Tetrahedron Letters 50 (2009) 1771–1776

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Proanthocyanidin glycosides from the leaves of *Quercus ilex* L. (Fagaceae)

Anastasia Karioti [a,b,*], Anna Rita Bilia [b], Chiara Gabbiani [c], Luigi Messori [c], Helen Skaltsa [a]

[a] Department of Pharmacognosy and Chemistry of Natural Products, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, 157 71 Athens, Greece
[b] Department of Pharmaceutical Sciences, University of Florence, via Ugo Schiff 6, Polo Scientifico, Sesto Fiorentino, 50019 Florence, Italy
[c] Laboratory of Metals in Medicine, Department of Chemistry, University of Florence, Via della Lastruccia 3, 50019 Sesto Fiorentino, Firenze, Italy

ARTICLE INFO

Article history:
Received 20 November 2008
Revised 20 January 2009
Accepted 28 January 2009
Available online 7 February 2009

Keywords:
Quercus ilex
Fagaceae
Oak-leaves
Proanthocyanidin glycosides
ROESY
Circular dichroism

ABSTRACT

From the polar extracts of the leaves of *Quercus ilex* L., two new proanthocyanidin glycosides, namely afzelechin-(4α→8)-catechin-3-*O*-β-glucopyranoside (**1**) and afzelechin-(4α→8)-catechin-3-*O*-α-rhamnopyranoside (**2**), were isolated in addition to catechin (**3**), proanthocyanidin B_3 (**4**), prodelphinidin C (**5**), dehydrodicatechin A (**6**), quercetin (**7**) and six known flavonol glucosides with their acylated derivatives (**8**–**13**) and ellagic acid (**14**). The structures of all isolated compounds were established by spectroscopic means, mainly 1D and 2D NMR, as well as LC/MS and HR-MS spectrometric analyses. The absolute configuration of compound **1** was determined by CD measurements. The proanthocyanidin glycosides are especially interesting, as they possess the sugar in the upper unit of the dimer, which is rare for this type of compounds.

Quercus ilex, also called holly oak or evergreen oak, is a common Mediterranean, medium-size, evergreen tree which is widely distributed along the Balkan Peninsula and the Mediterranean region to N. Spain and W. France.[1] Species of the genus *Quercus* have been for long used in traditional medicine as haemostatic, in the treatment of gastrointestinal disorders,[2] inflammations of the oral, genital and anal mucosa and externally against inflammation of the skin.[3] Polar extracts of the leaves, bark, wood and galls have shown antibacterial[4,5] and antiinflammatory activities[2,6] that explain their ethnopharmacological uses[7] and are attributed to their high phenolic content.

Due to the use of *Quercus* sp. in the construction of wine barrels and the interactions between wine and oak wood during the maturation of wine, these plants have been a subject of intensive research and have shown to possess a rich load of lignins, hydrolysable tannins, ellagitannins, flavano-ellagitannins, catechins, flavan and proanthocyanidin glycosides, flavonoids and simple phenols.[8–10] *Quercus* sp. have been used in the past in diverse ecological studies.[11–13] This work has been conducted in the framework of a project aiming at investigating possible qualitative and quantitative alterations in the phenolic content of healthy and mite-infected *Q. ilex* abaxial hairs. The lack of available standards of proanthocyanidins and acylated flavonol glucosides in the market made necessary the creation of such a database by extensive phytochemical investigations in the initial plant material so that reliable LC–DAD–MS analyses could be carried out. During the isolation process, 14 substances were isolated and characterised, among them two were naturally occurring proanthocyanidin glycosides. In this Letter, we report on the isolation and structure elucidation of the proanthocyanidin and flavonoid content of *Q. ilex* leaves.

For the initial extraction, which was all conducted in dark, a classical extraction scheme was applied that included organic solvents, such as cyclohexane and dichloromethane, and finally polar solvents like MeOH and MeOH/H_2O 70:30. This procedure was chosen in order to deactivate enzymes responsible for degradation, oxidation and polymerisation of the catechin/proanthocyanidin content.[14,15] Generally, the isolation protocol alternated between column chromatographies over Sephadex LH-20 and Sephadex LH-60 using hydroalcoholic mixtures or eluotropic mixtures of EtOH/MeOH/H_2O, which is a most suitable, gentle technique frequently applied for this type of secondary metabolites.[16,17] The isolation of these compounds is quite problematic and many authors[18,19] proceed to acetylation of the whole extracts and formation of the peracetates prior to chromatographic separation. In our case, the complexity of the mixtures due to the presence of rotamers of proanthocyanidins and cis and trans isomers of acylated flavonoid glycosides was evident not only in the TLC plates (tailing of spots) but also in the HPLC–DAD–MS analyses (double, triple, quadruple peaks; see Supplementary data), but the need to obtain the compounds in their natural form did not permit us for this approach.

Phytochemical investigations of the methanolic extract of the leaves of *Q. ilex* afforded two naturally occurring proanthocyanidin glycosides, namely afzelechin-(4α→8)-catechin-3-*O*-glucoside (**1**)

* Corresponding author. Tel.: +39 055457 3709; fax: +39 055457 3679.
E-mail address: anastasiakarioti@yahoo.it (A. Karioti).

0040-4039/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2009.01.158

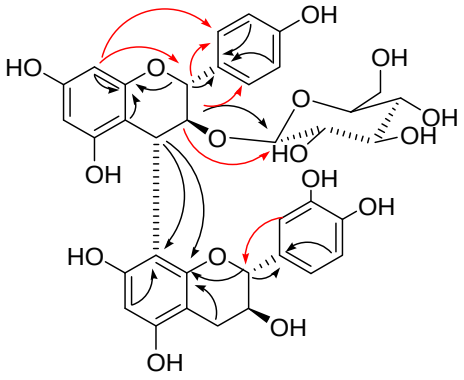
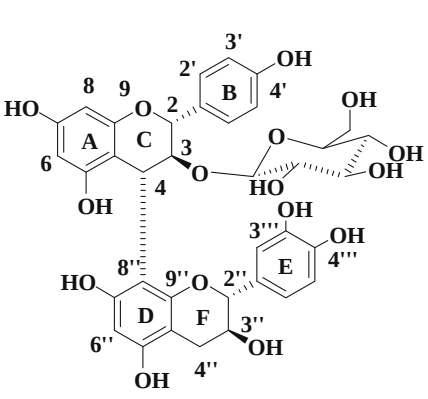
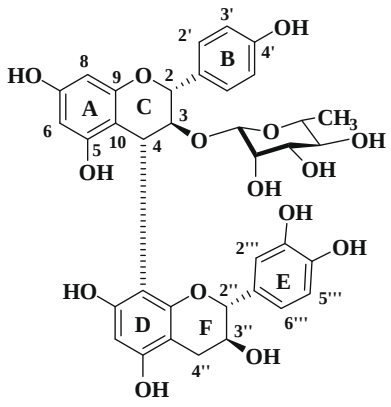


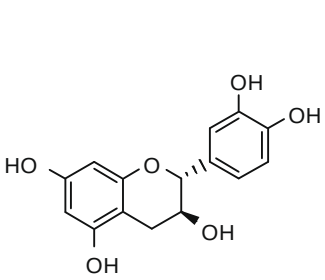
Figure 1. Selected HMBC and ROE correlations for compound **1**.

and afzelechin-(4α→8)-catechin-3-*O*-rhamnoside (**2**), catechin (**3**),[20] two known proanthocyanidins, proanthocyanidin B_3 (**4**)[19] and prodelphinidin C (**5**),[21] as well as small amounts of the oxidation product of catechin, dehydrodicatechin A (**6**).[22] The polar extracts were also abundant in flavonoids, in particular quercetin (**7**), two known flavonol glucosides, quercetin-3-*O*-glucopyranoside (**8**)[23] and isorhamnetin-3-*O*-glucopyranoside (**9**),[24] four flavonol acylated glucosides, namely kaempferol-3-*O*-(6′-galloyl)-glucopyranoside (**10**),[25] quercetin-3-*O*-(6′-galloyl)-glucopyranoside (**11**),[26] tiliroside (**12**),[27] kaempferol-3-*O*-(2″,6″-di-*E*-*p*-coumaroyl)-glucopyranoside (**13**)[28] and the phenolic ellagic acid (**14**). The known compounds **3**–**14** were identified by spectral analysis and direct comparison of their physical properties with those reported previously for these compounds.

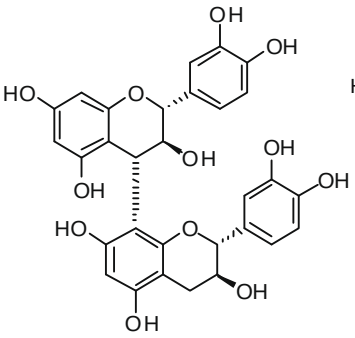
Compound **1** was obtained as amorphous yellow solid with $[\alpha]_D^{23}$ −26.9 (*c* 0.16, MeOH). The IR spectrum of **1** contained absorption bands characteristic of hydroxyl (3382 cm^{-1}), aliphatic groups (2920 cm^{-1}) and olephinic bonds (1630 cm^{-1}). The ESI mass spectrum (positive ion mode) of **1** showed molecular/pseudomolecular ion peaks at *m/z* 725.5 $[M+H]^+$, 747.5 $[M+Na]^+$, while in the negative ion mode exhibited a molecular peak at *m/z* 723.5 $[M-H]^-$ consistent with the molecular formula $C_{36}H_{36}O_{16}$. Its HR-ESI-MS spectrum exhibited pseudomolecular peak at *m/z* 747.1911 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_{16}Na$ 747.1890) and at *m/z* 725.2092 $[M+H]^+$, (calcd for $C_{36}H_{37}O_{16}$ 725.2070). It gave characteristic red colour after spraying with vanillin/sulfuric acid and positive blue with ferric ion reagent. Its UV spectrum (HPLC–DAD) presented a band with maximum at 278 nm. All the above data suggested that it belonged to the group of catechins/proanthocyanidins. Accordingly, the ^{1}H NMR spectrum (Table 1) showed signals characteristic of a flavan-3-ol skeleton (aromatic signals in the area of 6.0–7.0 ppm and aliphatic signals with large coupling constants in the region of 2.50–4.50), with catechin stereochemistry. Careful analysis of the ESI-MS spectrum provided us with more information about its structure. Positive ion mode MS spectra exhibited fragments at *m/z* 273.0 and 291.0, characteristic of the presence of afzelechin and catechin units, respectively, while a peak at 563.3 [M-163] suggested the loss of an hexose unit (possibly glucose) and gave evidence of the linkage between afzelechin and catechin. Furthermore, a peak at *m/z* = 435.4 indicated the linkage of the hexose to the afzelechin unit. Further 2D NMR experiments (COSY, HSQC, HMBC and ROESY) gave substantial evidence for these speculations. The ^{1}H NMR and COSY spectra of **1** exhibited signals of a *p*-disubstituted aromatic ring (AA′BB′ system with two doublets centred at δ_H 6.91 and δ_H 6.66, J_{ortho} = 8.4 Hz) which were attributed to the B ring of afzelechin. In the same area of the ^{1}H NMR spectrum, three aromatic protons resonating at δ_H 6.76 (d, *J* = 8.4), 6.69 (d, *J* = 1.8) and 6.45 (dd, *J* = 8.4, 1.8) forming thus an ABX system were observed and were assigned as H-5″, H-2″, H-6″, respectively, of the catechin moiety. The COSY permitted assignments of the aliphatic protons of rings C and F of the two flavan moieties and displayed the following connectivities: H-2″ (δ_H 4.49)/H-3″ (δ_H 3.76)/H-4a″ (δ_H 2.86), H-4b″ (δ_H 2.51) and H-2 (δ_H 4.38)/H-3 (δ_H 4.54)/H-4 (δ_H 4.48). The latter signals belonged to the upper unit of the dimer, as shown by the simplification in the correlation pattern due to the presence of one proton at C-4 instead of two. In the HSQC spectrum, the downfield shift of C-3 at δ 81.5 indicated that this carbon was the glycosylation site. This finding was further supported in both HMBC (crosspeak H-2/C-1 of glucose) and ROESY (H-3/H-1 of glucose) experiments. The HMBC spectrum was crucial for the identification of the afzelechin and catechin units. Common crosspeaks between the *meta* protons H-6 and H-8 (ring A) and the aliphatic protons H-4 & H-2 (ring C) with carbons C-10 and C-9, respectively, proved that these signals belonged to the same flavan nucleus, while crosspeaks between H-2, H-3 (ring C) and H-2′, 6′ (ring B) with C-1′ helped in the complete assignment of afzelechin (Fig. 1). Therefore, afzelechin was the upper part of the proanthocyanidin skeleton, bearing a sugar in position 3 and being linked to catechin at C-4. The interflavanoid bond was further proved in the HMBC spectrum by a crosspeak between H-4 of afzelechin and a quaternary aromatic carbon at δ_C 109.3 (C-8″ or C-6″) belonged to catechin. The interpretation of the HMBC spectrum was very problematic, since the key correlations[19] H-4/C-9″ and H-2″/C-9″ were observed but overlapping of protons H-4 and H-2″ rendered any assignment ambiguous. This problem was solved by re-measuring the spectra in acetone-d6. Indeed, protons H-4 and H-2″ were completely separated (at δ_H 4.39 and 4.53, respectively) and common crosspeaks between H-4, H-2″, H-4″ with C-9″ proved the 4→8 interflavanoid linkage.



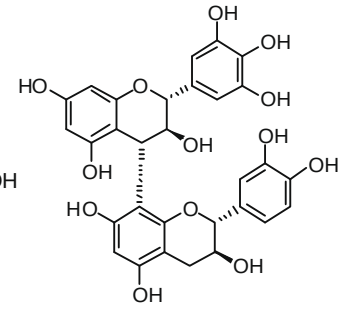




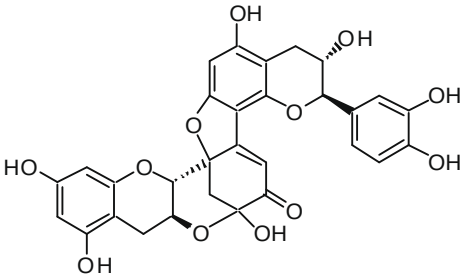
3



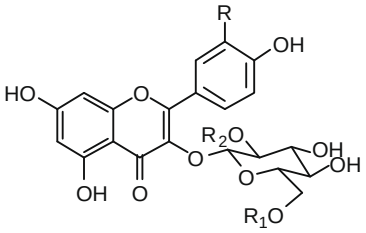
4



5



6

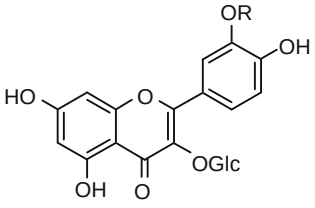


10 R = H, R_1 = A, R_2 = H

11 R = OH, R_1 = A, R_2 = H

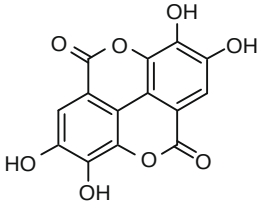
12 R = H, R_1 = B, R_2 = H

13 R = H, R_1 = B, R_2 = B

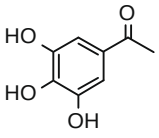


8 R = H

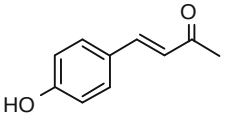
9 R = CH_3



14



A



B

At this point it is noteworthy to comment on the importance and necessity of ROESY experiments for this type of compounds. A literature survey showed confusion in the assignments of protons H-6 and H-8. This issue was thoroughly discussed by De Bruyne et al.,[19] who proved by detailed HMBC experiments the correct assignments for proanthocyanidin B_3, and showed that compared to usual flavonoids signals H-6 and H-8 are reversed, the latter being the one shielded. This was also observed in our case, but due to signal overlapping HMBC crosspeaks were unclear. A ROESY experiment, however, proved further this finding by clear interactions between H-8 and the aromatic protons H-2′, H-6′ of ring B. Motivated by this observation, we proceeded to the measurement of a series of ROESY experiments in the molecules of catechin, proanthocyanidin B_3 and prodelphinidin C, which were also isolated from the plant. In all cases, H-8/H-2′ and H-8/H-2 crosspeaks were observed. ROESY experiments present an alternative method to distinguish between H-6 and H-8 signals of ring A of proanthocyanidins in cases of signal confusion with the advantage of being easier and faster NMR experiments than the heteronuclear HMBC.

Coupling constants of the sugar protons (^{1}H NMR data in both methanol-d_4 and acetone-d_6–D_2O), as well as crosspeaks in the ROESY experiment between Glc-1/Glc-3/Glc-5 and Glc-2/Glc-4/Glc-6 confirmed the presence of β-glucose in the structure. In the same spectrum, lack of signals between protons H-2, H-3 and H-4 of the ring C suggested their trans disposition, while coupling constants ($J_{2,3}$ = 9.8 Hz; $J_{3,4}$ = 7.0 Hz) indicated a C-2 sofa conformation[16,29] (Fig. 2). Such conformation is expected when glycosylation follows the formation of the proanthocyanidin dimer. As a result of this conformation is the particular shielding of the glucose protons. A comparison of the chemical shifts of the glucose protons in **1** with those of other proanthocyanidin glucosides in the literature[30] makes evident a displacement of protons Glc-1 (3.36 vs 4.32),

Table 1
^{1}H and ^{13}C NMR spectroscopic data of compounds **1** and **2** (in CD_3OD; δ in ppm, J in Hz)

Position	1		2	
	δ_H	δ_C	δ_H	δ_C
Afzelehin unit				
2	4.38 (1H, d, 9.8)	82.2	4.26 (1H, d, 9.6)	83.3
3	4.54 (1H, dd, 9.8, 7.0)	81.5	4.54 (1H, dd, 9.5, 7.1)	80.3
4	4.48 (1H, d, 7.0)	37.4	4.41 (1H, d, 7.3)	37.2
5	–	157.1	–	158.6
6	5.89 (1H, d, 2.4)	96.9	5.87 (1H, br s)	96.9
7	–	157.3	–	158.3
8	5.82 (1H, d, 2.4)	96.8	5.86 (1H, br s)	97.5
9	–	158.5	–	157.2
10	–	107.2	–	107.0
1′	–	130.4	–	130.2
2′, 6′	6.91 (2H, d, 8.4)	130.5	6.82 (2H, d, 8.1)	130.3
3′, 5′	6.66 (2H, d, 8.4)	116.1	6.66 (2H, d, 8.1)	116.2
4′	–	158.3	–	158.3
Glucose unit			*Rhamnose unit*	
1	3.36[a]	103.4	3.48 (1H, br s)	101.3
2	2.86[a]	75.5	3.33[a]	71.9
3	2.92 (1H, t, 9.1)	77.4	3.49[a]	72.3
4	3.11 (1H, dd, 9.8, 9.1)	71.2	3.08 (1H, dd, 9.6, 9.1)	74.1
5	2.67 (1H, m)	77.3	3.13 (1H, m)	70.1
6a	3.57 (1H, dd, 11.2, 2.1)	62.8	0.71 (1H, d, 5.8)	18.1
6b	3.45 (1H, dd, 11.2, 4.2)		–	
Catechin unit				
2″	4.49 (1H, d, 7.7)	83.2	4.49 (1H, d, 7.8)	83.5
3″	3.76 (1H, ddd, 14.7, 7.7, 5.6)	69.4	3.75 (1H, m)	69.7
4″a	2.86[a]	29.9	2.95 (1H, dd, 16.2, 6.3)	31.0
4″b	2.51 (1H, dd, 16.8, 9.1)		2.48 (1H, dd, 16.4, 9.6)	
5″	–	155.7	–	155.7
6″	5.89 (1H, br s)	97.5	6.08 (1H, s)	96.9
7″	–	154.3	–	154.6
8″	–	109.3	–	109.0
9″	–	155.1	–	155.3
10″	–	102.8	–	103.9
1‴	–	131.8	–	131.7
2‴	6.69 (1H, d, 1.8)	116.2	6.69 (1H, d, 2.0)	116.4
3‴	–	146.0	–	145.9
4‴	–	146.2	–	146.3
5‴	6.76 (1H, d, 8.4)	116.3	6.78 d (1H, d, 8.3)	116.3
6‴	6.45 (1H, dd, 8.4, 1.8)	120.4	6.57 (1H, dd, 8.3, 2.0)	120.7

[a] Signal pattern unclear due to overlapping.

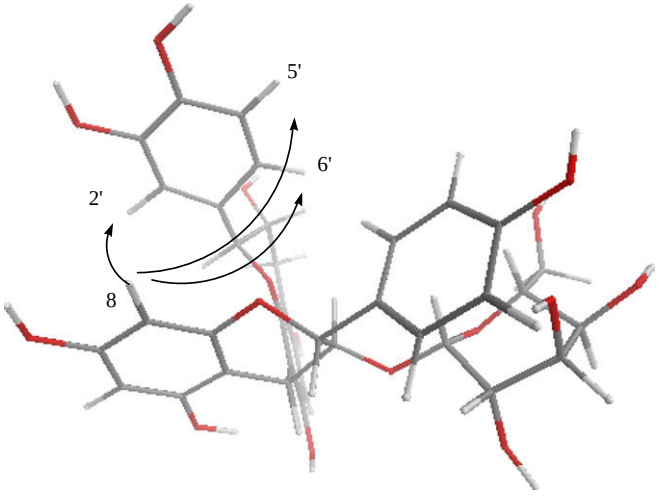


Figure 2. ChemDraw 3D model and key ROESY correlations of the rotameric form of compound **1**.

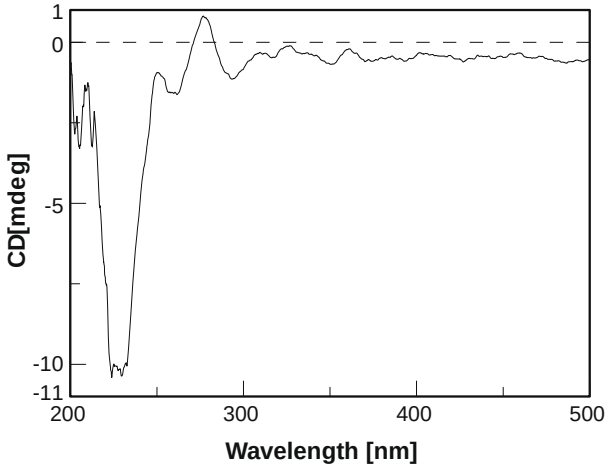


Figure 3. Circular dichroism (CD) spectrum (MeOH, 25 °C) of compound **1** (0.05 mg/ml).

Glc-2 (2.86 vs 4.92) and Glc-5 (2.67 vs δ 3.00–4.00), which is attributed to the magnetic anisotropy effect of ring B on the glucose group (Fig. 2). Interestingly, the anomeric proton gave strong interactions in the ROESY spectrum with both H-2′, 6′ and H-3′, 5′, while Glc-5 and Glc-2 of glucose gave weaker crosspeaks with H-3′, 5′ and H-2′, 6′, respectively.

Circular dichroism spectrum of compound **1**(Fig. 3) showed a very strong negative Cotton effect at a diagnostic[16,31,32] wavelength of 229 nm indicating the α orientation of the flavanyl substitutes, that is, a 4*S* configuration, at the C-4 (C ring) position. When taken into consideration the ^{1}H NMR coupling constants of the C ring protons, this Cotton effect establishes the 2*R*,3*S*,4*S* absolute stereochemistry in the upper subunit. However, for the lower subunit the sum of the CD data (positive Cotton effect +0.62 at 278 nm) could suggest the presence of ent-catechin.[33]

The presence of a sugar linked to the upper unit of the proanthocyanidin could also explain the fact that compound **1** shows only one rotational isomer. Opposite to other dimers (like proanthocyanidin B_3, prodelphinidin C) this compound did not exhibit any duplicate signals in its ^{1}H NMR spectrum. It seems that the concurrent presence of two bulky substituents at C-3 (glucose) and C-4 (catechin) forces them to assume spatial positions as far as possible, preventing rotation around the interflavanyl bond and enhancing thus one rotameric form in both methanol-d_4 and acetone-d_6–D_2O). Indeed, ROESY experiments revealed interactions between H-8 of the afzelechin with H-2″, H-5″ and H-6″ of the catechin. Therefore, compound **1** was identified as afzelechin-(4α→8)-catechin-3-*O*-β-glucopyranoside.

Compound **2** was obtained as yellowish powder with $[\alpha]_D^{23}$ −107.5 (*c* 0.35, MeOH). It was isolated from its mixture with **1** by repeated CC over Sephadex LH-20. Its spectral (UV and ^{1}H NMR) and chemical features were similar to those of compound **1**. Its ESI-MS spectrum exhibited pseudomolecular peaks at *m/z* 709.5 $[M+H]^+$ and 731.5 $[M+Na]^+$ in the positive mode which were compatible with the molecular formula $C_{36}H_{36}O_{15}$. Indeed, its HR-ESI-MS spectrum exhibited pseudomolecular peaks at *m/z* 709.2142 $[M+H]^+$ (calcd for $C_{36}H_{37}O_{15}$ 709.2121) and at *m/z* 731.1961 $[M+Na]^+$ (calcd for $C_{36}H_{36}O_{15}Na$ 731.1941). Its UV spectrum (HPLC-DAD) presented a band with maximum at 278 nm.

The ^{1}H NMR spectrum displayed signals belonging to two flavan-3-ol skeletons and a sugar moiety. Accordingly, the ESI-MS spectrum (positive ion mode) exhibited a fragment at *m/z* 275.0, characteristic of the presence of afzelechin, while a peak at 419.0 [M-290] suggested the loss of catechin and gave evidence of the linkage between afzelechin and a methylpentose. Detailed analysis of COSY, HSQC and HMBC spectra revealed the presence of an upper afzelechin unit, linked to a catechin unit at C-4 and glycosylated by a rhamnose at position 3. In particular, the spectra presented signals of an AA′BB′ aromatic system together with a pair of *meta* protons and three aliphatic protons (two of which oximethines) that corresponded to the afzelechin moiety. It also displayed signals belonging to an ABX system and signals typical of catechin relative stereochemistry.

The HSQC gave evidence of the presence of a rhamnose group: one anomeric carbon resonating at δ_H 101.3 (corresponding anomeric proton at δ_H 2.48, br s), four oximethines (resonating at δ_C 70.1, 71.9, 72.3 and 74.1) and one tertiary methyl group resonating as a doublet (J = 5.8 Hz) at δ 0.71 (18.1 for C-6). All rhamnose proton signals were shifted upfield indicating a similar substitution and conformation of the afzelechin unit, like in the previous case. A ROESY experiment played a pivotal role in the structure elucidation: crosspeaks between the anomeric proton (R-1) of rhamnose and H-2, H-3 and H-4 of afzelechin proved the linkage of the sugar at position 3, while interactions between H-6/H-8 with H-2‴ and H-6‴ suggested a 'compact'[34] rotameric form. Like in compound **1**, rhamnose is oriented below (in parallel) the aromatic ring B of afzelechin, which permits interactions between the protons of both units observed in the ROESY spectrum. Protons R-1 and R-2 interacted with H-5′ and H-6′ of afzelechin and R-3 and R-5, with H-2, H-3, H-4 and R-6 with H-3. The CD spectrum of compound **2** was similar to that of **1**. It presented a very strong negative Cotton effect at 232 nm which suggested a 4α orientation of the lower flavanyl moiety. Therefore, compound **2** was identified as afzelechin-(4α→8)-catechin-3-*O*-α-rhamnopyranoside.

An interesting feature of both substances is that they bare the sugar group in the upper unit of the dimer. A literature survey indicates that in the majority of procyanidin glycosides sugars are linked to the outer flavan rings.[35] Phytochemical investigations on the polar extracts of the plant, as well as HPLC–DAD–MS analyses revealed that catechin, proanthocyanidin B_3, glucosides of quercetin and isorhamnetin and acylated kaempferol glucosides are the main secondary metabolites in the leaves of *Q. ilex* as far the flavonoid content of the plant is concerned. LC–DAD–MS analyses also revealed the previously isolated kaempferol-3-*O*-(3″,4″-diacetyl-2″,6″-di-*E*-*p*-coumaroyl),[36] as well as a variety of hydrolysable tannins. As the focus of the present study was mainly on the proanthocyanidins and the flavonoids, fractions that contained hydrolysable tannins were not purified further. However, phytochemical investigations are still ongoing.

Acknowledgements

This project is co-financed within Op. Education by the ESF (European Social Fund) and National Resources. The authors are grateful to Professor G. Karabourniotis (Agricultural University of Athens) for the identification of the plant material and to Dr. Guido Mastrobuoni (Mass Spectrometry Center, University of Florence—CISM) for recording the HRESI mass spectra. The authors would also like to thank the diploma-student Eleni Angistrioti who during her diploma thesis re-isolated the compounds **3**, **4**, **5** and impure **1**, in the amounts of 14.3, 3.6, 4.3 and 5.0 mg, respectively.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.01.158.

References and notes

1. Schwarz, O., 2nd ed.. In *Flora Europaea*; Tutin, T. G., Burges, N. A., Chater, A. O., Edmonson, J. R., Heywood, V. H., Moore, D. M., Valentine, D. H., Walters, S. M., Webb, D. A., Eds.; Cambridge University Press, 1991; Vol. 1, p 74.
2. Kaur, G.; Hamid, H.; Ali, A.; Alam, M. S.; Mohammad, A. *J. Ethnopharmacol.* **2004**, *90*, 285–292.
3. König, M.; Scholz, E.; Hartmann, R.; Lehmann, W.; Rimpler, H. *J. Nat. Prod.* **1994**, *57*, 1411–1415.
4. Ito, H.; Yamagushi, K.; Kim, T. H.; Khennouf, S.; Gharzouli, K.; Yoshida, T. *J. Nat. Prod.* **2002**, *65*, 339–345.
5. Berahou, A.; Auhmani, A.; Fdil, N.; Benharref, A.; Jana, M.; Gadhi, C. A. *J. Ethnopharmacol.* **2007**, *112*, 426–429.
6. Hamid, H.; Kaur, G.; Abdullah, S. T.; Ali, M.; Athar, M.; Alam, M. S. *Pharm. Biol.* **2005**, *43*, 317–323.
7. Wellmann, M. Pedanii Dioscuridis. De Materia Medica. I, 107, 1.2. Berlin-Nieukölln, 1958.
8. Nonaka, G. I.; Nishimura, H.; Nishioka, I. *J. Chem. Soc., Perkin Trans. 1* **1985**, 163–172.
9. Conde, E.; Cadahía, E.; García-Vallejo, M. C.; De Simón, B. F.; Adrados, J. R. G. *J. Agric. Food Chem.* **1997**, *45*, 2695–2700.
10. Cantos, E.; Espín, J. C.; López-Bote, C.; De la Hoz, L.; Ordóñez, J. A.; Tomás-Barberán, F. *J. Agric. Food Chem.* **2003**, *51*, 6248–6255.
11. Feeny, P. P. *Ecology* **1970**, *51*, 565–581.
12. Faeth, S. H. *Oecologia* **1985**, *68*, 34–40.
13. Tikkanen, O. P.; Julkunen-Tiitto, R. *Oecologia* **2003**, *136*, 244–251.
14. Niehaus, J.; Gross, G. *Phytochemistry* **1997**, *45*, 1555–1560.
15. López-Serrano, M.; Ros Barceló, A. *J. Chromatogr., A* **2001**, *919*, 267–273.
16. Bae, Y. S.; Burger, J. F. W.; Steynberg, J. P.; Ferreira, D.; Hemingway, R. W. *Phytochemistry* **1994**, *35*, 473–478.
17. Lou, H.; Yamazakia, Y.; Sasaki, T.; Uchida, M.; Tanaka, H.; Oka, S. *Phytochemistry* **1999**, *51*, 297–308.
18. Hartisch, C.; Kolodziej, H. *Phytochemistry* **1996**, *42*, 191–198.
19. De Bruyne, T.; Pieters, L. A. C.; Dommisse, R. A.; Kolodziej, H.; Wray, V.; Domke, T.; Vlietinck, A. J. *Phytochemistry* **1996**, *43*, 265–272.
20. Foo, L. Y.; Lu, Y.; McNabb, W. C.; Waghorn, G.; Ulyatt, M. J. *Phytochemistry* **1997**, *45*, 1689–1696.
21. Tanaka, T.; Inoue, K.; Isao, K. *Nat. Prod. Res.* **2005**, *19*, 731–737.

22. Guyot, S.; Vercauteren, J.; Cheynier, V. *Phytochemistry* **1996**, *42*, 1279–1288.
23. Pakudina, Z. P.; Leont'ev, V. B.; Kamaev, F. G.; Sadykov, A. S. *Khimiya Prirodnykh Soedinenii* **1970**, *6*, 555–559. CHEMABS 74: 31932.
24. Rahman, W.; Ilyas, M. *J. Org. Chem.* **1962**, *27*, 153–155.
25. Romussi, G.; Sancassan, F. *Arch. Pharm.* **1983**, *316*, 648–649.
26. Braca, A.; Politi, M.; Sanogo, R.; Sanou, H.; Morelli, I.; Pizza, C.; De Tommasi, N. *J. Agric. Food Chem.* **2003**, *51*, 6689–6695.
27. Higuchi, R.; Donnelly, D. M. X. *Phytochemistry* **1978**, *17*, 787–791.
28. Yamashita, N.; Etoh, H.; Sakata, K.; Yagi, A.; Ina, H.; Ina, K. *Agric. Biol. Chem.* **1989**, *53*, 1383–1385.
29. Vdovin, A. D.; Kuliev, Z. A.; Abdullaev, N. D. *Chem. Nat. Comp.* **1997**, *33*, 11–24.
30. Zhang, B.; Nonaka, G. I.; Nishioka, I. *Phytochemistry* **1988**, *27*, 3277–3280.
31. Barrett, M. W.; Klyne, W.; Scopes, M. P. *J. Chem Soc., Perkin Trans. 1* **1979**, 2375–2377.
32. Lokvam, J.; Coley, P. D.; Kursar, T. A. *Phytochemistry* **2004**, *65*, 351–358.
33. Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177–2215.
34. Tarascou, I.; Barathieu, K.; Simon, C.; Ducasse, M. A.; André, Y.; Fouquet, E.; Dufourc, E. J.; De Freitas, V.; Laguerre, M.; Pianet, I. *Magn. Reson. Chem.* **2006**, *44*, 868–880.
35. Kolodziej, H.; Bonfeld, M.; Burger, J. F. W.; Brandt, E. V.; Ferreira, D. *Phytochemistry* **1991**, *30*, 1255–1258.
36. Romussi, G.; Parodi, B.; Caviglioli, G. *Pharmazie* **1991**, *46*, 679.