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# Plant Polyphenols: Extraction, Structural Characterization, Hemisynthesis and Antioxidant Properties

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## 1. Introduction

Many epidemiological studies showed that the consumption of diets consisting of fruits and vegetables offered protection against some chronic disease (Arts & Hollman, 2005; Chang-Claude et al., 1992; Graf et al., 2005; Phillips et al., 1978). This protection was suggested to be in part due to the intake of some beneficial nutrients including polyphenols which are the most abundant antioxidants in our diets. Indeed, polyphenols are natural products which are recognized as one of the largest and most widespread class of plant constituents occurring throughout the plant kingdom, and are also found in substantial levels in commonly consumed fruits, vegetables and beverages.

Polyphenols have recently aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others. Most of the experimental results confirmed that polyphenols have several biological activities including radical scavenging, anti-inflammatory, anti-mutagenic, anti-cancer, anti-HIV, anti-allergic, anti-platelet and antioxidant activities (Harborne & Williams, 2000).

Consumption of fruits and vegetables has thus been associated with protection against various diseases, including cancers (Steinmetz & Potter, 1996) and cardio-and cerebrovascular diseases (Rimm et al., 1996). This association is often attributed to the antioxidants in the fruits and vegetables such as vitamin C, vitamin E, carotenoids, lycopenes and polyphenols that prevent free radical damage (Steinberg, 1991). Fruits and vegetables consumption may also prevent stroke (Ness & Powles 1997), cancers (Yang & Wang 1993), coronary heart diseases (Tijburg et al. 1997), and osteoporosis (Adlercreutz & Mazur 1997). All these reasons make that polyphenols are receiving increasing interest from consumers and food manufacturers.

Chemically, polyphenols are diverse group of naturally occurring compounds containing multiple phenolic functionalities. They constitute a large and still expanding complex family of molecules, with diverse structures, properties and sizes ranging from monomers to polymers. Polyphenols gather a range of weakly acidic substances possessing aromatic rings

bearing hydroxyl substituents and constitute one of the most numerous and widely distributed groups of substances with more than 8000 phenolic structures currently known. Polyphenols can be divided into different classes depending on their basic structure. The main classes of polyphenols are phenolic acids and flavonoids while the less common are stilbenes and lignans. Major subclasses present in food correspond to anthocyanins, flavanols, flavones, flavanones, flavonols and isoflavones.

Several studies have been published about phenolic compounds and their role in the chemistry of fruits (Macheix et al., 1990). In particular, they are responsible for their color intensity, bitterness and their astringent perception. The amount and composition of phenolic compounds in fruit-based foods is dependent on a number of factors including species, varieties, geographical heritage, maturity, growing conditions, production area and yield of fruit as well as on technological processes. The age of fruit derived foods also influences the qualitative and quantitative composition of phenolic compounds in fruit derived foods. The chemical structure of polyphenols will also affect their biological properties such as bioavailability, antioxidant activity, specific interactions with cell receptors and enzymes and other properties.

Food organoleptic properties like color, taste and bitterness are obviously closely connected to the initial phenolic composition. It is also connected to environmental factors such as temperature, light and pH. In addition, the ability of these natural compounds to interact with various molecules is another way to modify these initial properties. Polyphenols are thus also responsible of the alterations usually observed during storage and ageing. While the color of new plant derived product is due to its initial chemical content, the subsequent color changes during storage and ageing involves generally condensation of phenolic compounds. These transformations generally result in browning, discoloration or darkening and this reactivity raises an important economic question. The sensory parameters usually altered during ageing include color and taste.

This chapter constitutes an overview of our findings in the field of polyphenols. Purification and characterization of some flavonoids and phenylethanoids from plants growing in Morocco will be presented. Hemisynthesis and structure determination of derivatized monomeric and dimeric flavanols will be also discussed. Application of spectroscopic methods for the analysis of these natural and derivatized products will be presented. In particular analysis of flavonoids, oligomeric and polymeric procyanidins using electrospray (ESI), matrix-assisted laser desorption ionization (MALDI), and tandem (MS-MS) mass spectrometry will be also reported. Finally the antioxidant properties of these natural/synthesized compounds will be discussed.

## **2. Results and discussion**

### **2.1 Purification and characterization of some natural phytochemicals**

Within a research program aimed at discovering new natural antioxidants, the aerial parts of *Globularia alypum* and *Salvia verbenaca* were extracted with various solvent mixtures, and the reduction of DPPH<sup>o</sup> was used to evaluate the antioxidant activity of the obtained extracts. The first obtained results showed that the hydromethanolic extracts demonstrated a notable antioxidant activity. The obtained fractions were subjected to SPE column

chromatography using a step gradient of MeOH/H<sub>2</sub>O mixture and each fraction was tested for its antioxidant activity.

Among the obtained fractions, the 50% aqueous methanolic fractions, which exhibited strong activity compared with negative control, were investigated for their phytochemical compositions. Analysis through analytical HPLC showed the presence of several compounds belonging to different families as demonstrated by their UV-visible spectra obtained through the used DAD detector coupled to the HPLC apparatus. Further fractionation by semipreparative HPLC allowed the isolation of sixteen compounds which were tested for their scavenging activity toward DPPH° radical.

The structures of the obtained compounds were elucidated through ESI-MS, CID MS, tandem MS-MS, 1D and 2D homonuclear and heteronuclear NMR analysis (Es-Safi et al., 2005a, 2006b). The phytochemicals isolated from *G. alypum* include syringin **1**, iridoids **2-7**, flavonoids **8-11**, and phenylethanoids **12-15** (Figure 1) while rosmarinic acid **16** was isolated from *S. verbenaca*.

Compound **1** was obtained as an amorphous powder. Its molecular weight 372 Da, determined through ESI-MS analysis ([M+Na]<sup>+</sup> and [M-H]<sup>-</sup> at *m/z* 395 and 371 Da respectively), was in agreement with the C<sub>17</sub>H<sub>24</sub>O<sub>9</sub> formula as confirmed through <sup>13</sup>C NMR analysis. The structure of compound **1**, was completely elucidated and was identified as syringin through 1D and 2D NMR analysis. Its spectral data were in agreement with those previously reported in the literature for the same compound (Sugiyama et al., 1993).

Among the isolated compounds, six iridoids **2-7** were isolated. The structure elucidation of the major one **6** was initiated through mass spectroscopy. Its molecular weight was determined as 492 amu by ESI-MS both at negative (*m/z* 491, [M-H]<sup>-</sup>) and positive (*m/z* 493 [M+H]<sup>+</sup>, 515 [M+Na]<sup>+</sup>) ion mode. Among the obtained fragmentation, the loss of a 162 amu was observed suggesting the probable presence of a glucosidic moiety. The structure of compound **6** was further elucidated by NMR analysis. Complete assignments were made using a combination of homonuclear and heteronuclear correlation experiments.

Construction of the iridoid skeleton started with the carbon at 96.5 ppm (C-1), which has an acetal proton at 5.36 ppm (*d*, *J* = 8.7 Hz, H-1). This acetal proton was coupled to the methine proton at 2.65 ppm (*dd*, *J* = 8.7 and 9.6 Hz, H-9), which in turn was coupled to the second methine proton located at 2.29 ppm (*dddd*, *J* = 1.5, 4.5, 7.8 and 9.6 Hz, H-5). H-5 was further coupled to an olefinic proton at 5.04 ppm (*dd*, *J* = 4.5 and 6.0 Hz, H-4), which in turn was coupled to another olefinic proton at 6.37 ppm (*dd*, *J* = 1.5 and 6.00 Hz, H-3). In the other direction, the proton H-5 was correlated to the oxymethine proton located at 3.96 ppm (*dd*, *J* = 1.2 and 7.8 Hz, H-6) which was also correlated, according to the COSY spectrum, with H-7 (3.50 ppm, *bs*). The absence of any other homonuclear coupling observed for H-7 and H-9 indicated a totally substituted C-8 (64.4 ppm). The chemical shift value and coupling constant of C-10 (65.3 ppm, 4.26 and 5.01 ppm, AB system, *J* = 12.6 Hz) also confirmed a C-8 to be a tertiary oxygenated carbon.

The presence of a trans cinnamoyl ester was confirmed by signals due to the five aromatic [7.39 (2H), 7.40 and 7.62 ppm (2H)] and two olefinic protons (6.56 and 7.72 ppm, AB system, *J* = 16.2 Hz) as well as observation of 6 aromatic carbons [130.2 (2C), 130.9 (2C), 132.4 and 136.7 ppm] in addition to the signals located at 119.5, 147.6 and 169.3 ppm corresponding

respectively to C( $\beta$ ), C( $\alpha$ ) and the carbonyl carbon. Spectral assignment of the glucopyranosyl moiety was easily attributed through 2D COSY experiment, starting from the anomeric proton H-1' which was attributed to the signal located at 4.72 ppm on the basis of its downfield chemical shift. The relative configurations of the stereogenic centres of compound **6** were clarified by 2D NOESY experiment suggesting  $\beta$  configurations for hydrogens at C-5 and C-9 and a  $\beta$ ,  $\beta$ ,  $\beta$  orientations for the substituents at C-6, C-7 and C-8 (Figure 1). The obtained results for compound **6** were in agreement with the spectral data of globularin previously reported in the literature (Faure et al., 1987). Having in hand the structure of compound **6**, the other known iridoids were easily identified by ESI-MS and NMR spectroscopy by comparison of their spectral data with those of globularin and the spectral data of the literature. Thus compound **4** was identified as globularicisin, compound **5** as globularidin, compounds **2** as globularinin and **3** and **7** as globularimin and globularioside respectively (Figure 1).

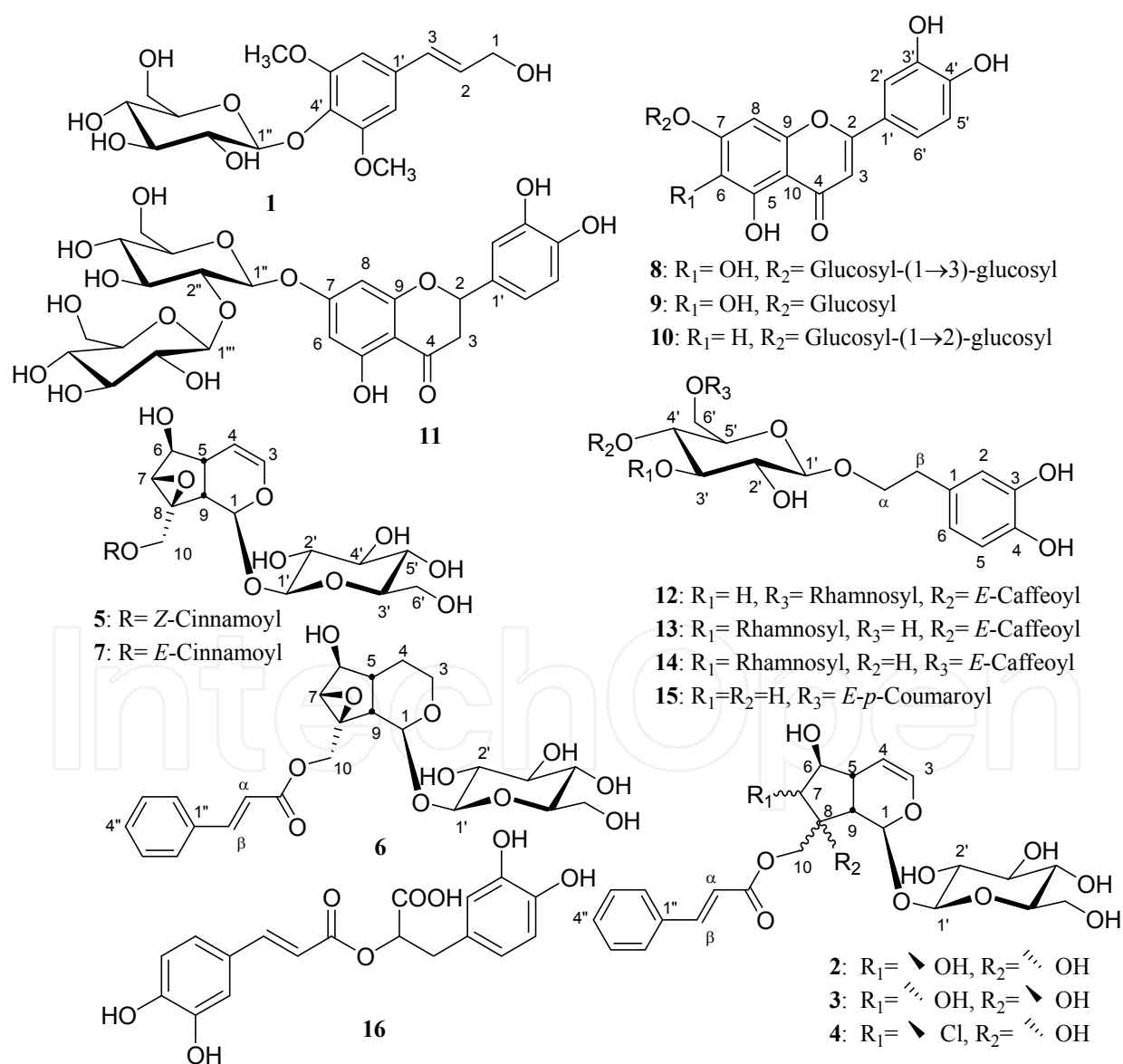


Fig. 1. Structure of the isolated natural compounds.



The  $^1\text{H}$  NMR signals of compound **9** between 3.50 and 4.00, at 5.06 ppm, and between 6.5 and 7.5 ppm, in connection with a corresponding pattern in the  $^{13}\text{C}$  NMR spectrum indicated typical features of a glycosylated flavonoid. Due to the fact that the  $^{13}\text{C}$  NMR spectrum showed 21 carbon atoms, the molecular formula  $\text{C}_{21}\text{H}_{20}\text{O}_{12}$  obtained through ESI-MS ( $m/z$  463  $[\text{M}-\text{H}]^-$ , 465  $[\text{M}+\text{H}]^+$ ) was retained. The positive ESI-MS spectrum also showed an ion signal at  $m/z$  303 which is likely to correspond to a fragment ion, presumably the aglycone. From the mass difference of  $m/z = 162$  between the  $[\text{M}+\text{H}]^+$  peak and the aglycone it was deduced that the sugar moiety was an hexose which was determined as glucopyranosyl unit through NMR analysis.

The  $^{13}\text{C}$  NMR spectrum displayed three peak signals in the upfield regions at 146.2, 151.1, and 150.4 ppm corresponding respectively to C-5, C-7 and C-9 in agreement with a 5,6,7-trihydroxylated A-ring of flavones (Horie et al., 1998). Compound **9** had almost identical NMR and MS-MS data for the aglycone 6-hydroxyluteolin as compared with those in **8** (Es-Safi et al., 2005a). The linkage between the sugar H-1'' (5.06 ppm) and the C-7 position (151.1 ppm) of the flavone was established by NOESY experiment showing correlation between the anomeric proton and the residual A ring aromatic proton H-8 (6.99 ppm). Compound **9** was thus identified as 6-hydroxyluteolin 7-*O*- $\beta$ -D-glucoside.

The ESI-MS spectra of compound **11** ( $m/z$  609  $[\text{M}-\text{H}]^-$ , 611  $[\text{M}+\text{H}]^+$ ) indicated a molecular weight of 610 in agreement with the  $\text{C}_{27}\text{H}_{30}\text{O}_{16}$  formula. The observation of a loss of a 324 neutral fragment in the mass spectra of compound **11** confirmed the presence of two hexose moieties. The CID MS-MS spectra of the  $[\text{M}-\text{H}]^-$  ion showed in addition to 609 an intense ion at  $m/z$  285 and 447. The latter was observed with a very low abundance in agreement with a diglucoside structure. The CID MS-MS spectra of the aglycone ion observed at  $m/z$  285 were in agreement with a luteolin unit. This was confirmed through NMR analysis where a singlet at around 6.7 ppm in its  $^1\text{H}$  NMR spectrum consistent with the H-3 of flavones was observed. This was also supported by the observation of carbon signal at ca 103.5 ppm associated with the C-3 in their  $^{13}\text{C}$  NMR spectrum. Compound **11** was concluded to be luteolin-7-*O*- $\beta$ -D-sophoroside through NMR analysis which results were in agreement with reported data (Imperato & Nazzaro, 1996).

Compounds **12-15** were obtained as colourless, amorphous compounds. Their UV spectra were very similar showing five absorption bands in the range 230-330 nm. Their IR spectra showed bands for hydroxyl groups and aromatic rings. These data indicated the presence of phenolic compounds with an unsaturated moiety outside the phenol ring. The ESI-MS data provided  $m/z$  values of 623  $[\text{M}-\text{H}]^-$ , and 625  $[\text{M}+\text{H}]^+$  ions giving a molecular weights of 624 consistent with the formula  $\text{C}_{29}\text{H}_{36}\text{O}_{15}$  for the three compounds. The CID-MS yielded an apparent peak at  $m/z = 448$ . From the mass difference of 146, the presence of a deoxyhexose was deduced.

The  $^1\text{H}$  NMR spectrum of compound **13** exhibited typical resonances arising from six aromatic protons (2 ABX systems, 6.56-7.05 region), two *trans*-olefinic protons (AB system, 6.27 and 7.59 ppm,  $J = 15.9$  Hz), a benzylic methylene at 2.78 ppm (2H, *t*,  $J = 7.2$  Hz) and two non-equivalent proton signals at 3.72 and 4.03 ppm (each 1H, *m*). These data were consistent with the presence of a (*E*)-caffeic acid unit and 3,4-dihydroxyphenethyl alcohol moiety. In addition, two anomeric proton signals at 4.38 (*d*,  $J = 7.9$  Hz) and 5.17 (*d*,  $J = 1.8$  Hz) were attributed to the  $\beta$ -D-glucose and  $\alpha$ -L-rhamnose units, respectively, indicating the disaccharide structure of **13**.

The presence of a doublet at 1.08 ppm in the  $^1\text{H}$  NMR spectrum, the  $^1\text{H}$ - $^1\text{H}$  COSY interactions, the  $^{13}\text{C}$  shifts deduced from 2D NMR experiments and the  $^1\text{H}$  NMR coupling constants made an  $\alpha$ -L-rhamnose-O- $\beta$ -D-glycoside likely. The acyl group was positioned at the C-4' position of the glucose unit, on the basis of the strong deshielding of the H-4' signal (4.91 ppm) of the glucose unit. In the  $^{13}\text{C}$  NMR spectrum, the C-3' (82.4 ppm) resonance of the glucose unit showed a remarkable downfield shift, indicating that the rhamnose moiety was attached to the C-3' position of the glucose. Therefore, based on the NMR data, the structure of **13** was identified as acteoside (Owen et al., 2003).

The proton and carbon resonances of **12** due to the aglycone and sugar moieties were in good agreement with those of **13**, indicating the similar substructures. However the caffeoyl residue was concluded to be attached to glucose C-6 instead of C-4 (normal chemical shift for H4, downfield shifts for H6a,b). Therefore, the structure of **12** was established as isoacteoside (Owen et al., 2003). The NMR data revealed that **14** had most of the structural features of **13** with the exception that the rhamnosyl residue is attached to glucose C-6 instead of C-3 as attested by the downfield shift (4 ppm) of the C-6' of the glucose moiety. Since the NMR data of **14** was in good agreement with the reported data, its structure was identified as forsythiaside (Shoyama et al., 1986).

Compound **16** which was isolated from *S. verbenaca* was concluded to be rosmarinic acid on the basis of its MS and NMR spectral data. Its ESI-MS spectrum recorded in the negative ion mode presents signal at  $m/z$  359 corresponding to the  $[\text{M-H}]^-$  ion in agreement with the proposed structure. The  $^1\text{H}$  NMR spectrum presents in particular two doublets ( $J=16$  Hz) at 6.2 and 7.5 ppm integrating one proton each and corresponding to the two olefinic protons. The two benzylic protons appeared as two double doublets at 2.9 and 3.1 ppm while the proton in the  $\alpha$  position of the carboxyl group appeared as a multiplet at 5.1 ppm. Finally the 6 aromatic protons appeared as multiplets between 6.5 and 7.1 ppm. The  $^{13}\text{C}$  NMR spectrum showed 18 signals in agreement with the structure of rosmarinic acid. The benzylic carbon atom appeared at 39 ppm and was confirmed through DEPT analysis. The two carbonyl carbon atoms were attributed to the signals located at 175 (COOH) and 169 (CO) ppm. The carbon in the  $\alpha$  of the carboxyl group signal was located at 76 ppm. The other observed signals were in agreement with the structure of rosmarinic acid and with previously published results (Mehrabani et al., 2005).

## 2.2 Hemisynthesis and structure determination of derivatized monomeric and dimeric flavanols

Natural antioxidants have been shown to enhance product stability, quality, and shelf life. Consequently, the development of antioxidants from natural origin has attracted considerable attention and many researchers have focused on the discovery of new natural antioxidants aimed at quenching biologically harmful radicals. However, natural antioxidants are usually difficult to isolate because they are often extracted in complex mixtures. Moreover, they generally occur in few amounts which did not allow achieving the desired biological activity. This prompted us to initiate an investigation program aimed at the hemisynthesis of monomeric and dimeric polyphenols.

The synthesis of some modified monomeric units derived from catechin was initiated with the objectives of exploring the impact of the A ring substitution on their antioxidant properties. For this study, the six 8-substituted derivatives of flavan-3-ols **19-24** in addition to taxifolin **25** (Figure 2) were synthesized and their antioxidative activity investigated.

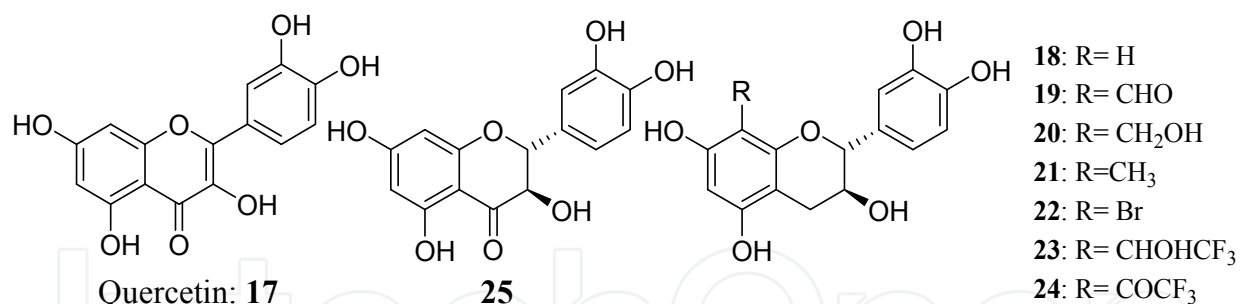
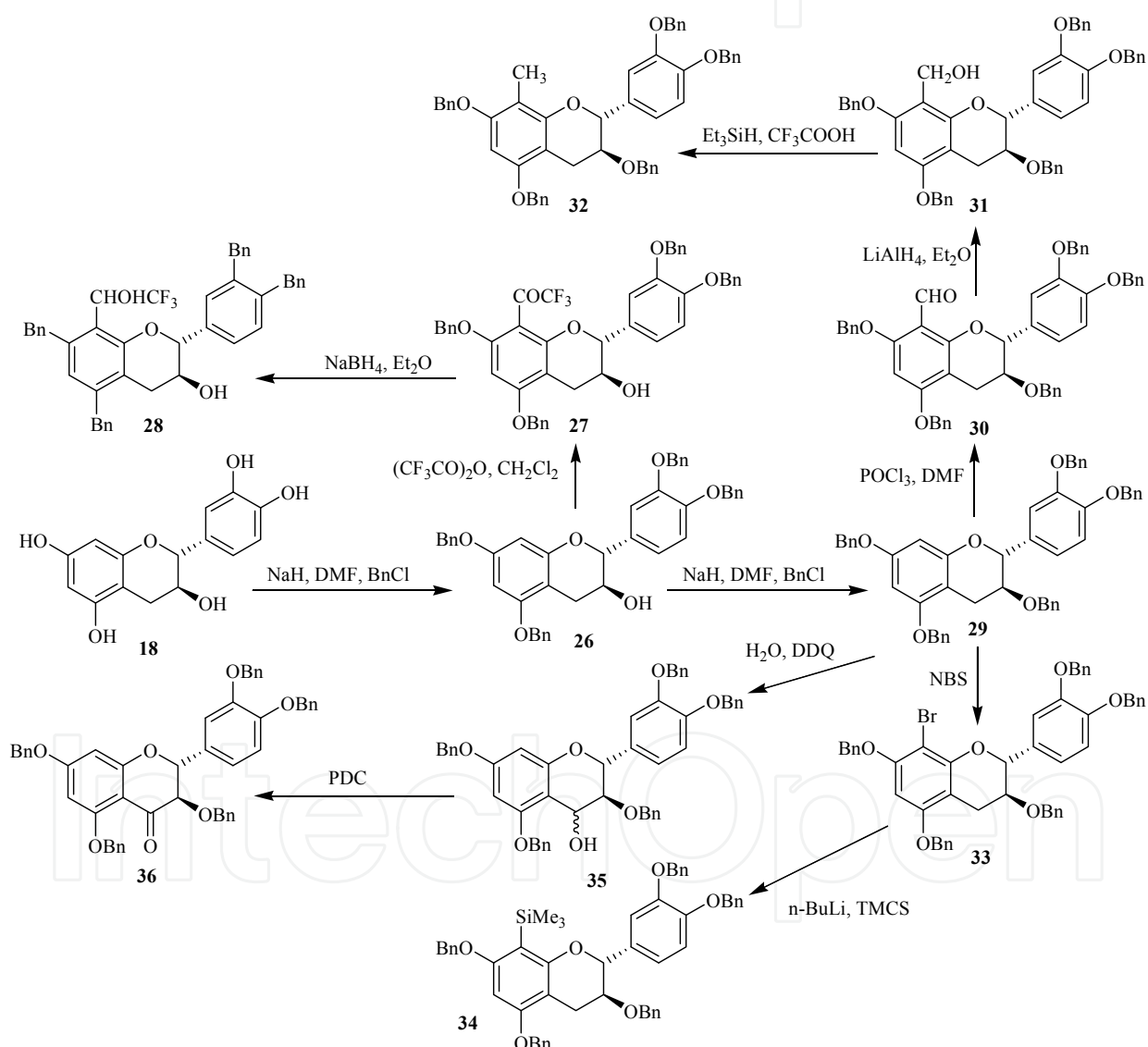


Fig. 2. Structures of the studied modified flavan-3-ol monomers **19-25**.



Scheme 1. Synthesis pathways of the studied modified flavanol monomers.

The modified flavan-3-ols monomer derivatives described in this work were synthesized according to the pathways depicted in Scheme 1. Compound **27** was prepared by action of trifluoacetic anhydride on tetrabenzylated catechin **26** following a Friedel-Craft's reaction on the 8 nucleophile position as previously described [Beauhaire et al., 2005; Es-Safi et al.,



2006c]. Further reduction by  $\text{NaBH}_4$  afforded compound **28**. Compound **30** was prepared starting from the pentabenzylated catechin **29**. After formylation through the classical Vilsmeier reaction, the obtained compound **30** was reduced by  $\text{LiAlH}_4$  giving the hydroxymethyl derivative **31**. Further reduction of the latter gave the target product **32** with a good yield. The bromided adduct **33** was obtained from **29** by action of NBS. Gram-scale of taxifolin **25** was prepared from (+)-catechin through reactions involving oxidation processes as previously described (Es-Safi & Ducrot, 2006a).

The structures of these modified catechin derivatives were determined through UV, MS and NMR spectroscopy. Structure elucidation of compound **32** will be detailed as example. The ESI-MS spectrum recorded in the positive ion mode showed signals located at  $m/z$  755, 772 and 777 corresponding to  $[\text{M}+\text{H}]^+$ ,  $[\text{M}+\text{NH}_4]^+$  and  $[\text{M}+\text{Na}]^+$  ions respectively and indicating a molecular weight of 754 amu in agreement with the structure of compound **32**. The usual flavan-3-ols characteristic RDA fragmentation was also observed at  $m/z$  423,  $[\text{M}+\text{H}-332]^+$  ion and corresponding to the protonated A moiety (Figure 3).

The remaining outstanding question that needed to be resolved was related to the position of the methyl group on the flavanol A ring. This constitutes the most encountered problem in flavanols structural characterization. Since the two positions 6 and 8 are almost magnetically equivalent, they could not be distinguished on the basis of their chemical shift. In our case, assuming that the substitution occurs at the more nucleophilic positions of the flavanol skeleton, i.e 6 or the 8 as confirmed through ES-MS spectrometry, determination of the residual proton (H6 or H8) could not be achieved based only on its chemical shift but would rather requires the use of 2D NMR analysis.

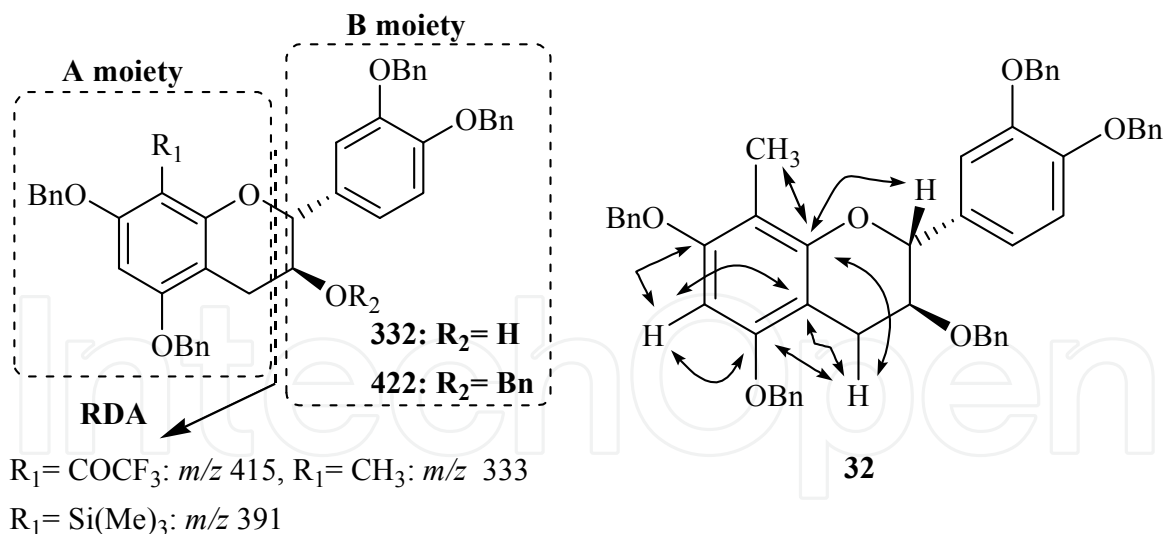
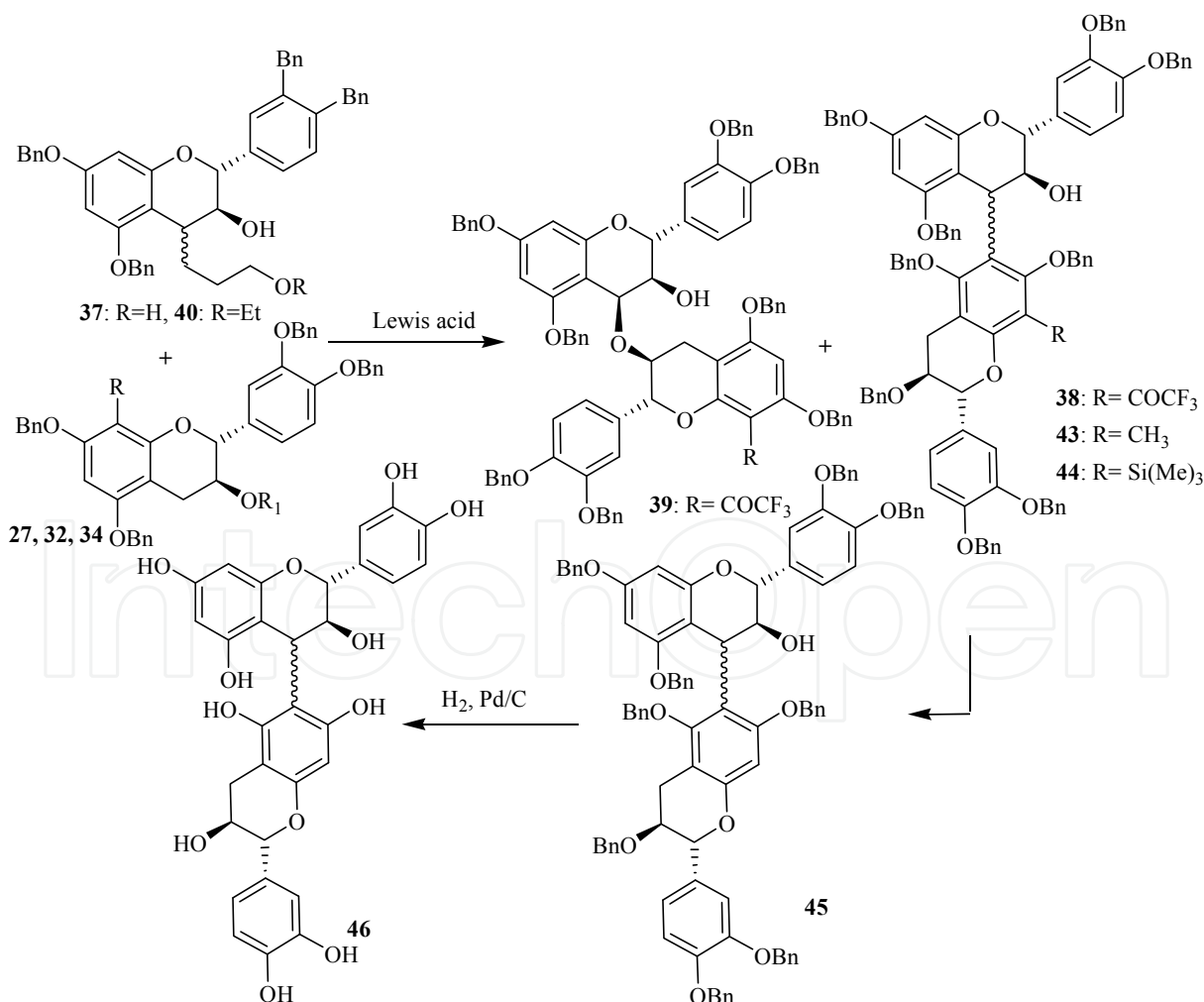


Fig. 3. Main fragmentations observed in compounds **27** ( $R_1 = \text{COCF}_3$ ), **32** ( $R_1 = \text{CH}_3$ ) and **34** ( $R_1 = \text{Si}(\text{Me})_3$ ) and main HMBC correlations observed for compound **32**.

The position of the  $\text{CH}_3$  group on the aromatic A ring was elucidated by long range distance carbon-proton correlations established by 2D NMR HMBC experiments through the following reasoning. The usual pyran ring protons H4 [(2.71 ppm, *dd*,  $J = 16.69$  and  $5.59$  Hz) and 3.03 ppm (*dd*,  $J = 16.69$  and  $8.73$  Hz)], H3 (6.60 ppm, *m*) and H2 (7.90 ppm, *d*,  $J = 7.99$  Hz) were easily assigned by  $^1\text{H}$  NMR analysis. The three B ring protons were observed between

6.93 and 7.00 ppm. For the aromatic A ring, only one proton signal appearing as a singlet at 6.21 ppm was present indicating a monosubstitution. The presence of the CH<sub>3</sub> group was confirmed through <sup>1</sup>H NMR analysis showing a singlet at 2.06 ppm. The protonated carbon chemical shifts were assigned through NMR HSQC analysis.

The definitive structure elucidation of compound **32** was achieved by HMBC experiment which allowed assignment of all hydrogen and carbon atoms. In addition to their correlations with C2 (79.84 ppm) and C3 (74.97 ppm), H4 protons (2.77 and 3.03 ppm) correlated with 3 carbons located at 102.74, 153.13 and 154.75 ppm. Carbons C4a, C8a and C5 are in a favorable position to give such correlations (Figure 3). The signal observed at 102.74 ppm was attributed to C4a due to its chemical shift position compared to C8a and C5 which are linked to an oxygen atom. The carbon signal located at 153.13 ppm also gave a correlation with H2, which pointed to the C8a carbon and thus the remaining signal observed at 154.75 ppm was attributed to C5. The C8a signal thus attributed did not show any correlation with the residual A ring aromatic protons which is thus H6. This was also confirmed by the presence of a correlation between C5 and the residual aromatic proton and between the methyl protons and the C8a (Figure 3). The position of the methyl group on the A ring 8 position was thus demonstrated.



Scheme 2. Lewis acids-catalyzed flavanols coupling reactions.

After having synthesized these modified (+)-catechin derivatives, we tried to synthesize some dimeric flavanols derivatives by coupling flavanols using Lewis acids as catalysts. Indeed lewis acids, like  $\text{TiCl}_4$ ,  $\text{AgBF}_4$ ,  $\text{SnCl}_4$ , TMSOTf have been employed in literature to synthesize dimeric and oligomeric procyanidins of (+)-catechin and (-)-epicatechin units (Arnaudinaud et al., 2001; Saito et al., 2002; Tückmantel et al, 1999). In these reactions, the role of Lewis acids is to promote the formation of the benzylic carbocation at C4 of a flavanol subunit starting from a C4 hetero substituted flavanol, which thereafter undergoes a Friedel-Craft-like addition on a second flavanol subunit. For this study, the Lewis acid  $\text{TiCl}_4$  was used as a carbocation promoting agent from the 4-(2-hydroxyethoxy) flavan-3-ol **37**. Coupling reaction between compound **27** and **37** in a 6/1 molar ratio was investigated in  $\text{CH}_2\text{Cl}_2$  according to Scheme 2.

The reaction was monitored by CCM and HPLC and showed the disappearance of compound **27** and appearance of new compounds. In order to verify the presence of compounds with the expected dimeric structures the mixture was explored by HPLC coupled to a mass spectrometry detection operating in the positive ion mode. An extracted ion current chromatogram recorded at  $m/z$  1395 and 1412 (Figure 4) and corresponding to a dimeric structure molecular weight showed the presence of a minor and a major compound. The UV spectrum of both compounds exhibited similar maxima (285 and 305 nm) to that of compound **27**, indicating that the original flavan structure with the  $\text{COCF}_3$  group was retained.

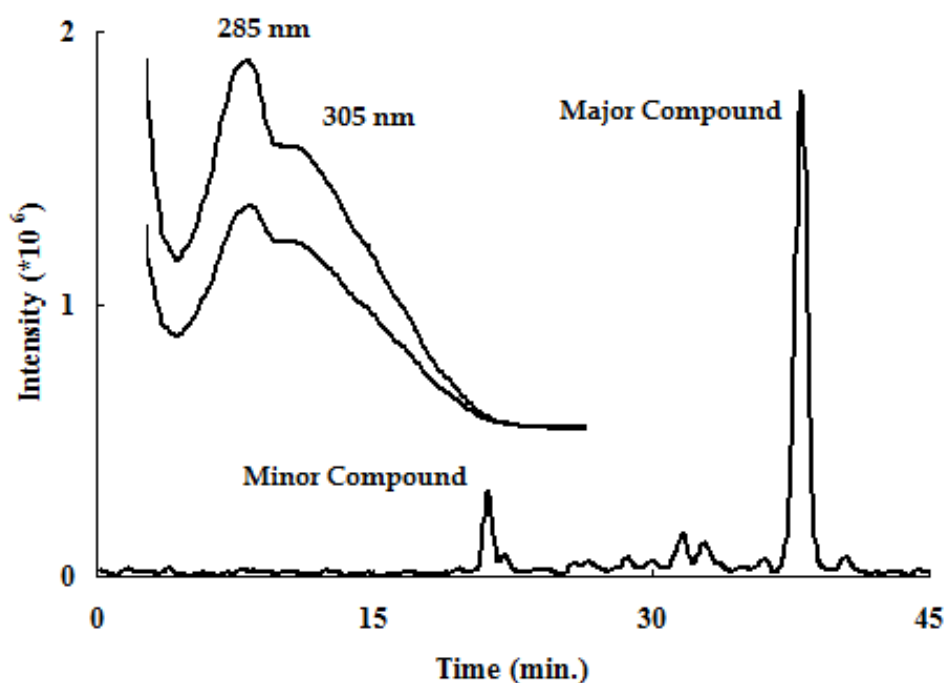


Fig. 4. Extracted ion chromatogram recorded at  $m/z$  1395.

ESI-MS analysis of both compounds showed that the fragmentations observed for minor compound **38** were in agreement with a dimeric structure consisting of tetrabenzylated (+)-catechin **26** coupled to its trifluoroacylated derivative **27** through a C4→C6 linkage (Figure 5A). Indeed, MS/MS fragmentation of the minor compound showed only one RDA fragmentation corresponding to the  $[\text{M}+\text{H}-332]^+$  ion.

The mass spectrum obtained of the major compound **39** in the positive ion mode (Figure 5B) showed signals at  $m/z$  1395, 1412, 1417 and 1433 corresponding respectively to  $[M+H]^+$ ,  $[M+NH_4]^+$ ,  $[M+Na]^+$  and  $[M+K]^+$  indicating a molecular weight of 1394 amu in agreement with a dimeric structure consisting of tetrabenzylated (+)-catechin **26** linked to its trifluoroacetylated derivative **27**. However, the remaining problem was the establishment of the position of linkage to compound **27**, as the tetrabenzylated catechin moiety is linked through its 4 position.

In addition to the signals indicated above, the mass spectrum of compound **39** also showed signals at  $m/z$  747 and 649 corresponding to the fission of the bond between the two constitutive units.

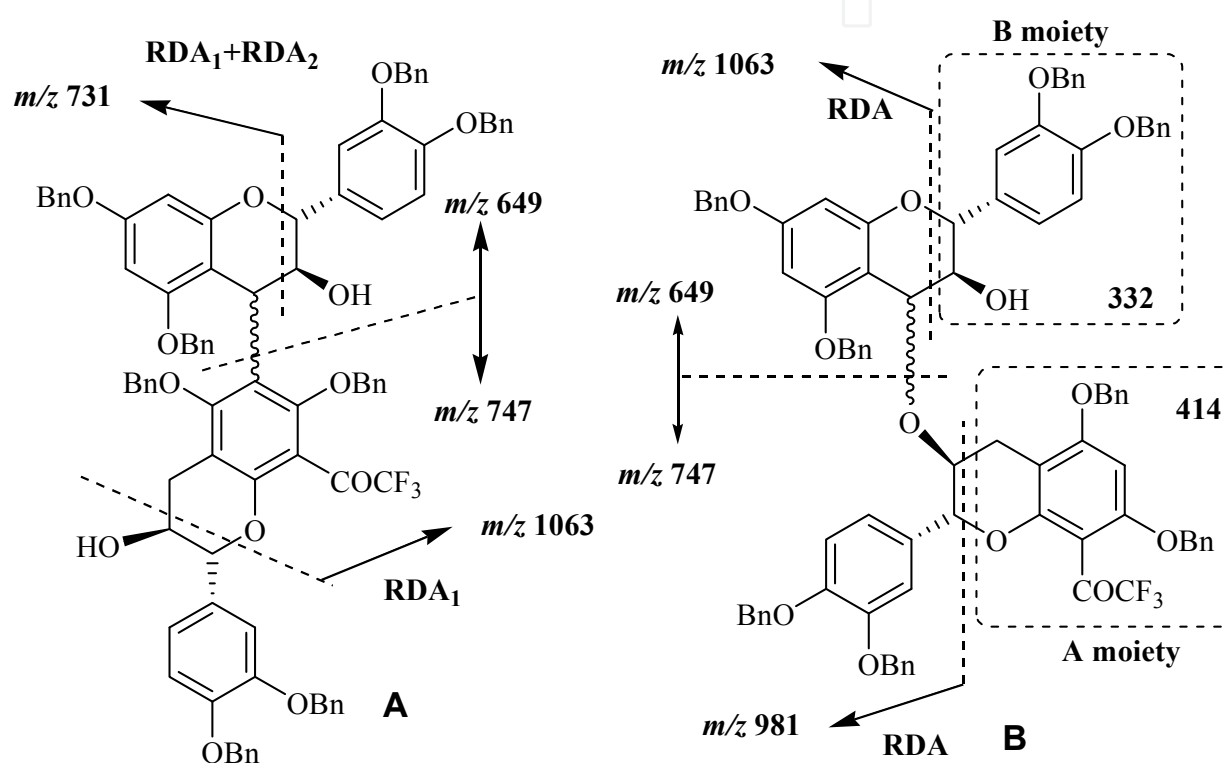


Fig. 5. MS/MS fragmentations observed for the minor (A) and the major compounds (B).

Among the other observed signals two of them were located at  $m/z$  1063 and 981 and were also observed in the spectrum obtained through positive ES CAD MS/MS fragmentation of the signal located at  $m/z$  1395 ( $[M+H]^+$  ion). The signal observed at  $m/z$  1063 was attributed to the characteristic RDA fragmentation corresponding to the  $[M+H-332]^+$  ion as what was observed for compound **27** through a loss of the B moiety. The second fragmentation observed at  $m/z$  981 correspond in fact to the  $[M+H-414]^+$  ion, meaning a loss of the A moiety of compound **27** unit (Figure 5B) and corresponding to another RDA fragmentation. The occurrence of this fission indicated the presence of the A moiety in the structure of compound **39**. In other words, this means that the isolated compound is not a C4→C6 dimer since only one RDA fragmentation corresponding to the  $[M+H-332]^+$  ion could be possible in this case as what was observed above for compound **38**. The possible linkage is thus expected to occur *via* the 2' or 3' position of the F ring or possibly the 2', 5' or 6' positions of the ring E.

Through NMR analysis and in conjunction with the absence of a doubly benzylic methylene proton characteristic of a C4→C6 linkage and taking into account the dimeric structure of the compound as supported by MS analysis, the NMR data collectively indicated a dimeric structure with an interflavanyl ether bond connecting the two heterocyclic C and F rings. Taking into account the fact that the linkage did not involve the H2, H3, H4 F ring protons since they were all evidenced through NMR analysis, a (4-O-3) mode of linkage was thus concluded to occur between the two flavan-3-ols units. This was also confirmed by comparison of the chemical shifts of the H4 and H3 resonances of both the C and the E rings with those of their precursors. Finally the structure of compound was univocally confirmed through HMBC analysis where several long range correlations were observed. In particular correlations involving proton and carbon of both C and F rings via the oxygen atom were observed and confirmed thus the ether linkage involved in compound **39**. Full assignment of the protons and carbon chemical shifts was achieved through HMBC analysis (Figure 6) where the main correlations involving H/C of the C and F rings were showed in agreement with the proposed structure.

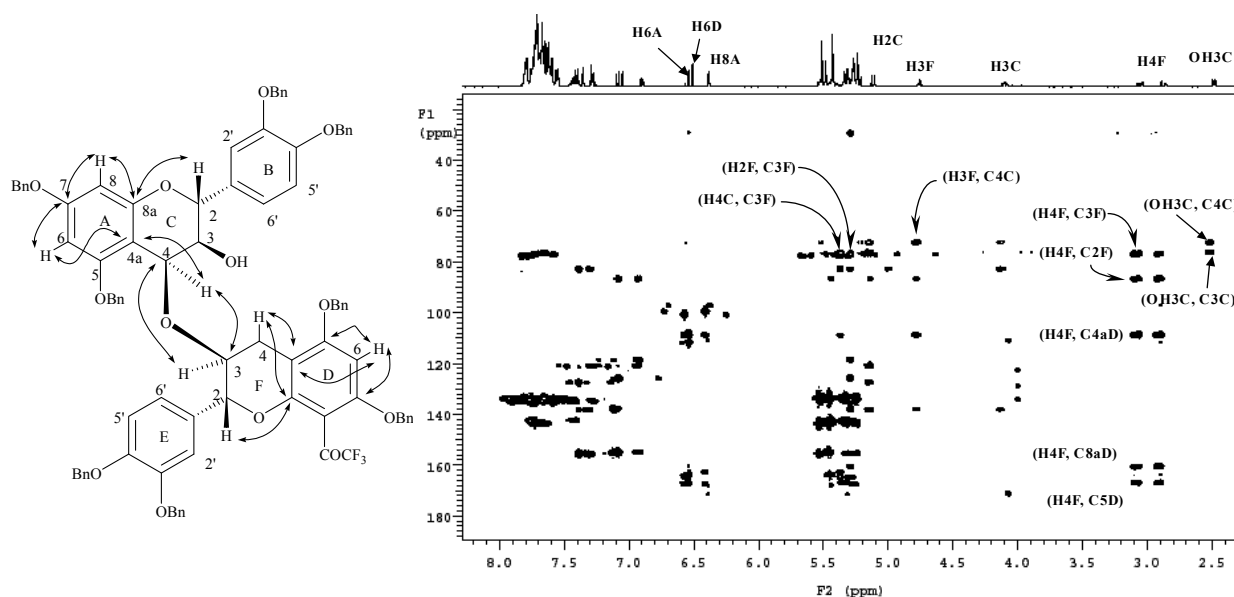


Fig. 6. Main  $^1\text{H}$ - $^{13}\text{C}$  long range correlations observed in compound **39**.

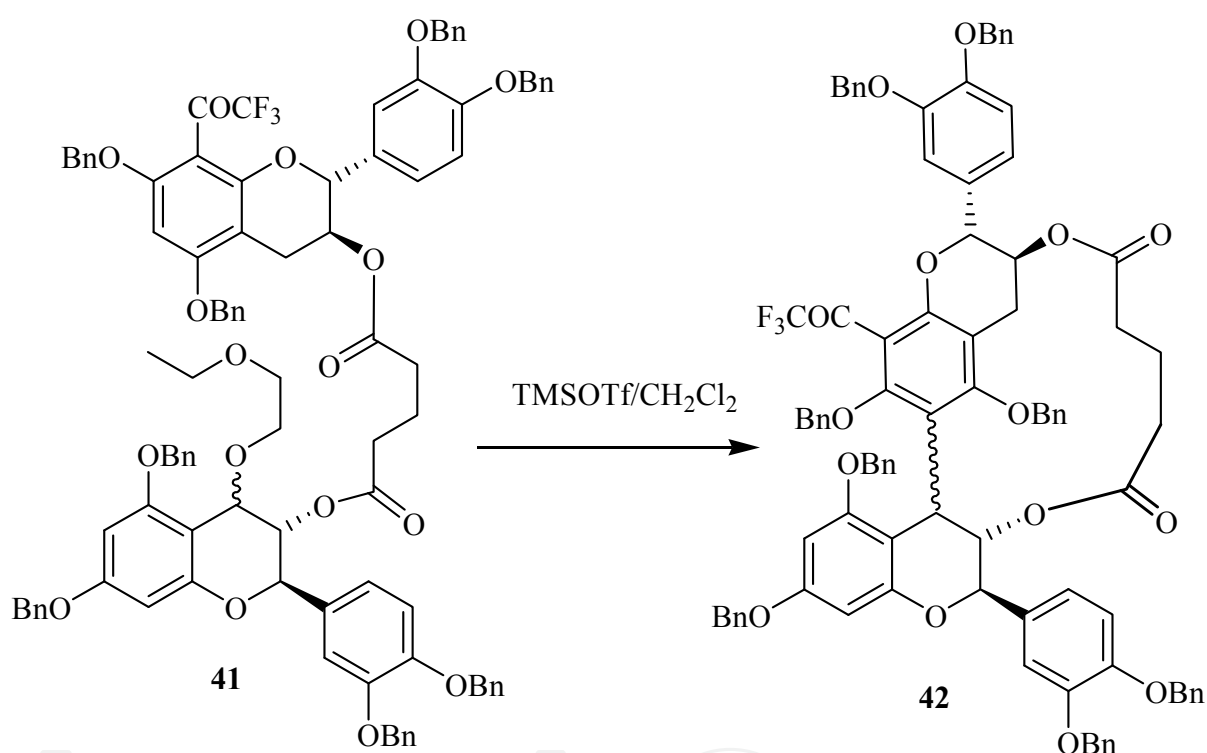
It was concluded that a (4-O-3) linkage was occurred between the two flavan-3-ol units. Moreover, coupling constants for the AMX spin system of the C-ring protons ( $J_{3,4} = 3.2$  Hz) indicated a 3,4 *cis* relative configuration for this ring, that is a  $4\beta$  linkage between both flavanol units ( $J_{3,4} = 3.2$ ), which was determined through homonuclear decoupling experiment. The complete stereoselectivity of the reaction remains, however, to be explained and should presumably be due to a participation of the hydroxy group at C3 of **11**. However, its involvement in the stereochemical course of the reaction cannot be, in our case, related to the formation of a protonated epoxide similar to that reported by Bennie et al., 2001 in a work dealing with the dimerization of epioritin-4-ol derivatives.

In addition to  $\text{TiCl}_4$ , TMSOTf was also used to catalyze intermolecular flavanol coupling reactions. When the reaction was conducted between compounds **27** and **40** in the presence of TMSOTf as Lewis acid (Scheme 2), results similar to that obtained with  $\text{TiCl}_4$  were



obtained giving the C4→C6 dimer as minor compound. Intramolecular flavanol coupling reaction was also assayed using the previously reported strategy (Saito et al., 2003). The synthesized compound **41**, which contains both the electrophilic and the nucleophilic parts, was submitted to the flavanol coupling reaction using TMSOTf as Lewis acid (Scheme 3). However, the reaction was difficult to achieve may be due to steric hindrance and the expected C4→C6 **42** was obtained with lower yield.

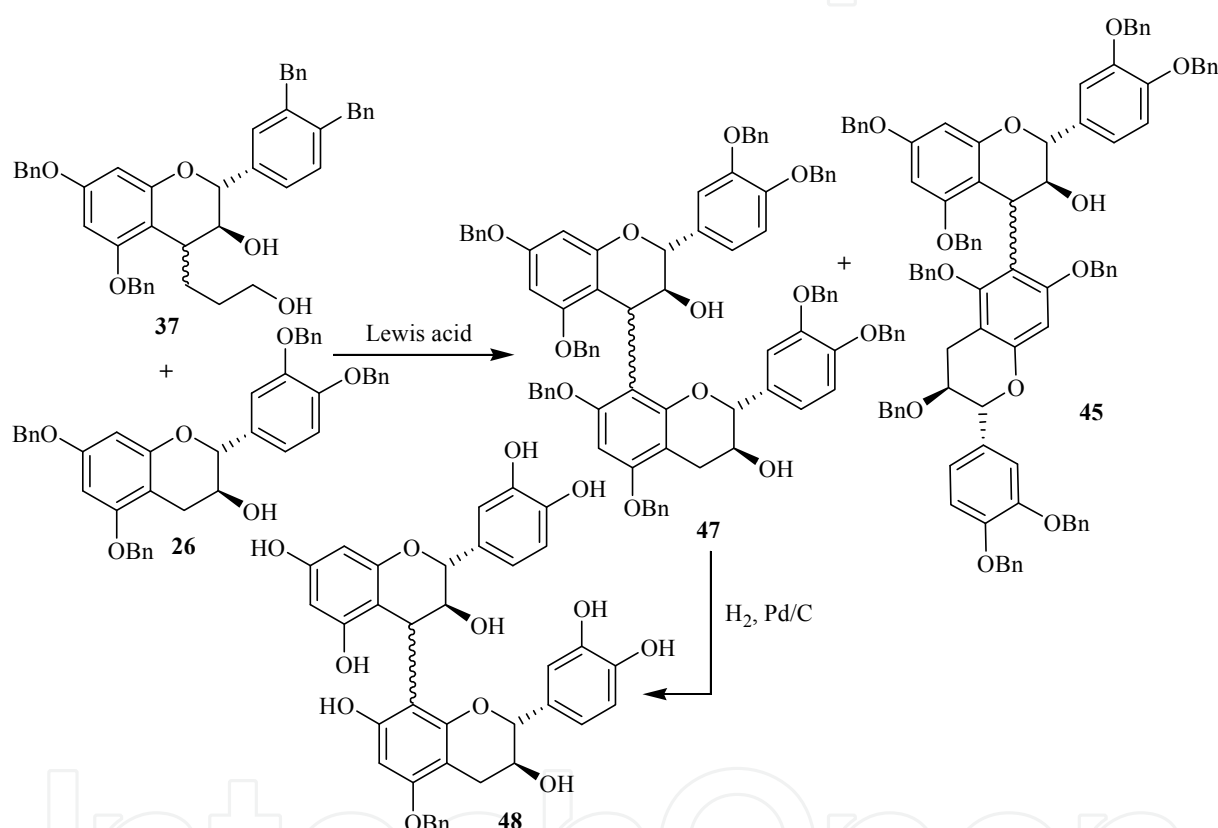
The almost exclusive, high yielding formation, in these conditions of the novel ether-linked procyanidins as main compound rather than its carbon-carbon C4→C6 coupled analogue reflects the importance of electronic features in the formation of flavan-3-ol dimers. The poor nucleophilicity of the A ring monomeric precursor, caused by the presence of the COCF<sub>3</sub> group, permits alternative centers to participate in the interflavanyl bond formation.



Scheme 3. TMSOTf catalyzed intramolecular flavanol coupling.

The obtained results discussed above showed that the interflavanic carbon-carbon linkage formation was largely inhibited during Lewis acid induced flavanols coupling reactions by the presence of an electron-withdrawing group, while this was not the case with an electron-donor group like the methyl substituent. This could constitute thus a stereoselective method for the formation of C4→C6 procyanidin dimers which are usually obtained as minor compounds compared to the C4→C8 ones. In order to apply this technique to the stereoselective preparation of natural dimeric procyanidin of the C4→C6 type, we unsuccessfully tried to remove the methyl group from the modified derivatives **43** (Scheme 2). Then we used the trimethylsilyl group which is easily removable by hydrolysis. Indeed, during purification of compound **34** on silicagel chromatography column, we saw that this compound was easily transformed to **29** by loss of the 8 substituent group. This prompts us to use this compound as nucleophile acceptor unit in the Lewis acid coupling reaction. The

reaction was monitored through CCM and LC-MS showing the presence of compound with a molecular mass of 1460 amu corresponding to the dimeric compound **44**. After hydrolysis and hydrogenolysis, the corresponding product **46** was separated and analyzed through mass spectroscopy. The obtained results indicated a molecular mass of 578 amu in agreement with the dimeric structure of compounds **46**. Indeed, the mass spectrum of compound **46** showed procyanidin characteristic RDA fragmentation giving an ion fragment by a loss of 152 mass units. We tried to proof the interflavanic linkage of the obtained compound through 2D NMR technique, but due to degradation of the obtained compound, our attempt was unsuccessful. It may be noted that the lewis acid induced flavanol coupling using compounds **26** and **37** give the C4→C8 adduct **47** as major dimer while the C4→C6 **45** was obtained as minor one (Scheme 4).

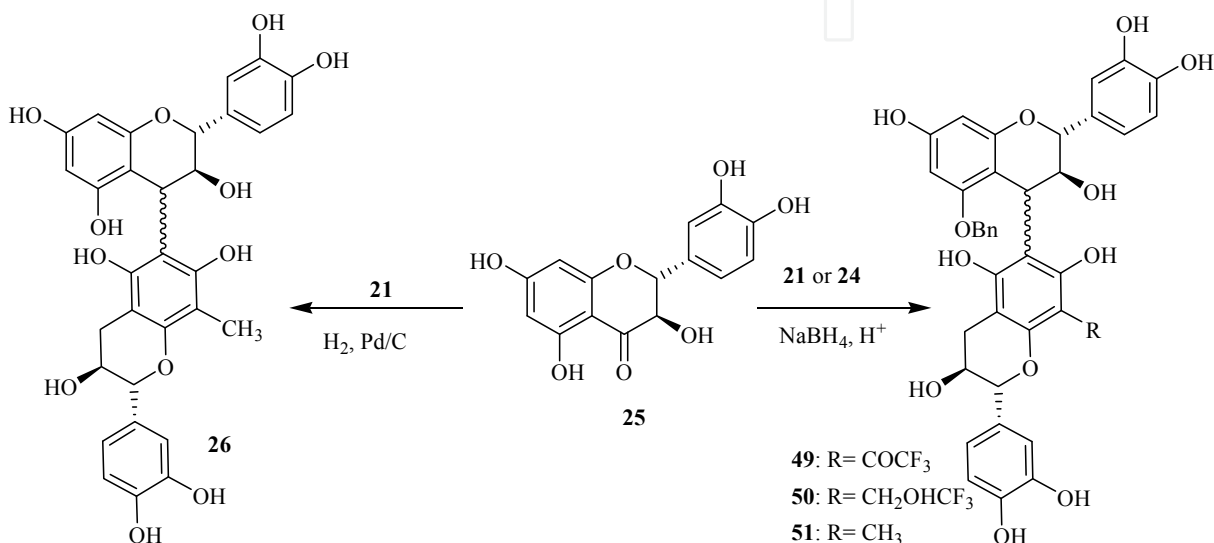


Scheme 4. Formation of dimeric C4→C8 and C4→C6 procyanidins.

After having investigated the Lewis acid induced flavanol coupling reaction involving 4-activated catechin derivatives, we were interested to use other precursors. Experiments were achieved using taxifolin **25**, a natural product often used as starting material in the hemisynthesis of procyanidins (Balas & Vercauteren, 1994). It was prepared as previously described (Es-Safi & Ducrot, 2006a) and was used through two pathways for the preparation of dimeric flavanols (Scheme 5).

In the first pathway, taxifolin was used as precursor of the electrophilic unit by action of  $NaBH_4$  giving an 4-hydroxy group which release a 4-carbocation adduct in a mildly acidified medium. The compounds **21** and **24** were used in their free form as nucleophilic units and the reactions were studied through LC-MS techniques (Scheme 5). When the

reaction was assayed with compound **24**, new compounds showing a mass signals at  $m/z$  673 and 675 in the negative ion mode were detected. These compounds were interpreted as the dimeric modified procyanidins **49** and **50** formed by action of the carbocation  $cat^+$  on the 8-substituted catechin compounds used as nucleophile units. The  $cat^+$  unit is issued from the reduction of taxifolin into a flavan-3,4-diol, followed by protonation and dehydration. The action on compound **24** gave thus the dimer **49** while compound **50** was formed after reduction of the carbonyl group by  $NaBH_4$  and further action of the  $cat^+$  adduct. This was confirmed by detection of compound giving a signal peak at  $m/z$  591 corresponding to the reduced 8-substituted monomer. The same reaction was investigated using the **21** monomer and the corresponding methylated dimer **51** was detected through LC-MS analysis.



Scheme 5. Flavan-3-ol coupling reactions using taxifolin **25** as starting material.

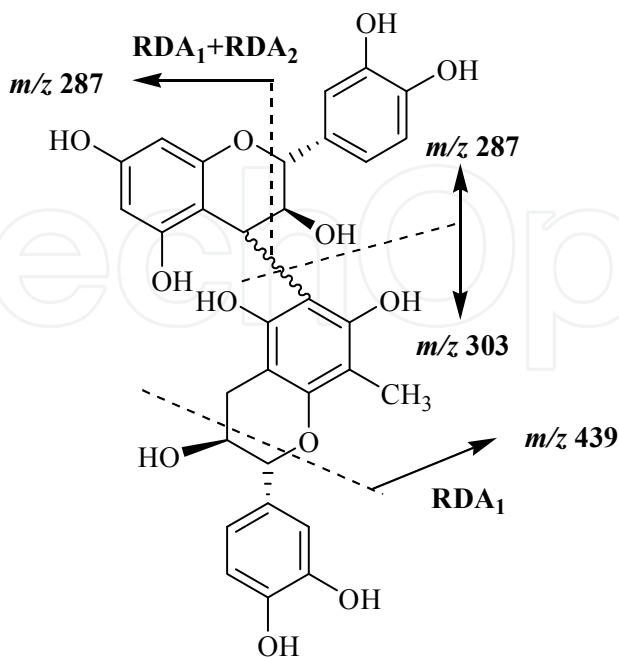


Fig. 7. Mass spectrum and main fragmentations observed in compound **51**.

The second pathway using taxifolin was adapted from the recently described methylene linked flavanol dimers synthesis (Boyer & Ducrot, 2005). The application of such reaction to procyanidin synthesis was achieved through hydrogenation of taxifolin **25** in the presence of an excess of the methylated catechin **21** used as nucleophile (Scheme 5). The reaction was explored through LC-MS analysis and the formation of new compounds initially absent in the mixture was observed. Among the obtained products, compound with a molecular mass of 592 amu corresponding to the dimeric procyanidin **51** was observed. The mass spectrum of compound **51** recorded in the negative ion mode showed a molecular ion at  $m/z$  591 and characteristic fragment signals as shown in figure 7.

### 2.3 Structural elucidation of natural and derivatized polyphenols through spectroscopic methods

The structural elucidation of polyphenols is difficult because of their heterogeneous character. The diversity of polyphenols is given by the structural variability of their basic skeletons with different hydroxylation patterns of the aromatic rings, different configurations at the chiral centers, distinct regio and stereochemistry, .... Due to this complexity and diversity, the characterization of polyphenols remains thus very challenging, and less is known regarding structure-activity relationship.

Various spectroscopic techniques including UV visible, NMR and mass spectroscopy have been used to characterize polyphenols. NMR spectroscopy could be considered as a powerful analytical technique for the determination of polyphenol structures even its poor sensitivity, slow throughput, and difficulties in analysis of mixtures. Technological developments in the field of NMR analysis, such as the LC-NMR analysis, have made of it the most important tool for complete structure elucidation of polyphenols. In addition to NMR, mass spectrometry has also been used for the characterization of polyphenols from various plant sources using various techniques including fast atom bombardment, liquid secondary ion, electrospray, and matrix-assisted laser desorption time of flight.

The examples on the structural elucidation of the compounds described above confirm the usefulness of the different spectroscopic techniques including 1D, 2D NMR and MS analysis. As examples of the usefulness of mass spectroscopy in polyphenols analysis, we gave below two examples. The first one concern the application of MS/MS techniques to the fragmentation study of flavonoids and the second is an application of ESI-MS and MALDI-TOF techniques for the study of a polymeric tannin fraction extracted from pear juice.

Various approaches have been proposed to use mass spectrometric fragmentations for structural characterization of flavonoid aglycones and glycosides (Cuyckens & Claeys, 2004; Es-Safi et al., 2005b; Feketeova et al., 2011; Ma et al., 1997; March & Brodbelt, 2008; Vukics & Guttman, 2010). It has been demonstrated that fragment ions provide important structural information for flavonoids and can be used to establish the distribution of the substituents between the A- and B-rings. A careful study of the fragmentation patterns in CID MS/MS can also be of a particular value in the structural elucidation of glycosides. Even many advances have been achieved in the field of structural identification of flavonoids, it is still a challenge in food chemistry to identify these compounds in foodstuff or those derivatives arising during biotransformation. We present here our results on the MS fragmentation study carried out on natural flavonoids achieved through a combinatory method using

positive and negative ESI/MS, CID/MS and tandem MS/MS analysis. The flavonoid glycoside 6-hydroxyluteolin 7-*O*-glucoside **9** isolated from *Globularia alypum* (Es-Safi et al., 2005a) is given as example.

In order to study the fragmentations of the flavonoid **9**, the CID spectra of the protonated ion  $[M+H]^+$  located at  $m/z$  465 were recorded and the obtained result is shown in Figure 8. It showed fragment ions with a relatively high intensity in the higher mass region and corresponding to the glucose moiety fragmentations or loss of small molecules ( $H_2O$ , CO,  $CH_2O$ ). In addition to the parent ion observed at  $m/z$  465, the spectrum showed an ion signal at  $m/z$  303 which presumably corresponds to the aglycone. The mass difference of  $m/z$  162 Da between the  $[M+H]^+$  peak and the aglycone is in agreement with the glucoside structure of compound **9**. The complete obtained MS data and the corresponding fragmentation pathways are gathered in Figure 8.

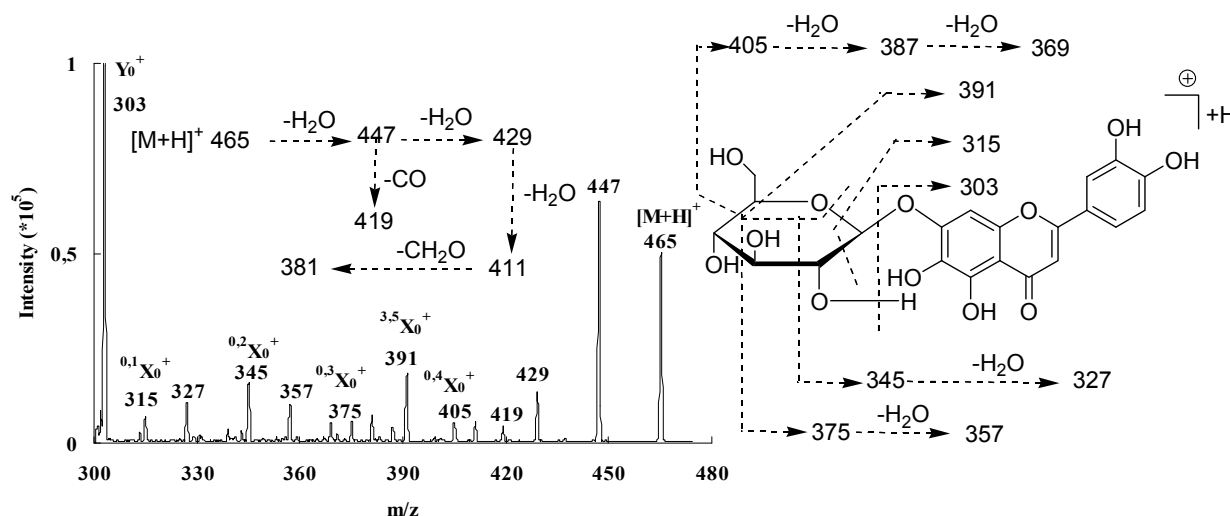


Fig. 8. CID-MS of protonated flavonoid **9** ( $m/z$  465) and the corresponding fragmentations.

In order to characterize the aglycone part of the flavonoid **9**, the  $Y_0^+$  CID spectra are more suited providing data similar to those of free flavonoid aglycones. Low-energy CID spectra of the  $[M+H]^+$  ion of 6-hydroxyluteolin ( $m/z$  303) showed various fragment signals. The detected ion species are formed according to the fragmentation pathways shown in Figure 9. The cleavage of the 1 and 3 bonds gave rise to the  $^{1,3}A^+$  ( $m/z$  169) and  $^{1,3}B^+$  ( $m/z$  135) ions. This pair of product ions clearly provides the substitution pattern in the A (3 OH) and B (2 OH) rings. The obtained ions can also undergo further fragmentations by loss of small molecules giving rise to other ions. By successive losses of 18 ( $H_2O$ ) and 28 (CO) mass units, the ion at  $m/z$  123 which is the base peak could be obtained from that at  $m/z$  169, while that observed at  $m/z$  117 could arise from the ion  $m/z$  135 by loss of a water molecule. An other RDA-type fragmentation corresponding to the cleavage of the 0 and 4 bonds, results in  $^{0,4}A^+$  ( $m/z$  125) and  $^{0,4}B^+$  ( $m/z$  179) ions that can further fragment by loss of small molecules giving rise to other ions at  $m/z$  161 ( $^{0,4}B^+ - H_2O$ ),  $m/z$  151 ( $^{0,4}B^+ - CO$ ) and  $m/z$  123 ( $^{0,4}A^+ - H_2$ ). Cleavage of the 0 and 2 bonds leads to the formation of  $^{0,2}B^+$  at  $m/z$  137. Cleavage of the 1 and 2 bonds leading to the formation of  $^{1,2}B^+$  and  $^{1,2}A^+ + 2H$  ions were also observed giving signals at  $m/z$  123 and 183 respectively. Finally cleavage of the 0 and 3 bonds giving rise to the formation of  $^{0,3}B^+$  and  $^{0,3}A^+$  ions at  $m/z$  153 were also observed even at low relative intensity.



Mass spectrometry has also been used for the characterization of condensed tannins. In particular electrospray (Cheynier et al., 1997; Hayasaki et al., 2003) and MALDI-TOF (Behrens et al., 2003; Kruger et al., 2000) were used to characterize the degree of polymerization and structure of proanthocyanidins. The lyophilized proanthocyanidin fraction of a pear juice was investigated through mass spectroscopy. After extraction, the purified condensed tannin fraction was first initiated by ESI-MS and MALDI-TOF techniques and the obtained results are discussed below.

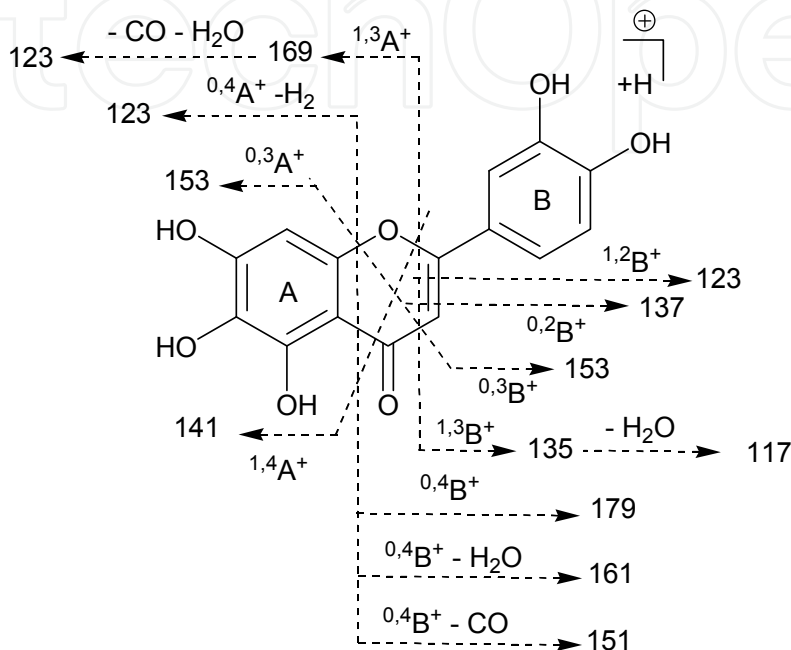


Fig. 9. CID MS-MS data and proposed fragmentation cleavage of the  $[M+H]^+$  ion of compound 9 aglycone.

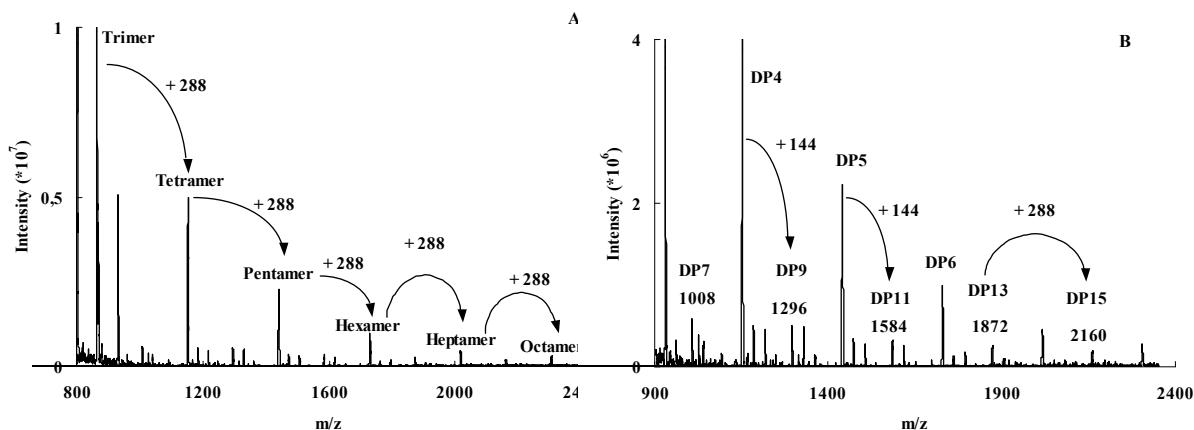


Fig. 10. ESI-MS spectra showing monocharged (A) and doubly charged (B) procyanidin ions.

ESI-MS analysis was performed in the negative ion mode as proanthocyanidin molecules are thereby better detected than in the positive ion mode due to the acidity of the phenolic protons. They are also more negatively charged as the chain length increases. An example of the obtained results is shown in figure 10.

Among the observed peak signals, figure 10A showed molecular ion species consistent with procyanidin oligomers containing singly linked units. A first series of abundant ions separated by 288 Da were observed from  $m/z$  865 to 2306 Da corresponding to the molecular masses of procyanidins with DP3 to DP8. Indeed these signals could be interpreted as  $[M-H]^-$  ion peaks of trimeric ( $m/z$  865 Da), tetrameric ( $m/z$  1153 Da), pentameric ( $m/z$  1441 Da), hexameric ( $m/z$  1729 Da), heptameric ( $m/z$  2017 Da), and octameric ( $m/z$  2305 Da) procyanidins respectively. In addition to the signals indicated above, other less intense signals were also observed in the higher  $m/z$  region values. These peaks could correspond to nonameric ( $m/z$  2593 Da), decameric ( $m/z$  2881 Da), and undecameric ( $m/z$  3169 Da) proanthocyanidins or to doubly charged ions  $[M-2H]^{2-}$  of DP6, DP8, DP10, DP12, DP14, DP16, DP18, DP20, and DP22 species.

Mass spectra also proved evidence for a series of compounds that are 144 mass units higher than those described above. Compounds of this series were separated by 288 mass units with the most intense signals at  $m/z$  1008, 1296, 1584, 1872, and 2160 Da (Figure 10B). These signals were attributed to doubly charged ions due to their narrower signal width compared to the singly charged species. Existence of the doubly charged ions was proven by the presence of additional signals that can be unambiguously attributed to the doubly charged ions  $[M-2H]^{2-}$  of odd polymerization degree, starting from DP7. Such multiply charged species are reported to be more frequently observed in ES-MS and became more intense as the molecular weight increases, probably as a result of longer chain length which allows a better charge separation, thus minimizing the electrostatic repulsive forces. The ion peaks at  $m/z$  1008, 1296, 1584, 1872, and 2160 Da were attributed to the doubly charged species of heptameric, nonameric, undecameric, tridecameric, and pentadecameric procyanidins respectively. No clear multiply charged species beyond the doubly charged ones were detected, presumably because of the lower concentration of larger tannin molecules. However, the apparent decrease of polymer concentration as the molecular weight increases may also be due to an increased dispersion of signal among variously charged ions, including large ones that cannot be detected.

This study shows the importance of ESI-MS analysis in determining the molecular weight of condensed tanins revealing the presence of various oligomeric proanthocyanidins detected as singly and doubly charged ions. However, the limited range imposed by the quadrupole analyzer as well as the easy generation of multiple ions for the larger molecules, inducing peak dispersion and frequent overlapping, result in an increased difficulty of interpretation and quantification of the signals due to higher DP procyanidins.

In order to overcome the problem related to the detection of higher molecular weight proanthocyanidins with a good precision, MALDI-TOF analysis was used. Since its introduction this technique has revealed itself as powerful method for the characterization of synthetic and natural polymers and has been recently introduced for the analysis of condensed tannins in food science (Kruger et al., 2000).

The obtained MALDI-TOF mass spectra of the studied polymeric mixture, recorded as sodium adducts in the positive reflectron ion mode and showing a series of repeating procyanidin polymers. The polymeric character is reflected by the periodical occurrence of peak series representing different chain lengths. The obtained results indicated that pear juice condensed tannins are characterized by mass spectra with a series of peaks with

distances of 288 Da corresponding to a mass difference of one catechin/epicatechin between each polymer. Therefore prolongation of condensed tannins is due to the addition of catechin/epicatechin monomers.

Higher molecular weight ions but with significantly less signal intensity were also observed and were attributed to procyanidin consisting of 20 to 25 flavanol units. These observations fully corroborated the interpretation accorded to the ESI-MS data and demonstrated that both techniques were comparable in usefulness for the analysis of low to moderate size proanthocyanidin polymers.

For the condensed tannins indicated above, each peak was always followed by mass signals in a distance of 152 Da corresponding to the addition of one galloyl group at the heterocyclic ring C. Thus peak signals corresponding to monogalloylated derivatives of various procyanidin oligomers were easily attributed. No procyanidin containing more than one galloyl group was detected. Therefore, MALDI-TOF mass spectrometry indicates the simultaneous occurrence of pure procyanidin polymers and monogalloylated polymers. This showed that only monogalloylation occurs in pear juice procyanidin oligomers. To our knowledge, this is the first mass spectrometric evidence confirming the existence of galloylated procyanidin oligomers in pear fruits.

#### 2.4 Antioxidant properties of natural and synthesized compounds

Free radicals are known to be a major factor in biological damages, and DPPH° has been used to evaluate the free radical-scavenging activity of natural antioxidants (Yokozawa et al., 1998). DPPH°, which is a molecule containing a stable free radical with a purple color, changes into a stable compound with a yellow color by reacting with an antioxidant which can donate an electron to DPPH°. In such case, the purple color typical of the free DPPH° radical decays, a change which can be followed spectrophotometrically at 517 nm. This simple test can provide information on the ability of a compound to donate an electron, the number of electrons a given molecule can donate and on the mechanism of antioxidant action. In cases where the structure of the electron donor is not known (e.g. a plant extract), this method can afford data on the reduction potential of unknown materials. The DPPH° test is a very convenient method for screening small antioxidant molecules because the reaction can be observed visually using common TLC and also its intensity can be analysed by simple spectrophotometric assays (Sanchez-Moreno et al., 1998). The DPPH° radical is scavenged by antioxidants through the donation of hydrogen to form the stable reduced DPPH molecule.

Because of the ease and convenience of this reaction, this technique was thus used for exploring the antioxidant activity of the natural and synthesized compounds described above. The natural isolated compounds described in the first part of this chapter were tested for their antioxidant scavenging effects on DPPH radical and their activity was compared to the synthetic antioxidant BHT and quercetin used as positive control. The free radical scavenging activity is usually expressed as percentage of DPPH° inhibition but also by the antioxidant concentration required for a 50 % DPPH° reduction (IC<sub>50</sub>). IC<sub>50</sub> value is considered to be a good measure of the antioxidant efficiency of pure compounds and extracts.

The obtained results are summarized in figure 11. It showed that 6-hydroxyluteolin derivatives **8** (6.6  $\mu\text{M}$ ) and **9** (7.1  $\mu\text{M}$ ) were the most potent radical scavenging compounds. The little differences found in the radical scavenging activities among these compounds is may be due to the presence of an additional glucose moiety in compound **8**. Among the flavonoid derivatives, eriodictyol- and luteolin-diglucosides **11** and **10** also showed strong activity compared to the positive control (7.8 and 12.2  $\mu\text{M}$ ). Figure 11 also showed that the four phenylethanoids **12-15** possessed the ability to act as a hydrogen donors with an  $\text{IC}_{50}$  around 12-15  $\mu\text{M}$  due to their ortho-dihydroxy structures. The results showed that acteoside, isoacteoside and forsythiaside exhibited higher activity than the positive control BHT, but there was no significant difference between them ( $\text{IC}_{50}$  around 12  $\mu\text{M}$ ). Within the phenylethanoid family compound **15** (15.5  $\mu\text{M}$ ) was slightly less active than the three others. The obtained results are in agreement with previous reports, where phenyl propanoid glycosides are described as potent antioxidant agents (Aligiannis et al., 2003; Wang et al., 1996), as well as flavonoids (Bors et al., 1990) due to their catechol groups. On the other hand, figure 11 showed that the iridoid derivatives **2-7** exhibited moderate to weak DPPH-scavenging activities compared to the tested flavonoids and phenylethanoids.

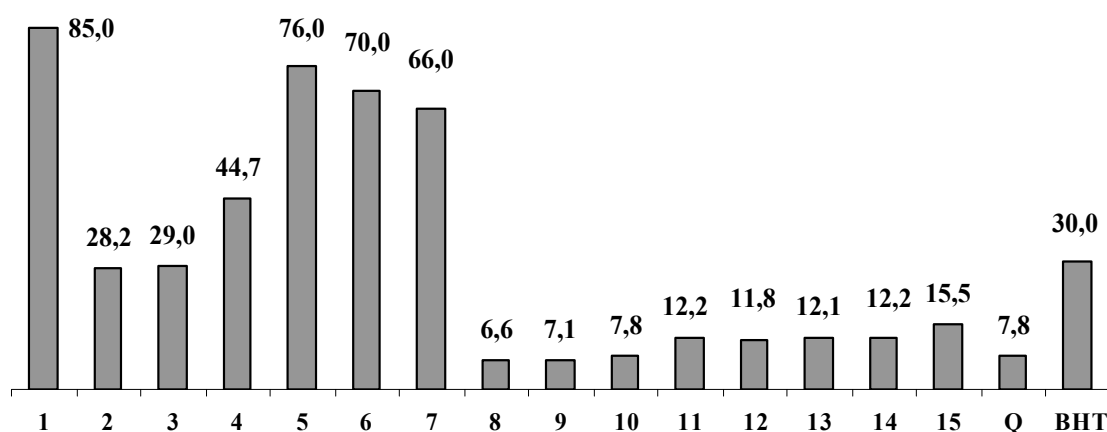


Fig. 11.  $\text{IC}_{50}$  ( $\mu\text{M}$ ) of the natural compounds **1-15** compared to that of quercetin (Q) and BHT.

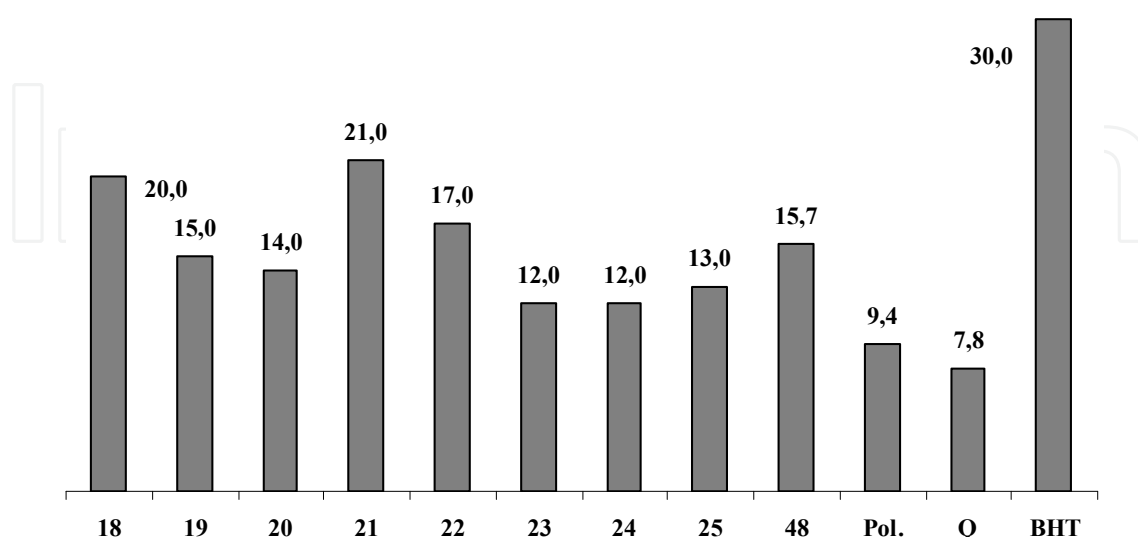


Fig. 12.  $\text{IC}_{50}$  ( $\mu\text{M}$ ) of the hemisynthesized polyphenols and the polymeric fraction (Pol.) compared to that of quercetin (Q) and BHT.

The results concerning the antioxidant activity of the synthesized modified (+)-catechin using the DPPH° method are summarized in Figure 12 which showed that the new flavan-3-ol derivatives are potent free radical scavenging agents in the DPPH° free radical assay. It can be noticed that some of the tested compounds were more efficient than (+)-catechin **18** and BHT. Compound **25** is clearly the most efficient of the new tested molecules. Among all the tested compounds, only compound **21** with a methyl group as substituent was less active than (+)-catechin.

The proanthocyanidins pear juice fraction investigated above through ESI-MS and MALDI-TOF analysis was tested in an *in vitro* free radical scavenging assay as well as the monomeric catechin and its dimer B3 and their antioxidant power were compared to BHT and quercetin used as positive control. The obtained results are summarized in Figure 12. It showed that the polymeric procyanidin was the most potent radical scavenging fraction followed by the dimer B3 and catechin. The polymeric procyanidin fraction and the dimer B3 are as effective as quercetin and significantly better than BHT.

### 3. Conclusion

This chapter showed the importance of phenolic compounds as one of the largest and most widespread class of plant constituents occurring throughout the plant kingdom. The use of a combination of chromatographic and spectroscopic methods, allowed the purification and structural elucidation of several compounds and their capacity to scavenge the stable DPPH° free radical was evaluated.

The use of various synthesis pathways allowed the hemisynthesis of derivatized monomeric and dimeric flavanols. The obtained results showed that the introduction of a substituent onto position 8 of (+)-catechin yielded compounds with improved antiradical efficacy in solution. The usefulness of mass spectrometry in the analysis of polyphenols was demonstrated on a flavonoid glycoside and a polymeric condensed tannin fraction. Detailed informations were obtained through tandem mass spectrometry (MS/MS) in combination with collision-induced dissociation (CID) and various oligomeric procyanidins with increasing polymerization degree were thus detected. The antioxidant activity of the studied polymeric fraction was also investigated.

In summary, this chapter showed that the field of polyphenols remains an open research area of a good interest. Given their importance in food industry, it is interesting to know which factors can influence their use and in which manner this could be achieved. It is noteworthy to mention that the possible use of the studied phytochemicals could not be envisaged without taking into account the toxicological aspect and current legislative rules, in addition to the influence of these compounds on the organoleptic properties of food like taste, color, odor, and stability.

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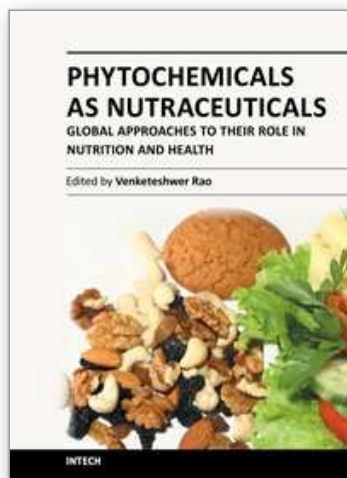
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## **Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health**

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Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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