/locate/phytochemGlycosides of arylnaphthalene lignans from *Acanthus mollis* having axial chiralityTomáš Řezanka^{a,*}, Pavel Řezanka^b, Karel Sigler^a^a Institute of Microbiology, Vídeňská 1083, Prague 142 20, Czech Republic^b Institute of Chemical Technology Prague, Dept. of Analytical Chemistry, Technická 5, Prague 166 28, Czech Republic

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ABSTRACT

Glycosides of arylnaphthalene lignans having axial chirality were isolated from *Acanthus mollis*. Owing to the axial chirality, their structure, including absolute configuration, was determined by means of extensive spectroscopic data such as UV, IR, MS, 1D and 2D NMR spectra, and computational chiroptical methods. A compound, 2',4-dihydroxyretrohelioxanthin (2'-hydroxy-justirumalin), has a structure containing two aromatic moieties with substituents hindering rotation about the biaryl axis. The aglycone was connected to a saccharide moiety linked at C-4 or C-2' and made up of one or four sugars (rhamnose or quinovose, and tetrasaccharide 4-O-β-D-xylopyranosyl-(1'''-6'')-O-[β-D-rhamnopyranosyl-(1'''-3'')]-O-β-D-apiofuranosyl-(1'''-2'')-O-β-D-glucopyranoside and quinovose). Two mono- and one tetraglycoside gave positive results in the sea urchin eggs test (*Paracentrotus lividus*) of cytotoxicity and in a crown gall tumor on potato disks test (*Agrobacterium tumefaciens*).

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1. Introduction

Acanthaceae is a large family comprising, according to Mabberley (1987), 4300 species in 346 genera. Members are found mostly in the tropics, but occur also in the temperate region, mainly in the Mediterranean. The Acanthaceae are mostly herbs or shrubs, including twining forms. The genus *Acanthus* as perennials grows wild in the grasslands, woods, scrub and rocky hills. *Acanthus mollis*, commonly known as Bear's Breeches, is an herbaceous perennial plant, native to the Mediterranean region from Portugal and northwest Africa to Balkan, and is one of the earliest cultivated species of garden plants. The leaves of this plant are generally considered by historians to have been the design inspiration for the Corinthian column capitals of Roman architecture.

Lignans are a group of naturally occurring products found in plant material that are characterized by the union of two phenylpropanoid (C₆–C₃) units with β–β' linkages. The phenylpropanoid dimer can be post-processed in the plant to varying degrees of oxidation. Further cyclization can also arise via the introduction of a C₆–C₇ or C₆–C₆ linkage. Accordingly, lignans can be divided into several subgroups based on their general structure. The more common subclasses of lignans are the aryltetralins and arylnaphthalenes. There are many common variations in the substitution pattern on the aryl rings that occur in nature, including methylene-dioxy, methoxy and hydroxy groups.

Thus, there is an incredible diversity in lignan structure that arises from the construction of the lignan skeleton, the substitu-

tions on the aryl rings and the various states of oxidation of the terminal groups. More than 2000 members of this class of natural products have been identified and there are several reviews available that catalog many of the known compounds (Ward, 1999; Umezawa, 2003).

Lignans are typical chemical constituents of the family Acanthaceae, especially those of the arylnaphthalene and arylnaphthalide types (Day et al., 2000; Liu et al., 2008). Lignans have important antineoplastic and antiviral properties (Gordaliza et al., 2000), and also exhibit many diverse biological activities, such as phosphodiesterase inhibition (Ukita et al., 1999), leukotriene biosynthesis inhibition (Therien et al., 1993), and antiviral activities (Yeo et al., 2005; Charlton, 1998). Further, arylnaphthalide lignans were shown to be cytotoxic towards several cancer cell lines (Day et al., 1999, 2002).

To the best of our knowledge, the species *A. mollis* has not been investigated previously regarding its natural lignan product content. Other groups of potentially interesting compounds found in this species include flavonoids (Reynaud et al., 1988), cyclic hydroxamic acids (Pratt et al., 1995), and derivatives of benzoxazolinone (Wolf et al., 1985). Lignan glucosides, all aryltetralin lactones, were identified only in the genus *Acanthus ilicifolius* (Kanchanapoom et al., 2001; Wu et al., 2004).

This report is part of our investigation of compounds having axial chirality (Řezanka and Dembitsky, 2006; Řezanka and Sigler, 2007; Řezanka et al., 2008) within the framework of a comprehensive program on the chemistry and biological activity of natural compounds. In this study, we report the isolation of new glycosidic lignans related to retrohelioxanthin (2'-hydroxy-justirumalin) moiety.

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2. Results and discussion

The ethanolic extract of the leaves of *A. mollis* was chromatographed on Sephadex LH-20. Compound **1**, i.e. 2',4-dihydroxyretrohelioxanthin (2'-hydroxy-justirumalin) was obtained as a pale-yellow microcrystals with the molecular formula $C_{20}H_{12}O_8$, as determined by positive-ion HR-ESI-MS (m/z 403.0435 ($[M+Na]^+$; calc. 403.0430)).

The UV maxima at 252 and 313 nm and bluish fluorescence under UV light of compound **1** similar to lignans described in the literature (Innocenti et al., 2002) indicated an aryl-naphthalene nucleus. It also exhibited an 18 nm bathochromic shift in the UV spectrum under alkaline conditions, indicating the presence of a phenolic hydroxyl (Rajasekhar et al., 1999). The IR spectrum contained bands associated with hydroxyl group (3390 cm^{-1}), an α,β -unsaturated γ -lactone (1764 cm^{-1}), an aromatic ring (1623 and 1507 cm^{-1}) and a methylenedioxy group (940 cm^{-1}), which were similar to those of justirumalin (Rajasekhar et al., 1999) and retrohelioxanthin (Charlton et al., 1996), indicating again an aryl-naphthalene nucleus (Weng et al., 2004).

The ^1H NMR data (Table 1) of **1** contained the signals of two singlet aromatic protons (δ 6.54 and 7.10 ppm), two doublet aromatic protons (7.45 and 7.97 ppm, $J = 8.9\text{ Hz}$), two methylenedioxy groups (δ 5.89 and 5.91 ppm, δ 6.02 and 6.04 ppm), and a γ -lactone methylene group (δ 5.54 ppm, 2H, broad s). The ^1H NMR data of **1** were similar to those of justirumalin except for the absence of an aromatic proton that led to the disappearance of an ABX system seen in its ^1H NMR spectrum. The above observation suggested that an oxygenated group might be substituted at the C2' of the 1-aryl group of **1**. Considering the elemental composition of **1**, this group was characterized as a hydroxyl.

^{13}C NMR spectral data (Table 1) of compound **1** contain five oxygenated aromatic carbons at δ 145.1, 142.8, 146.7, 143.1, corresponding to two methylenedioxy groups, δ 144.9 and 150.8 due to the phenolic hydroxyls.

In the HMBC correlations (Fig. 1), the correlations CH_2 (δ 5.89 and 5.91, respectively) to C-8 (145.1) and C-9 (142.8) confirmed the presence of an OCH_2O moiety. Further the correlations CH_2 (δ

6.02 and δ 6.04), respectively to C-4' (δ 146.7) and C-5' (δ 143.1) confirmed the presence of a second OCH_2O moiety (on ring C). The HMBC cross-peak CH_2 (H-12) δ 5.54 to C-11 (δ 169.6) confirmed the presence of a γ -lactone.

The information obtained from 2D NMR and ^{13}C NMR data, and a comparison with previously reported data for justirumalin, further supported the characterization of **1**.

Based on the above, compound **1** could be identified as 2',4-dihydroxyretrohelioxanthin, a new aryl-naphthalide lignan, named 2'-hydroxy-justirumalin **1**.

The CD spectrum of compound **1** (see Fig. 2) showed an optical activity. This is due to the rotational barrier of biaryl derivatives, which depends on the nature, position, and number of the substituents. The unsubstituted biphenyl has a rotational barrier of approximately 8.4 kJ/mol (2 kcal/mol). Increasing steric bulk of substituents in the *o*-positions causes increasing conformational stability as a result of steric interactions in the coplanar transition state. The majority of tetra-*o*-substituted biaryls have a rotational barrier sufficiently high to prevent racemization of the atropisomers at room temperature. The separation of stereoisomers at room temperature requires energy barriers of at least 92.1 kJ/mol (22 kcal/mol) (Leroux, 2004). For this reason, we computed (B3LYP functional and 6-31G** basis set, see below) the rotational barrier of our biaryl derivative (**1**) by computing the energy of conformers whose dihedral angle of aryls differed by 10 energy of 36 conformers was optimized with the appropriate dihedral angles of aryls. The rotational barrier was then calculated as the difference between the maximum and minimum of conformer energy and the result for (**1**) was calculated to be 141 kJ/mol. This clearly shows that at room temperature compound **1** cannot isomerize from *M* to *P* atropisomer and vice versa. This is in conformity with previously published results (Charlton et al., 1996; Wolf et al., 1997) for similar compounds.

Therefore, to elucidate the absolute configuration of **1** a quantum mechanical calculation (Diedrich and Grimme, 2003) of CD was carried out with Gaussian 03 software (Gaussian 03) using time-dependent density functional theory with B3LYP functional and 6-31G** basis set with 40 electronic transitions (Frisch et al.,

Table 1
 ^1H and ^{13}C NMR data (measured in perdeuterated DMSO) of (*R*)-2'-hydroxy-justirumalin (**1**) and its monoglycosides (**2**, **3**).

No.	1 ^1H NMR	2 ^1H NMR	3 ^1H NMR	1 ^{13}C NMR	2 ^{13}C NMR	3 ^{13}C NMR
1	–	–	–	129.6 (s)	129.6 (s)	129.5 (s)
2	–	–	–	121.7 (s)	121.7 (s)	121.6 (s)
3	–	–	–	119.9 (s)	126.0 (s)	120.0 (s)
4	–	–	–	144.9 (s)	146.2 (s)	145.0 (s)
5	–	–	–	120.8 (s)	126.1 (s)	120.7 (s)
6	7.97 (1H, d, $J = 8.9$)	8.01 (1H, d, $J = 8.9$)	7.96 (1H, d, $J = 8.9$)	116.5 (d)	116.9 (d)	116.6 (d)
7	7.45 (1H, d, $J = 8.9$)	7.50 (1H, d, $J = 8.9$)	7.45 (1H, d, $J = 8.9$)	112.0 (d)	112.2 (d)	112.1 (d)
8	–	–	–	145.1 (s)	145.0 (s)	144.9 (s)
9	–	–	–	142.8 (s)	142.7 (s)	142.8 (s)
10	–	–	–	123.8 (s)	123.9 (s)	123.7 (s)
11	–	–	–	169.6 (s)	169.5 (s)	169.4 (s)
12	5.54 (2H, brs)	5.61 (2H, brs)	5.55 (2H, brs)	66.8 (t)	69.1 (t)	66.7 (t)
8,9- OCH_2O	5.89 (1H, s); 5.91 (1H, s)	5.90 (1H, s); 5.92 (1H, s)	5.91 (1H, s); 5.93 (1H, s)	101.1 (t)	101.1 (t)	101.1 (t)
1'	–	–	–	113.6 (s)	113.5 (s)	117.0 (s)
2'	–	–	–	150.8 (s)	150.9 (s)	152.7 (s)
3'	6.54 (1H, s)	6.53 (1H, s)	6.62 (1H, s)	99.1 (d)	99.0 (d)	101.6 (d)
4'	–	–	–	146.7 (s)	146.9 (s)	147.4 (s)
5'	–	–	–	143.1 (s)	143.2 (s)	143.3 (s)
6'	7.10 (1H, s)	7.11 (1H, s)	7.17 (1H, s)	110.1 (d)	110.2 (d)	111.0 (d)
4',5'- OCH_2O	6.02 (1H, s); 6.04 (1H, s)	6.01 (1H, s); 6.04 (1H, s)	6.05 (1H, s); 6.08 (1H, s)	101.3 (t)	101.3 (t)	101.3 (t)
1''	–	5.01 (1H, d, $J = 1.9$)	4.84 (1H, d, $J = 8.3$)	–	104.1 (d)	106.6 (d)
2''	–	3.89 (1H, dd, $J = 1.9, 2.5$)	3.73 (1H, dd, $J = 8.3, 9.9$)	–	72.1 (d)	76.6 (d)
3''	–	3.71 (1H, dd, $J = 2.5, 9.5$)	3.56 (1H, dd, $J = 9.9, 9.7$)	–	71.3 (d)	77.0 (d)
4''	–	4.32 (1H, t, $J = 9.5$)	3.39 (1H, t, $J = 9.7$)	–	73.2 (d)	76.1 (d)
5''	–	4.13 (1H, dq, $J = 9.5, 6.5$)	3.48 (1H, dq, $J = 9.7, 6.6$)	–	69.7 (d)	74.6 (d)
6''	–	1.31 (3H, d, $J = 6.5$)	1.39 (3H, d, $J = 6.6$)	–	18.6 (q)	17.9 (q)

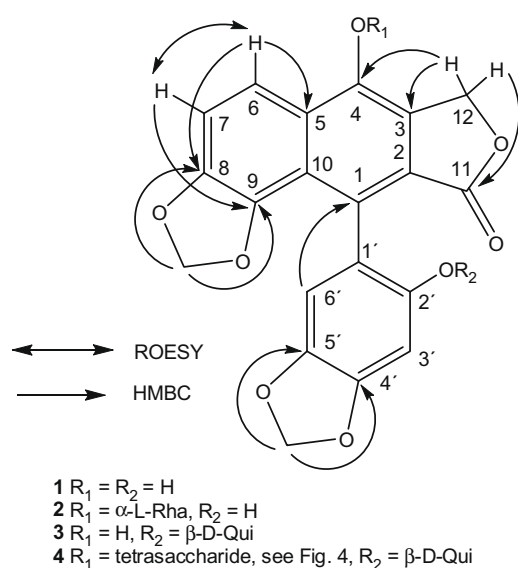


Fig. 1. Structures of new compounds (**1–4**), only important HMBC and ROESY correlations are shown.

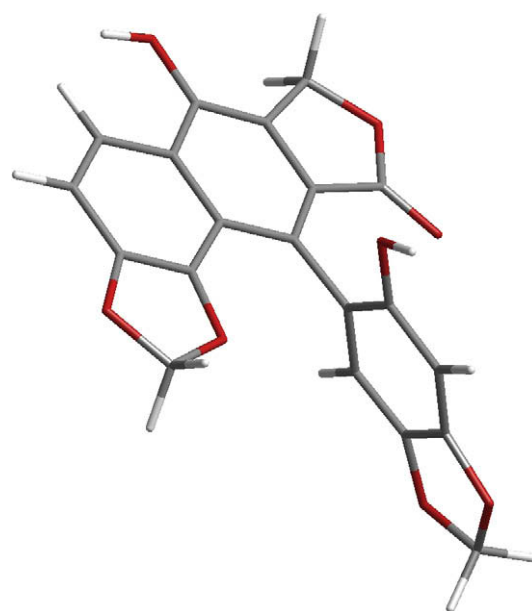


Fig. 3. Model of (*R*)-2'-hydroxy-justirumalin (**1**) shows that rotation about the 1,1' bond is hindered.

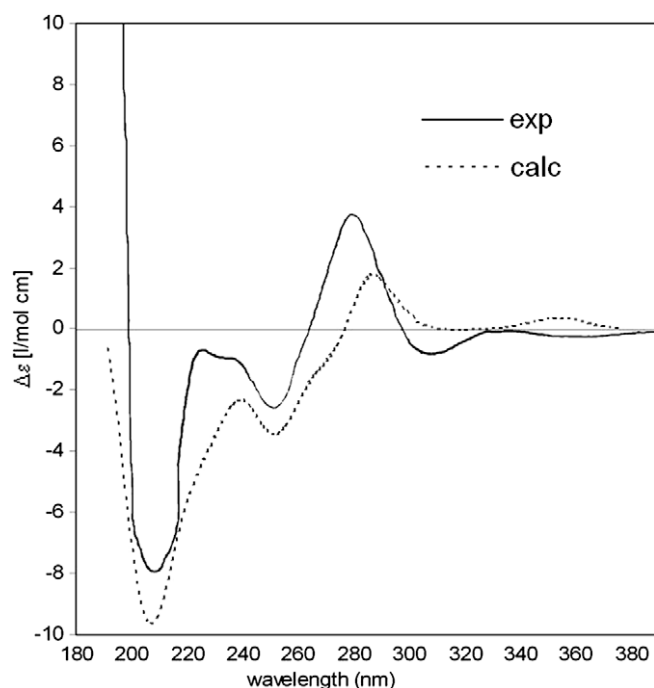


Fig. 2. B3LYP/6-31G**.-calculated CD spectrum for (*R*)-**1** and experimental CD spectrum of **1**.

2004). The quantum mechanical calculation has already been used for this type of compounds (Charlton et al., 1996; Wolf et al., 1997). The conformer used for CD calculation was the minimum-energy conformer. A comparison of theoretically predicted data with the corresponding experimental data has allowed us to elucidate the absolute configuration of **1**. Experimental (Fig. 2) and calculated spectra (Fig. 3) are in good agreement. Hence the absolute configuration of **1** is *M* (i.e. *R*).

The positive HRFABMS of compound **2** showed an $[M+Na]^+$ peak at m/z 549.1012 (calc. 549.1009) corresponding to the molecular formula $C_{26}H_{22}O_{12}Na$, deduced also by ^{13}C NMR and DEPT analyses. The negative FABMS spectrum of **2** showed a peak $[M-H]^-$ at m/z

525 that gave the fragment ions at m/z 379 $[M-H-146]^-$ and m/z 361 $[M-H-H_2O-146]^-$, which were formed by the loss of the deoxyhexose unit. The FABMS of compounds **2** and **3** were identical, which corresponds to the same molecular weight and compounds **2** and **3** are consequently isomers.

The NMR data of glycoside **2** (see Table 1) showed the presence of one *O*-glycosidic deoxyhexopyranose, i.e., one anomeric carbon resonance at δ 104.1 and one anomeric proton (δ 5.01). The key resonance is at δ 18.6, which represents C-6'' of a 6-deoxy sugar. This resonance was identified as a methyl group from the DEPT spectrum and the corresponding 1H NMR chemical shift (δ 1.31). It was used as a starting point in the homonuclear correlated spectra to determine all glycosidic protons. The $J_{H-1''-H-2''}$ value (1.9 Hz) of compound **2**, and the NOE correlation between H-1'' and H-2'', H-3'' and H-5'', H-4'' and H-5'', and H-5'' and H-6'', observed in the NOESY experiment, further confirmed that the deoxyhexose sugar was α -rhamnopyranose (Breitmaier and Voelter, 1987). The coupling constant ($^1J_{CH}$) between C-1'' and H-1'' in pyranose derivatives of carbohydrates is useful in the assignment of anomeric configuration since pyranoses with an axial H-1'' have a $^1J_{CH}$ value that is approximately 10 Hz lower than the corresponding value in compounds with an equatorial H-1 (Bock et al., 1973). On the basis of literature data, the anomeric configuration can be seen to be α because the $^1J_{CH}$ value in our glycoside was 170.9 Hz (Podlasek et al., 1995). Enzymatic hydrolysis of **2** with hesperidinase (EC 3.2.1.40), an enzyme specifically catalyzing the hydrolysis of α -L-rhamnose, yielded a sugar (3.5 mg) that had $[\alpha]_D^{25} +9.0$, compared to the literature data, $[\alpha]_D +9.1$ and/or $+8.9$, for L-rhamnose (6-deoxy-L-mannose) (Johnson and Liu, 1999). HMBC connectivities from C-4 and H-1'' indicated the position of attachment of the α -rhamnopyranose moiety.

The coupling constants of the sugar moiety for compound **3** show that the vicinal protons are diaxially oriented with coupling constants varying from 6.6 to 9.9 Hz. These data together with the shift of the anomeric proton at 4.84 ppm indicate that the sugar is a 6-D-deoxyglucose (D-quinovose). This was confirmed by acid hydrolysis of **3** leading to aglycone and a sugar identified with a standard by TLC and optical rotation as D-quinovose. Therefore, the structure of **3** was established as 2'-*O*- β -D-quinovopyranosylaglycone.

Table 2¹H and ¹³C NMR data (measured in perdeuterated DMSO) of compound **4**.

No.	4 ¹ H NMR	4 ¹³ C NMR
1	–	129.6 (s)
2	–	121.7 (s)
3	–	126.0 (s)
4	–	146.2 (s)
5	–	126.1 (s)
6	7.97 (1H, d, <i>J</i> = 8.9)	116.9 (d)
7	7.45 (1H, d, <i>J</i> = 8.9)	112.2 (d)
8	–	145.0 (s)
9	–	142.7 (s)
10	–	123.9 (s)
11	–	169.5 (s)
12	5.54 (2H, brs)	69.1 (t)
8,9–OCH ₂ O	5.89 (1H, s); 5.91 (1H, s)	101.1 (t)
1'	–	117.0 (s)
2'	–	152.7 (s)
3'	6.54 (1H, s)	101.6 (d)
4'	–	147.4 (s)
5'	–	143.3 (s)
6'	7.11 (1H, s)	111.0 (d)
4',5'–OCH ₂ O	6.02 (1H, s); 6.04 (1H, s)	101.3 (t)
Glc		
1''	4.41 (1H, d, <i>J</i> = 8.1)	104.0 (d)
2''	3.48 (1H, dd, <i>J</i> = 8.1, 9.2)	82.1 (d)
3''	3.44 (1H, dd, <i>J</i> = 9.2, 8.9)	84.5 (d)
4''	3.32 (1H, dd, 8.9, 9.5)	70.6 (d)
5''	3.38 (1H, ddd, <i>J</i> = 9.5, 4.6, 2.2)	75.3 (d)
6''	3.78 (1H, dd, <i>J</i> = 12.0, 4.6); 3.66 (1H, dd, <i>J</i> = 12.0, 2.2)	69.3 (t)
Api		
1'''	5.38 (1H, d, <i>J</i> = 2.3)	110.8 (d)
2'''	3.94 (1H, d, <i>J</i> = 2.3)	78.6 (d)
3'''	–	80.6 (s)
4'''	4.06 (1H, d, <i>J</i> = 9.5); 3.71 (1H, d, <i>J</i> = 9.5)	75.3 (t)
5'''	3.61 (2H, s)	66.0 (t)
Rha		
1''''	5.14 (1H, d, <i>J</i> = 1.4)	102.7 (d)
2''''	3.92 (1H, m)	72.3 (d)
3''''	3.38 (1H, m)	72.2 (d)
4''''	3.35 (1H, m)	73.9 (d)
5''''	3.95 (1H, m)	70.0 (d)
6''''	1.23 (3H, d, <i>J</i> = 6.0)	17.9 (q)
Xyl		
1'''''	4.63 (1H, d, <i>J</i> = 7.6)	105.1 (d)
2'''''	3.26 (1H, m)	74.7 (d)
3'''''	3.40 (1H, m)	77.5 (d)
4'''''	3.34 (1H, m)	71.4 (d)
5'''''	3.24 (1H, dd, <i>J</i> = 13.0, 3.2); 3.91 (1H, dd, <i>J</i> = 13.0, 10.5)	66.7 (t)
Qui		
1''''''	4.84 (1H, d, <i>J</i> = 8.3)	106.6 (d)
2''''''	3.73 (1H, dd, <i>J</i> = 8.3, 9.9)	76.6 (d)
3''''''	3.56 (1H, dd, <i>J</i> = 9.9, 9.7)	77.0 (d)
4''''''	3.39 (1H, t, <i>J</i> = 9.7)	76.1 (d)
5''''''	3.48 (1H, dq, <i>J</i> = 9.7, 6.6)	74.6 (d)
6''''''	1.39 (3H, d, <i>J</i> = 6.6)	17.9 (q)

spectrum of **4** in C₅D₅N shows five anomeric proton signals [δ 4.41 (*d*, *J* = 8.1 Hz; Glc), 4.63 (*d*, *J* = 7.6 Hz; Xyl), 4.84 (*d*, *J* = 8.3 Hz; Qui), 5.14 (*d*, *J* = 1.4 Hz; Rha), and 5.38 (*d*, *J* = 2.3 Hz; Api)] and five anomeric carbons 102.7 (*d*) Rha, 104.0 (*d*) Glc, 105.1 (*d*) Xyl, 106.6 (*d*) Qui, and 110.8 (*d*) Api, see Table 2. The chemical shift of the anomeric carbon of apiose (δ 110.8) indicated its furanose form (Lamidi et al., 2006).

The HMBC correlations between H-1'' of β -Glc (δ = 4.41) and C-4 of aglycone (δ = 146.2), H-1''' of β -Api (δ = 5.38) and C-2'' of β -Glc (δ = 82.1; glycosidation shift +8.7 ppm), H-1'''' of α -Rha (δ = 5.14) and C-3'' of β -Glc (δ = 84.5; glycosidation shift +8.0 ppm), H-1''''' of β -Xyl (δ 4.63) and C-6'' of β -Glc (δ = 69.3 glycosidation shift +8.3 ppm) are clearly apparent. Furthermore, the HMBC experiment displayed a long-range correlation from the anomeric proton at δ 4.84 (*d*, *J* = 8.3 Hz) of β -quinovose unit to C-2' (δ 152.7) of the aglycone. The linkage of each monosaccharide was also confirmed by NOESY spectral data. Accordingly, the structure of oligosaccharide was determined as shown in Fig. 4.

Compounds **2–4** showed significant inhibition of the growth of crown gall tumors on potato disks (Table 3). They also exhibited significant antiproliferative activity with *Paracentrotus lividus*. Their biological activities were determined as described previously (Řezanka et al., 2008). As has been proposed, different lignans, e.g. justicidin A–C, have antiviral activity against cultured rabbit lung cells (RL-33) (Asano et al., 1996), patentiflorin A or B and their derivatives showed cytotoxicity for the human carcinomas (Susplugas et al., 2005), cleistanone was tested against MT2 cell lines and showed an LD₅₀ value of 38.1 μ M (Ramesh et al., 2003). Phyllanthusmin A exhibited cytotoxicity against P-388 cells, with IC₅₀ value of 0.13 μ g/ml (Wu and Wu, 2006), while cleistanone showed LD₅₀ 38.1 μ M against LoVo cell line *in vitro* (Innocenti et al., 2002).

Compound **1** is the first aryl-naphthalene lignan having biaryl chirality, which was isolated from nature, since chiral aryl-naphtha-

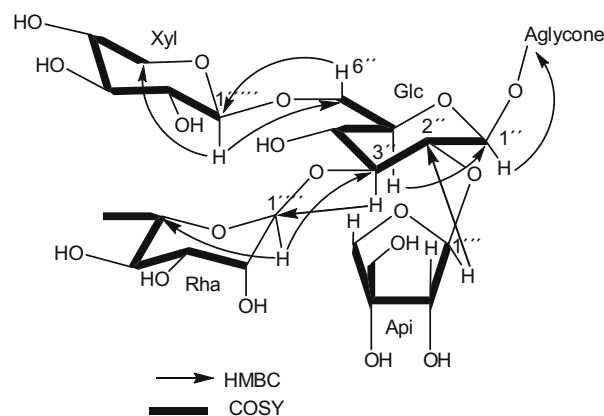


Fig. 4. The HMBC and COSY correlations of tetrasaccharide moiety of glycoside **4**, only important correlations are showed.

Table 3Tumor inhibition and sea urchin (*P. lividus*) eggs test of compounds **2–4**.

Compound	Crown gall tumor 10 ppm (inhibition in %) ^a	Crown gall tumor 100 ppm (inhibition in %) ^a	<i>Paracentrotus lividus</i> ^b
2	45 ± 7	99 ± 1	0.11 ± 0.03
3	32 ± 6	90 ± 6	0.41 ± 0.09
4	9 ± 3	54 ± 9	0.58 ± 0.15
DMSO	0	0	0

^a Value ± S.D., *n* = 10.^b IC₅₀ ± S.D. (μ g/ml), *n* = 10, the compounds were used dissolved in DMSO at 50 μ g/ml.

HR-FAB-MS showed the molecular formula of **4** to be C₄₈H₅₈O₂₉, which suggested that it has five saccharides. On acid hydrolysis, **4** afforded apiose, glucose, rhamnose, xylose, and quinovose, which were identified by TLC and comparison of the optical rotation with authentic samples, as the component sugars together with **1**. The negative FAB-MS spectrum shows pseudo-molecular ion peaks at *m/z* 1097 [M–H][–], together with fragment ion peaks, due to the cleavage of the monosaccharide moieties at *m/z* 965 [M–H–132; pentose] and *m/z* 951 [M–H–146; deoxyhexose].

These data indicate that **4** consists of an aglycone, a tetrasaccharide (4-*O*- β -D-xylopyranosyl-(16)-[α -L-rhamnopyranosyl-(13)]-*O*-[β -D-apiofuranosyl-(12)]- β -D-glucopyranose) and monosaccharide (β -D-quinovose). The sequence of these monosaccharides was determined using 1D and 2D NMR as follows: The ¹H NMR

lene lignans (only as aglycones) have so far been known only as synthesized compounds (Charlton et al., 1996).

3. Experimental

UV–vis spectra were measured in MeOH within the range of 220–550 nm in a Cary 118 (Varian) apparatus. A Perkin–Elmer (Perkin–Elmer, Norwalk, CT, USA) model 1310 IR spectrophotometer was used for scanning IR spectroscopy as neat films. Circular dichroism (CD) measurement was carried out under dry N₂ on a Jasco-500A spectropolarimeter at 24 °C. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (¹H) and 125.7 MHz (¹³C). High- and also low-resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HRFABMS (positive-ion mode) were obtained with a PEG-400 matrix. All following compounds were purchased from Sigma–Aldrich (Prague, Czech Republic).

Aerial parts of flowering *A. mollis* were collected in the Botanical Garden, Charles University, Prague in summer 2008. A voucher specimen is kept in our institute (voucher No. TR-AM-020708a). The leaves (3 kg, wet weight) were extracted with ethanol (triplicate) and the extracts were further chromatographed by means of Sephadex LH-20 columns with methanol, and then separated by RP–HPLC on a Discovery C18 column (Supelco) particle size 5 mm, length × I.D. (250 × 21.2 mm) using a linear gradient from 90% H₂O and 10% acetonitrile to 10% water and 90% acetonitrile over 50 min, with a flow rate of 9.0 ml/min and monitored by a variable wavelength detector at 254 nm, to give compound **1** (7.1 mg), compound **2** (2.8 mg), compound **3** (3.9 mg), and compound **4** (6.1 mg) as yellow powders.

A solution of glycoside **2** (2.4 mg) in acetate buffer (pH 4.4, 1 ml) was treated with hesperidinase (EC 3.2.1.40) for 48 h at 37 °C. The reaction solution was extracted by a mixture of CH₂Cl₂–MeOH (90:10) and was chromatographed on a column of silica gel (10 g), using CH₂Cl₂–MeOH–H₂O (90:10:1) to provide 1.2 mg (96.1%) of compound **1** for further analysis. The aqueous phase was lyophilized and the residue obtained after lyophilization was purified on a Sepharon SGX NH₂ column (7 μm, 3 × 150 mm) eluted with 90% MeCN (flow rate 0.7 ml/min) to yield 0.9 mg of L-rhamnose [α]_D²² +9.0 (equilib.).

A solution of glycoside **3** (3.5 mg) in acetate buffer (pH 5.0, 1 ml) was treated with β-D-glucosidase (EC 3.2.1.21) for 6 h at 37 °C. The reaction solution was extracted and after chromatography, see above, the aglycone **1** (1.2 mg) and β-D-quinovose (0.9 mg; [α]_D²² +28 (equilib.)) was identified.

Compound **4** (5.0 mg) was treated with a mixture of 1:1 M HCl and 1,4-dioxane (5 ml) at 100 °C for 3 h. The reaction mixture was neutralized by addition of Ag₂CO₃ and filtered. The filtrate was concentrated and the residue suspended in water (10 ml) was extracted with diethyl ether (10 ml, twice). Then the extract concentrated to dryness afforded the aglycone **1**. The aqueous layer containing monosaccharides was concentrated and subjected to TLC (silica gel impregnated with AcONa (0.2 M), EtOAc–i-PrOH–AcOH–H₂O (100:60:35:30) to afford D-glucose (*R*_f 0.18, [α]_D²⁵ +50°), D-quinovose (*R*_f 0.31, [α]_D²⁵ +28°), D-xylose (*R*_f 0.35, [α]_D²⁵ +19°), D-apiose (*R*_f 0.45, [α]_D²⁵ +7°), and L-rhamnose (*R*_f 0.52, [α]_D²⁵ +8°), the optical rotation was compared with authentic samples.

Compound (1): Pale-yellow microcrystals m.p. 251–253 °C; yield 7.1 mg (~0.00024%); [α]_D²³ 156 (c 0.01, MeOH); UV $\lambda_{\max}$ (MeOH, nm) (log ϵ): 252 (4.67), 313 (3.91) nm; IR (KBr, cm^{−1}): $\nu_{\max}$ 3390, 1764, 1623, 1507, and 940 cm^{−1}; CD (c, 0.3 dioxan): λ ($\Delta\epsilon$) 212 (−9.75), 240 (−2.34), 251 (−3.48), 281 (1.82), 310 (0), 338 (0.39), 360 (0.04) nm, see also Fig. 2; HRFABMS (*m/z*): 403.0435 [M+Na]⁺, calc. for [C₂₀H₁₂O₈Na]⁺ 403.0430; NMR data see Table 1.

Glycoside (2): Amorphous pale-yellow powder (2.8 mg). [α]_D²⁰ 165 (c 0.02, MeOH). HRFABMS (*m/z*): 549.1012 [M+Na]⁺, calc. for [C₂₆H₂₂O₁₂Na]⁺ 549.1009; negative FABMS *m/z* 525 [M–H][−], *m/z* 379 [M–H–146][−], *m/z* 361 [M–H–H₂O–146][−]; NMR data see Table 1.

Glycoside (3): Amorphous pale-yellow powder (3.9 mg). [α]_D²⁰ 164 (c 0.02, MeOH). HRFABMS (*m/z*): 549.1012 [M+Na]⁺, calc. for [C₂₆H₂₂O₁₂Na]⁺ 549.1009; negative FABMS [M–H][−] at *m/z* 525, *m/z* 379 [M–H–146][−], *m/z* 361 [M–H–H₂O–146][−]; NMR data see Table 1.

Glycoside (4): Amorphous pale-yellow powder (6.1 mg). [α]_D²⁰ 160 (c 0.02, MeOH); HRFABMS (*m/z*): 1121.2965 [M+Na]⁺, calc. for [C₄₈H₅₈O₂₉Na]⁺ 1121.2960; negative FABMS [M–H][−] at *m/z* 1097, *m/z* 965 [M–H–132][−], *m/z* 951 [M–H–146][−]; NMR data see Table 2. The signals in the ¹H and ¹³C spectra of aglycone were identical with values measured for glycosides **2** and/or **3**, see Table 1.

3.1. Computations

The calculated CD spectrum presented in Fig. 2 was scaled down by a factor of 0.95 to the wavelength scale. The spectral intensity remains unchanged. Gaussian band shapes and 15 nm half-width (at 1/e of peak height) were used to simulate the predicted spectrum.

3.2. Biological tests

The antiproliferative and cytotoxic effects of compounds **2–4** were evaluated by the sea urchin eggs test using eggs and sperm from the sea urchin (*P. lividus*) gonads. The crown gall tumor potato disk test (*Agrobacterium tumefaciens*) bioassay used to test the compounds for biological activity. Both tests were performed essentially according to our previously published paper (Rezanka et al., 2008).

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