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Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans

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The antioxidant activity, in terms of radical scavenging capacity, of altogether 15 different lignans was measured by monitoring the scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The effect of differences in skeletal arrangement or the degree of oxidation of the lignans was investigated in a structure–activity relationship study. A large variety in the radical scavenging capacities of the different lignans was observed and related to some structural features. Lignans with catechol (3,4-dihydroxyphenyl) moieties exhibited the highest radical scavenging capacity, while the corresponding guaiacyl (3-methoxy-4-hydroxyphenyl) lignans showed a slightly weaker scavenging capacity. In addition, the butanediol structure was found to enhance the activity, whereas a higher degree of oxidation at the benzylic positions decreased the activity. Additionally, the readily available lignans (−)-secoisolariciresinol, a mixture of hydroxymatairesinol epimers and (−)-matairesinol were studied in more detail, including kinetic measurements and identification of oxidation products in the reactions with DPPH and ABAP (2,2-azobis(2-methylpropionamidine) dihydrochloride. The identification of reaction products, by GC–MS, HPLC–MS and NMR spectroscopy, showed that dimerisation of the two aromatic moieties was the major radical termination reaction. Also, the formation of adducts was a predominant reaction in the experiments with ABAP. The kinetic data obtained from the reactions between the lignans and DPPH indicated a complex reaction mechanism.

Introduction

Natural phenolic compounds have recently received much attention due to their antioxidant properties. In foods, polyphenolic substances protect against the oxidation of unsaturated fatty acids, and other oxidative processes, which lead to off-flavours and to the formation of undesired chemical components causing nutritional alterations and deterioration of the foods.[1] The growing interest in natural antioxidants of plant origin is also due to the fact that epidemiological studies have indicated, although with some controversial results, that the dietary intake of phenolic compounds is associated with a lower risk of age-related health problems including cancer and coronary heart diseases.[2–4] Therefore, natural phenolic compounds may be used, not only in food protection, but also as nutra- and pharmaceuticals. Also, the demand for natural antioxidants has increased because of questions about the long-term safety and negative consumer perception of the commonly used synthetic antioxidants BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisol).

There is a general opinion among scientists that natural phenolic antioxidants are health-promoting substances and that their antioxidant mechanisms and their biological activity should be investigated at a fundamental scientific level.[5] The main objective of these studies has been to find the correlation between the antioxidant activity and the prevention of diseases. However, several questions need to be answered. What is a good antioxidant, how does it work, and how does it function in the human body? Are certain biological effects of natural phenolic compounds correlated with their antioxidant activity? Many of these questions are gradually being answered by scientists in the field of medicine, however, some of the answers need to be supplied by chemists able to study the mechanisms of pure compounds at the molecular level.

One of nature's most readily available and most valuable sources of phenolic antioxidants is wood, especially the wood knots of several tree species.[6,7] The amount of extractable phenolic compounds in, for example, Norway spruce (*Picea abies*) knotwood can be up to 30% of the dry weight, almost exclusively consisting of lignans.[6] Lignans are biologically active polyphenolic compounds, which are defined by the β–β linkage between two phenylpropane units or between their biogenetic equivalents. These compounds are found in the plant kingdom in various structures with different degrees of oxidation of the side chain or with differences in the aromatic substitution pattern.[8] The main lignans found in knotwood extracts are, for example, hydroxymatairesinol (**1**), secoisolariciresinol (**3**), nortrachelogenin (**4**), pinoresinol (**5**), and lariciresinol (**6**), of which some are important dietary lignans found also in our everyday diet. Methods for the separation of knots and for isolation of lignans in wood pulping processes have recently been developed, making these natural antioxidants available on a large scale for various applications.[6,9]

The antioxidant potency and the radical scavenging capacity of knotwood extracts have recently been studied.[10] However, correlations between the structures and the antioxidant effects were not studied, and no mechanisms of antioxidant action were discussed.

According to their mode of action, natural antioxidants can be classified as primary (chain-breaking) antioxidants, which can react directly with lipid radicals and convert them into stable products, or as secondary (preventive) antioxidants, which can lower the rate of oxidation by different mechanisms. Primary antioxidants most often act by donating a hydrogen atom,

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while secondary antioxidants may act by binding metal ions able to catalyse oxidative processes, by scavenging oxygen, by absorbing UV radiation, by inhibiting enzymes or by decomposing hydroperoxides.[1] It is known that different natural phenolic compounds function as both primary and secondary antioxidants by different mechanisms.[11,12]

The free radical scavenging effect of phenolic compounds has mostly been ascribed to the ability of the compound to donate a phenolic hydrogen radical to the free radical. In turn, the phenolic compound forms a stabilised phenoxyl radical, while the free radical is scavenged. This reversible radical trapping stage, which is highly dependent on the molecular structure of the phenolic compound and the bond dissociation energy of the phenolic O–H bond, is followed by a termination stage. In the second stage the generated phenoxyl radical combines with another radical species, to produce stable termination products. The self-redox reaction (disproportionation) of the phenolics should also be considered as an alternative termination stage. Structural identification of these termination products, as well as kinetic studies of the radical reactions, may provide us with an insight into the antioxidant mechanisms involved at the molecular level.

The antioxidant activity (chain breaking) of natural antioxidants can be expressed in terms of radical scavenging capacity, by reacting the antioxidant with a specific radical under controlled conditions. This reaction is fundamentally the same as radical-mediated lipid peroxidation and other radical-mediated reactions *in vivo*. Monitoring of either the decrease of the radical or the antioxidant, or the formation of products can be used for assessing the antioxidant activity. One well-established method for determining the antioxidant activity of various phenolic compounds is the spectrophotometric monitoring of the concentration of the highly coloured and stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fig. 1).[13] During recent years, a tremendous increase in the number of publications reporting the antioxidant activity of lignans or lignan-rich extracts can be noticed. However, only a few studies of pure lignans using the DPPH method have been published.[14–18] The results of these studies are difficult to compare due to differences between the compounds tested, the initial concentrations, the reaction times, and the ways of expressing the antioxidant activity.

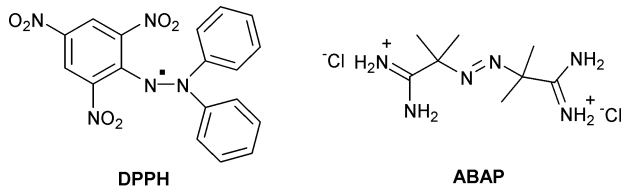


Fig. 1 Structures of the stable free radical DPPH and the radical initiator ABAP.

Yamauchi and co-workers recently reported the antioxidant activity of synthesised oxygenated furofuran- and olivil-type lignans, using a Tween 20 micelle system.[19,20] It was shown that a tertiary hydroxyl group on the tetrahydrofuran ring (aliphatic part) affected the antioxidant activity.

However, in order to correlate the antioxidant effects with structural features, and to elucidate the mechanisms behind the antioxidant effects, it is necessary to collect more data from different lignans. In this study, we have investigated and compared the radical scavenging capacity of several natural and semisynthetic lignans (of different oxidation degrees) using the DPPH method. For some lignans, the kinetics of the reaction was studied in more detail.

We propose here a general antioxidant mechanism, based on the products formed in the reactions between the lignans and the free radical DPPH. In addition to studies with DPPH, the lignans were reacted with the water-soluble radical initiator ABAP (2,2-azobis(2-methylpropionamidine) dihydrochloride) (Fig. 1) under aqueous conditions. Contrary to the nitrogen-centered stable free radical of DPPH, ABAP undergoes thermal decomposition to carbon-centered radicals.

However, it should be kept in mind that both the free radical DPPH and the radical from ABAP are synthetic radicals used as hydrogen radical acceptors, and that peroxyl radicals involved in natural oxidation processes may show rather different electronic demands, resulting in other kinds of reactions and termination products.

Results and discussion

For large-scale reactions and for a more detailed mechanistical study, the three most common and readily available lignans hydroxymatairesinol (**1**), matairesinol (**2**) and secoisolariciresinol (**3**) were chosen (Fig. 2). These lignans were treated either with DPPH in THF or with ABAP in aqueous THF, and the outcomes of the reactions were investigated using chromatographic and spectroscopic methods. Azo compounds such as ABAP are radical initiators, which undergo thermal unimolecular decomposition into nitrogen gas and carbon radicals. The rate of the decomposition can be controlled by temperature, but the stoichiometry of the reaction cannot be totally controlled, because of the partial recombination of two carbon radicals. However, azo compounds have previously been successfully used in antioxidant studies of various natural products.[21,22] For kinetic studies and investigation of reaction stoichiometry, the stable free radical DPPH was used conveniently.

The reactions were performed with 1–3 equivalents of DPPH or ABAP. Preliminary experiments revealed that a larger amount of the radicals afforded complex mixtures and polymeric products, and that the primary products and information on the initial mechanisms were destroyed. Under the conditions used, approximately 30% of the starting material was normally unreacted. Despite the fact that both products and starting material were recovered from the reaction, a large amount of material was lost during the reaction and workup, which indicated either polymerisation or decomposition of the starting material. In the reactions with DPPH, the dominating products were identified as 5–5′-coupled lignan dimers (Scheme 1, pathway a, and Scheme 2). After fractionation by column chromatography, the dimers were analysed by HPLC–MS, which gave a single broad peak containing chromatographically inseparable compounds with m/z 745 $[M - H]^-$ for **1** and m/z 713 $[M - H]^-$ for **2**, respectively. EIMS data of the matairesinol dimers (**16**) gave m/z 714 and the fragmentation peaks m/z 493 and m/z 271, the latter arising from the 5–5-coupled moiety of the guaiacyl units. The ^{1}H-NMR data of these dimers showed typical signals for one of the aromatic rings, whereas in the other ring the doublet of H-5 or H-5′, and the corresponding coupling of H-6 or H-6′, had disappeared. Correlation spectroscopy (COSY, HMBC, HSQC) facilitated the identification of the signals, enabling confirmation of the structure.

In conclusion, the dimers were isomers in a statistical distribution (1 : 2 : 1) of 5–5, 5–5′ = 5′–5, and 5′–5′ coupled lignans and no preference for a particular aromatic ring, in the radical abstraction stage, was therefore observed. The corresponding NMR data of dimeric hydroxymatairesinol (**17**) could not be extracted, because of severe overlapping of the signals from a multitude of isomers due to the fact that the starting material consisted of two diastereomers. Compound **3** gave only small amounts of dimeric structures (**18**) (Scheme 3), which were identified solely by HPLC–MS analyses. The peak corresponding to the dimeric structure gave m/z 721 $[M - H]^-$, which showed a similar fragmentation pattern to the previously reported, tentatively identified, dilignans of **3**.[23]

In addition to the dilignan structures, two-electron oxidised products were separated and identified (Scheme 1, pathway b and Scheme 2) from the reactions with DPPH. **1** yielded oxomatairesinol (**7**) (by comparison with the spectral data of

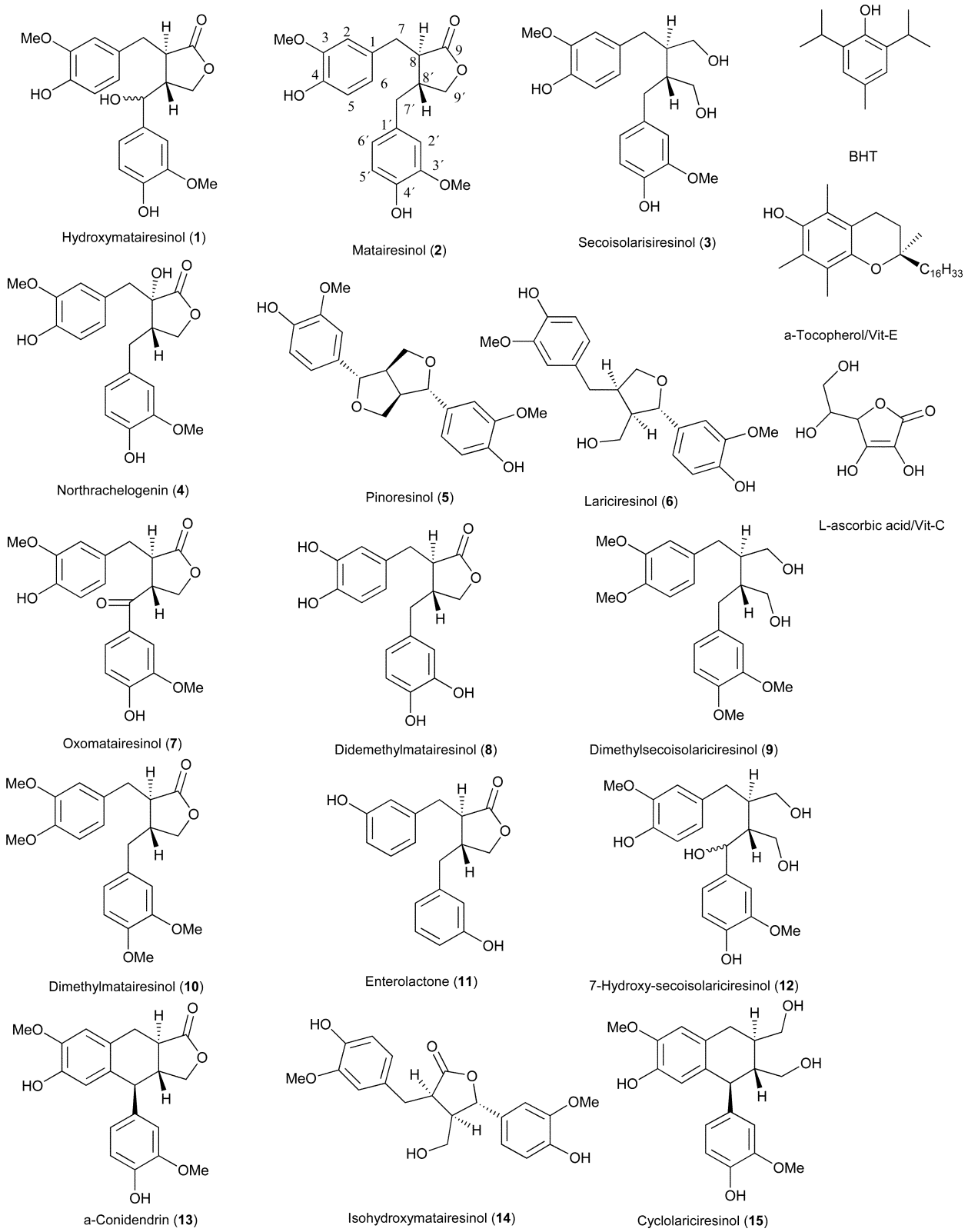
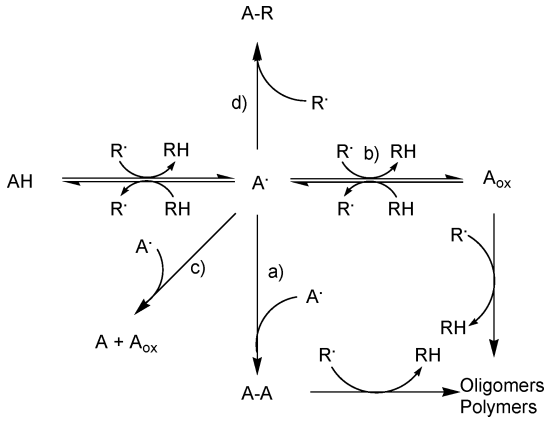


Fig. 2 Structures of the lignans and reference compounds tested.

pure **7**), presumably *via* a quinone methide intermediate (**QM1**). Compound **2** yielded the highly reactive quinone methide structure (**QM2**), as shown by the addition of nucleophiles (methanol) under workup procedures resulting in 7-methoxymatairesinol (**19**) (by comparison with the spectral data of pure **19**). These intermediate quinone methide structures have previously been demonstrated to add nucleophiles.[24] Isomers of 7′,8′-dehydro-7-hydroxymatairesinol (**20**) were also detected as minor reaction products in the reaction of **1**. These compounds have previously been reported as oxidation products in the reaction between **1** and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.[24] **1** and **7** were also detected as minor oxidation products of **2**. In the reactions between DPPH and **3**, no oxidised monomeric structures were detected.

HPLC–ESI–MS analyses, using both normal chromatographic methods and the direct infusion technique (introducing the sample directly into the ion-trap detector) of the crude reaction mixtures and of different fractions, resulted, in

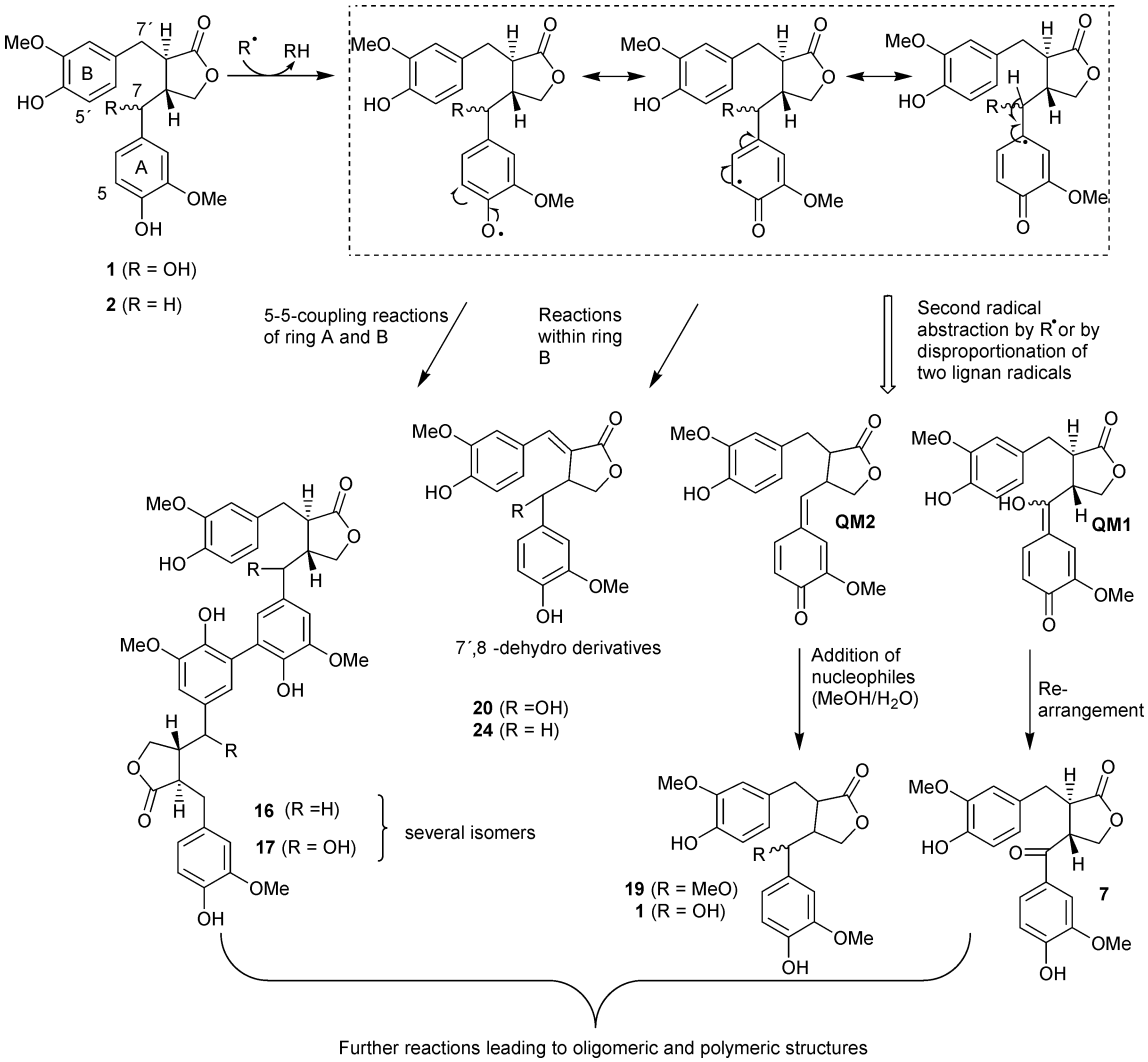


Scheme 1 Possible reaction pathways in the reaction between the antioxidant (AH) and the free radical (R•): a) dimerisation; b) donation of a second hydrogen atom; c) disproportionation of two lignan radicals; d) combination.

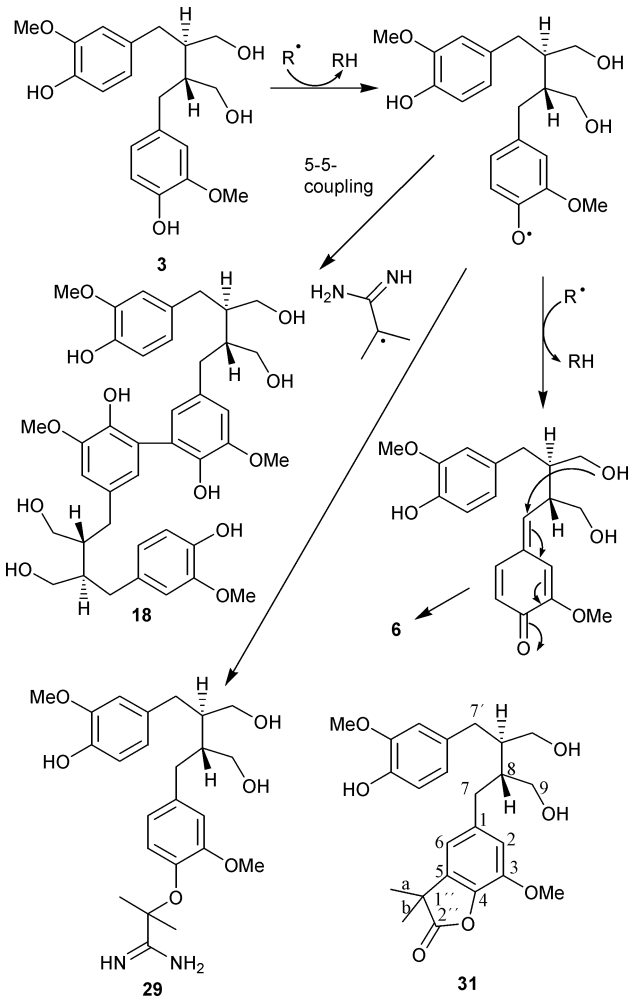
combination with GC–MS analyses, in a tentative identification of several products (solely based on mass spectra). In the reactions with **1**, dehydro derivatives of both dimeric (**21** and **22**) and trimeric structures were inferred from the *m/z* 743 $[M - H]^-$ and 1115 $[M - H]^-$ (didehydro) respectively (Fig. 3). Similarly, dehydro dimeric structures (**23**) with *m/z* 711 $[M - H]^-$ were also detected in the reactions of **2**. Also 7′,8′-dehydromatairesinol (**24**) was tentatively identified by the *m/z* 500 (M^+) peak and the fragments *m/z* 209 and 291 (GC–MS) (Scheme 2).

The reactions with ABAP afforded the same dimeric structures as discussed above, but the predominant products were different adducts according to HPLC–MS analyses. **1** yielded products with *m/z* 457 $[M - H]^-$, 440 $[M - H]^-$, and 441 $[M - H]^-$. The molecular weight of an amidino adduct of **1** (*m/z* 458) was well in accordance with *m/z* 457 $[M - H]^-$ obtained in negative mode. Fragmentation of the molecular ion gave *m/z* 439 $[M - 18]^-$, by the loss of water from **1**, *m/z* 372 $[M - (1\text{-methyl-1-amidinoethyl})]^-$ and *m/z* 354 $[M - H_2O - 1\text{-methyl-1-amidinoethyl}]^-$. These fragmentations allowed us to tentatively identify this adduct as 4′-*O*-(1-methyl-1-amidinoethyl)hydroxymatairesinol (**25**) (Fig. 3). The second adduct was clearly a less polar compound, which eluted several minutes later than the amidino adduct. Upon fragmentation, this compound gave *m/z* 422 $[M - H_2O]^-$ and *m/z* 413 $[M - HCN]^-$ but not $[M - 1\text{-cyano-1-methylethyl}]^-$. This compound was therefore tentatively identified as 5′-(1-cyano-1-methylethyl)-hydroxymatairesinol (**26**) (Fig. 3).

Correspondingly, **2** and **3** yielded both the 2-methyl-2-propionamidino and 1-cyano-1-methylethyl adducts (**27–30**) (Fig. 3 and Scheme 3). Several attempts to isolate these adducts by column chromatography were unsuccessful, and these compounds were identified solely from MS data. The third adduct of **1–3** was, in contrast to the other adducts, also detectable by GC–MS as the trimethylsilyl ether derivative. In addition, we were able to isolate this adduct of secoisolariciresinol. GC–MS analyses showed the molecular ion at *m/z* 646 (trimethylsilyl ether), and HPLC–MS at *m/z* 429 $[M - H]^-$. HRMS analyses gave *m/z* 430.1989 with the composition $C_{24}H_{30}O_7$. ^{1}H NMR



Scheme 2 Reaction mechanisms of **1** and **2** with DPPH, based on the detection of reaction products.



Scheme 3 Reaction products formed in the reaction between **3** and ABAP.

data showed two methyl groups at $\delta = 1.43$ ppm. These methyl groups were correlated to C-5, to a carbonyl group, and to a quaternary carbon at $\delta = 43.9$ ppm in the HMBC spectrum. This compound was thereby identified as the dimethyl furanone derivative of secoisolariciresinol (**31**) (Scheme 3). Likewise **3**, **1** and **2** yielded the furanone derivatives **32** and **33** (Fig. 3). However, due to the lack of symmetry in the parent lignan, **32** and **33** were present as several regio- and stereoisomers. This was shown both by splitting of the chromatographic peaks and by complex overlapping in the NMR spectra. Unfortunately, we were unable to separate these isomers by chromatography, and the structures of **32** and **33** could not be verified by NMR spectroscopy. The formation of similar furanone adducts in the reactions between catechins and azobisisobutyronitrile (AIBN) have previously been reported.[22d] In our experiments, we could not detect 4-*O*-(1-cyano-1-methylethyl) or 5-(1-methyl-1-amidinoethyl) adducts. This indicates that the 5-amidino adduct readily rearranges to the cyano and furanone derivatives. The loss of NH_3 and the transformation into the furanone derivative seems to be promoted by the adjacent hydroxyl group. A reasonable explanation may be the neighboring group participation, in which protonation of the amidino moiety by the hydroxyl group promotes the loss of an ammonium ion, resulting in the formation of a cyano group. The cyano group can then be attacked by the phenoxyl group, which resuts in a furanone moiety, as shown in Fig. 4.

In the reactions with ABAP, only small amounts of oxidised monomeric lignan structures were detectable. **1** yielded detectable amounts of **7** and **3** gave **6** as the major two-electron oxidised product. In the reaction with **2**, no products beside dimers and adducts were identified.

In addition to the identified products, quantitative analyses by long- and short-column GC indicated the presence of unidentified oligomeric and polymeric structures. It was shown, from the quantitative GC analyses and from the isolated amounts of material, that almost 50% of the initial material was undetectable by GC and was lost during column chromatography. Also, numerous still-unidentified minor products were detectable by GC and GC–MS. The fact that considerable amounts of the starting material could be recovered, and that oligomeric and further oxidised products could be detected, indicates that the primary products are targets for further oxidation instead of the parent lignans. By comparison of the reactions with DPPH and ABAP, it seems that the reactions with DPPH resulted in more unidentified polymeric material than the corresponding reactions with ABAP. This can be explained by the fact that DPPH does not form adducts, which is one of the most important termination reactions with ABAP. Instead, polymerisation seems to be the dominating termination reaction with DPPH.

Based on the products formed, it seemed quite safe to assume that lignans react by pathways similar to those previously discussed for phenolic compounds. The initial stage involves a (theoretically) reversible donation of a phenolic hydrogen radical from the lignan to the free radical, which results in a scavenged radical and a lignan phenoxyl radical. The thermodynamic equilibrium favours the reaction, partly because of the highly resonance-stabilised phenoxyl radical. The outcome of the reactions shows that the free electron is readily delocalised to the *ortho* or *para* positions (Scheme 2). One major termination reaction is the radical coupling at position 5, resulting in dimeric structures after intramolecular hydrogen transfer (Scheme 1, pathway a). Alternatively, an abstraction of a second hydrogen radical from the benzylic position occurs, as shown by pathway b (Scheme 1). The second radical abstraction results in a quinone methide, which is unstable and undergoes nucleophilic addition or rearrangement reactions. If the oxidation takes place at the benzylic position located on the same side as the lactone carbonyl group, the compound can rearrange to the α,β-conjugated product, by intramolecular hydrogen transfer.

Combination of the lignan radical and the radical initiator, pathway d (Scheme 1), was only observed in the reactions with ABAP. Azo compounds, with a carbon-centered radical, have previously been reported to undergo such reactions,[22c,25] while DPPH, with a nitrogen-centered radical, reacts poorly. The poor combination ability of DPPH has been ascribed to the steric hindrance at the radical centre.[13b] Disproportionation of two lignan radicals, in which donation of a hydrogen radical from one lignan radical to another occurs, should also be considered as an alternative termination route (Scheme 1, pathway c). Regardless of the pathway, the phenolic system is regenerated and the antioxidant can again scavenge a new radical by donating its phenolic hydrogen atom.

In order to further investigate the mechanisms and to study the kinetic profile and the reaction order of the DPPH reactions with the three most readily availablable lignans **1**, **2**, and **3**, we measured the decrease of the DPPH concentration as a function of time. The initial ratios of antioxidant and DPPH were 1 : 1, 1 : 2, 1 : 3.

The reaction between DPPH and β moles of a lignan (AH) can be described by a reversible reaction as:

$$\mathrm{DPPH} + \beta\mathrm{AH} \underset{k_-}{\overset{k_+}{\rightleftharpoons}} \mathrm{P} \qquad (1)$$

The kinetic data obtained from the reactions of **1**, **2**, and **3** with DPPH, support a complex reaction mechanism, as the kinetics do not support any usually applied kinetic model. The kinetics can be expressed by the exponent law as:where k_+ and k_-

$$r = k_+ c_{\mathrm{AH}} c_{\mathrm{DPPH}}^{2\left(\frac{c_{0,\mathrm{A}}}{c_{0,\mathrm{DPPH}}}\right)^{\alpha}} - k_- c_{\mathrm{P}}$$

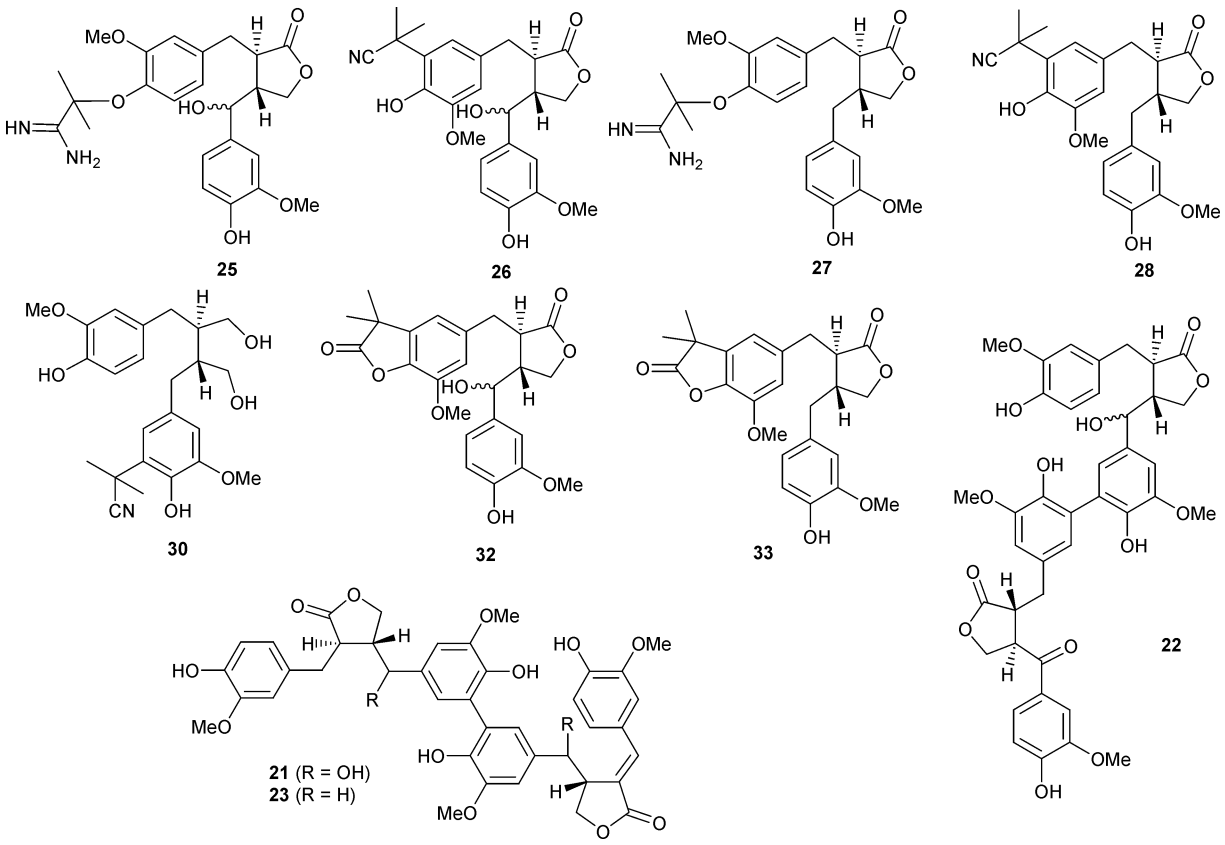


Fig. 3 Proposed structures for the tentatively identified adducts and dehydrodimer products.

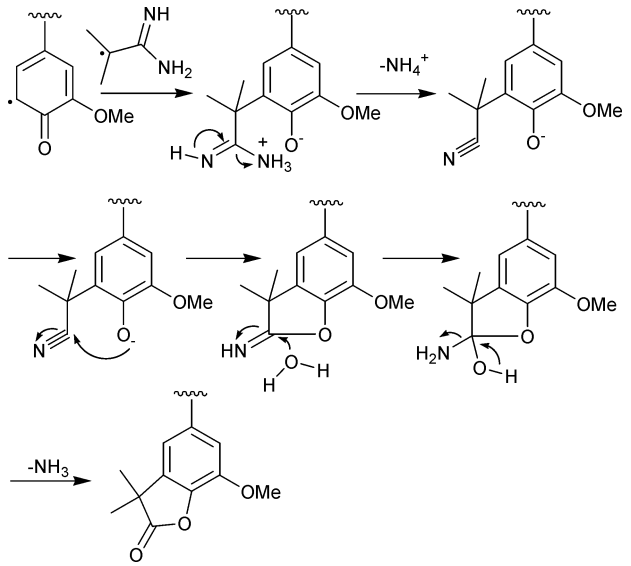


Fig. 4 A possible mechanism for the formation of the 5-(cyanopropyl) and furanone derivatives.

are the forward and backward rate constants, respectively, and a is an exponent. Some initial parameter estimations revealed that the reaction order for the lignan AH and the products P are best described with a reaction order of 1. The reaction order of DPPH was found to be close to 2 but it appeared to change as a function of the initial concentration ratio of the lignan and DPPH ($c_{0,\mathrm{AH}}/c_{0,\mathrm{DPPH}}$). Therefore the reaction order of DPPH was described as $2(c_{0,\mathrm{AH}}/c_{0,\mathrm{DPPH}})^{a}$.

The disappearance of the reactants and the generation of the products can be calculated by the solution of the molar balances for the components:

$$\frac{\mathrm{d}c_{\mathrm{AH}}}{\mathrm{d}t} = -\beta r\frac{\mathrm{d}c_{\mathrm{DPPH}}}{\mathrm{d}t} = -r\frac{\mathrm{d}c_{\mathrm{P}}}{\mathrm{d}t} = r \qquad (2)$$

The differential eqn. (2) was solved numerically with the backward difference method using the Odessa solver in the Modest program package.[26] The differential equation solver operated under a parameter estimation routine, which minimised an objective function, the residual sum of squares:

$$Q = \sum_{t}(c_{\mathrm{DPPH}}(t) - \hat{c}_{\mathrm{DPPH}}(t))^2$$

where c_{DPPH} and $\hat{c}_{\mathrm{DPPH}}$ denote the experimental and predicted activities. A hybrid Simplex–Levenberg–Marquardt algorithm was used in the minimisation of the objective function. The numerical algorithm is included in the program package Modest. The quality of the model fit and the model parameters were tested by calculating the standard deviations of the parameters and the degree of explanation of the model. The estimated 4 parameters (k_+, k_-, a and β) and their estimated values are listed in Table 1.

The fit of the model to experimental data was rather good with degrees of fit, in the range 92–96%. The standard errors of the estimated parameters were about 5%, except for the backward rate constant which obtained a standard error of 22%. The fit of the model to the experimental data are displayed in Fig. 5.

Theoretically, one lignan should scavenge two equivalents of DPPH, by the donation of each of the two phenolic hydrogen atoms. The obtained estimated value for β was approximately 0.5 for all lignans, which agrees well with the above statement. With equimolar concentrations (in fact 1 : 2 excess of radical scavenging groups) the reaction order seems to be 1 for the lignans and 2 for DPPH, and the reaction can be described by the reversible reaction (1). However, when the lignan is treated with an excess of DPPH, the reaction order of DPPH appears to decrease and the concentrations of lignan becomes more significant. This indicates that the reactions do not proceed as in eqn. (1), and that a more complex mechanism with a possible "feedback" activity of the products, disproportionation

Table 1 Estimated parameter values

Compound	$k_+/(\mathrm{l\,mol^{-1}})^{\delta-1}\,\mathrm{s^{-1}}$	$k_-/\mathrm{s^{-1}}$	α	β	$\delta = 2(c_{0,\mathrm{AH}}/c_{0,\mathrm{DPPH}})^{\alpha}$ 1 : 1	1 : 2	1 : 3	Degree of fit (%)
1	8.78×10^{-7}	4.16×10^{-5}	0.15	0.53	2	1.8	1.7	92.4
2	1.28×10^{-6}	1.52×10^{-5}	0.14	0.56	2	1.8	1.7	93.4
3	6.9×10^{-6}	5.8×10^{-6}	0.22	0.46	2	1.7	1.5	96.5

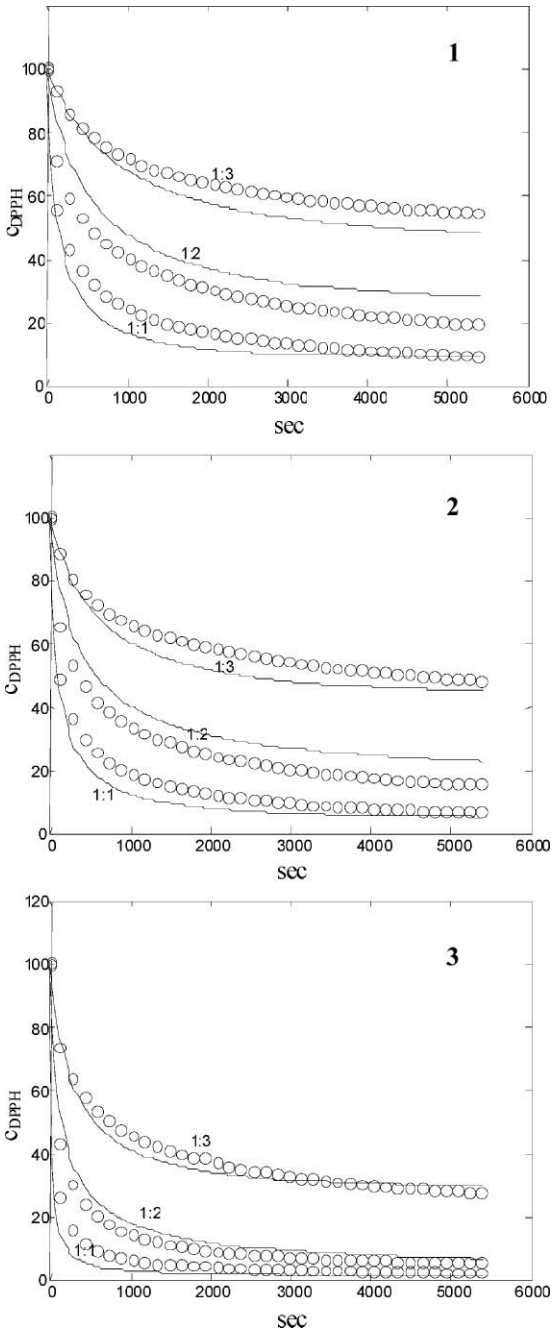


Fig. 5 Fit of model to experimental data (**1** = hydroxymatairesinol, **2** = matairesinol, **3** = secoisolariciresinol). DPPH concentration (initial ratios AH : DPPH 1 : 1, 1 : 2, 1 : 3) as a function of the reaction time (s). — = Model prediction. ○ = Experimental data.

reactions (inter- and intramolecular) and regeneration of active species may be involved. In fact, when the initial ratio was 1 : 8 (AH : DPPH), **3** was able to scavenge 2.5 equivalents of DPPH per phenolic OH group (discussed below). It is clearly shown that the radical scavenging capacity of the lignans exhibits a nonlinear relationship with respect to the initial concentration (Fig. 6).

To further investigate the stoichiometric factors and the scavenging capacity of the lignans, the radical scavenging capacity of altogether 15 different lignans was measured with the DPPH method, essentially as described by Sánchez-Moreno *et al.*[13b] Several different guaiacyl–lignan structures, including dibenzylbutyrolactone (**1**, **2**, **4**, **7**, **8**, **10**, **11**, **13**, **14**) dibenzylbutanediol (**3**, **9**, **12**), tetrahydrofurano- (**6**), furofurano- (**5**), and aryltetralin (**13**, **15**) skeletons, were chosen for a structure–activity relationship study. Additionally, 3,3′-*O*-didemethylated (**8**), 4,4′-methylated (**9** and **10**), and *meta*-hydroxy (**11**) derivatives were included in the study. The concentration of lignans able to reduce by 50% (EC_{50}) the initial concentration of DPPH (*i.e.* 40 μM) was determined from the curve of %-neutralised DPPH *vs.* lignan concentration (Fig. 6). In kinetic studies, the more rapidly the absorbance decreases, the more potent is the antioxidant in terms of hydrogen-donating ability (see Fig. 7). However, when determining EC_{50} values, the rate of the reaction is normally not considered, and the measurements are made when the reaction is virtually finished (90 min). Instead, the stoichiometry, *i.e.* the number of moles of DPPH neutralised per mole of antioxidant, is a more important factor. This number was dependent of the initial molar ratio between DPPH and the antioxidant. When the initial lignan concentration was 10% of that of DPPH this number was 3.4, and at 50% it was 1.5 for **1**. At an EC_{50} of 15.7 μM the value was 2.6 (Table 2). One should therefore clearly state at which concentration, preferably EC_{50}, this number is given.

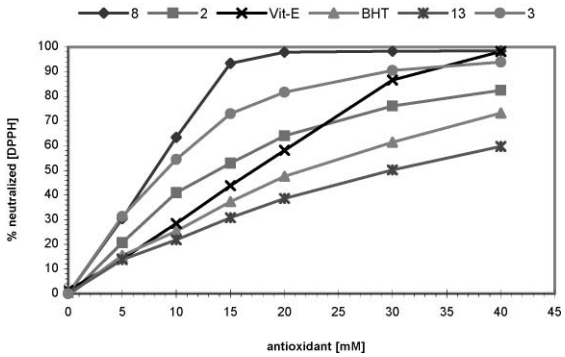


Fig. 6 Percent scavenged DPPH radicals as a function of the lignan concentration, exemplified with compounds **2**, **3**, **8**, **13**, Vit-E and BHT with EC_{50} values ranging from 8.5 to 31.

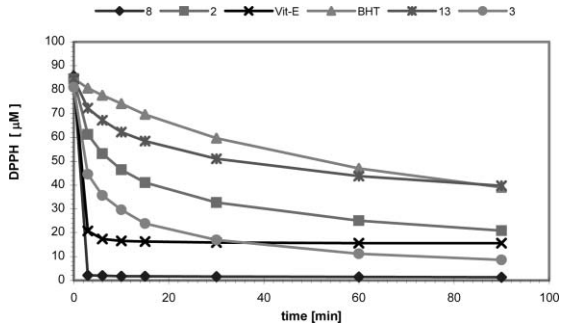


Fig. 7 Examples of the reaction kinetics showing the decrease of [DPPH] as a function of time. Initial concentrations of 80 μM for DPPH and 30 μM for antioxidants were used.

Table 2 Radical scavenging capacity (EC_{50}) of lignans and reference compounds, in order of radical scavenging capacity. DPPH/AH = stoichiometric factor at EC_{50}. $T50\%_{30\,\mu M}$ = the time taken to scavenge 50% of DPPH at 30 μM.

Compound	EC_{50}/μM for AH[a]	DPPH/AH	$T50\%_{(30\,\mu M)}$/min
8	8.5 ± 0.7	4.7	2
3	9.0 ± 1.0	4.5	5
6	10.7 ± 1.2	3.8	8
12	12.7 ± 1.5	3.1	10
15	13.5 ± 0.7	3.0	13
2	14.0 ± 0.0	2.9	16
1	15.7 ± 0.6	2.6	10
Vit-E	17.3 ± 1.5	2.3	2
4	17.7 ± 1.5	2.3	27
5	17.7 ± 0.6	2.3	31
Vit-C	18.7 ± 3.0	2.1	2
BHT	21.0 ± 2.8	1.9	81
13	21.3 ± 2.3	1.9	65
14	26.7 ± 3.8	1.5	90
7	31.3 ± 2.1	1.3	90
11	>500[b]	—	—
10	>500[b]	—	—
9	>500[b]	—	—

[a] Each value represents the mean ± SD of three determinations. [b] Compounds with no significant radical scavenging effect at 500 μM.

The rate of the reaction is also an important parameter when determining the antioxidant activity. This parameter can be taken into account by using the parameter antiradical efficiency (AE), introduced by Sánchez-Moreno *et al.*[13b]

However, the time needed to reach steady state at the concentration corresponding to EC_{50} is necessary for determining AE. In the reactions between lignans (except for **8**) and DPPH, no steady state was observed as the reaction progressed with a continuous decrease of the rate. For antioxidants showing a slow kinetic profile the steady state is difficult to define and, for example, BHT has been reported to reach a steady state after several hours. For antioxidants with intermediate or fast kinetics, the time taken to reach a steady state can easily be determined graphically from time scan experiments, as shown by **8** and Vit-E in Fig. 7. As the determination of the steady state is undoubtedly difficult for the lignans, we simply avoided this by measuring the time taken to scavenge 50% ($T50\%_{30\,\mu M}$) of the initial DPPH concentration (80 μM) by 30 μM of the antioxidant. The $T50\%$ value was used as a comparable measurement for the rate and the efficiency of the antioxidants tested (Table 2).

When comparing the results of the radical scavenging capacity of the different lignans, large differences were observed. The most effective radical scavenger, with an EC_{50} value of 8.5 μM and a stoichiometry of 4.7, was compound **8** (Table 2). **8** possesses four phenolic hydroxyl groups in its catechol moieties. This structural feature is known to have a strong antioxidative effect.[22c,27] However, **3** was almost equally effective as compound **8**. Compound **3** has two *para* phenolic and two primary aliphatic hydroxyl groups, but also two *meta* methoxyl groups due to the guaiacyl substitution pattern in the aromatic rings. By comparison of compound **2** and **3**, it seems that the butanediol structure is more effective than the butyrolactone structure. This is also clearly shown by comparison of **13** with **15**. Again, comparison of **2** and **8** shows that the catechol structure is more effective than the guaiacyl structure. One additional hydroxyl group, either in a benzylic position or adjacent to the carbonyl group, decreased the activity. This can clearly be shown by comparison of **3** with **12**, and **2** with **1** and **4**. Thus, it seems that the nature of the hydroxyl groups is far more important than the number of them. Clearly, a *para* hydroxyl group in combination with a *meta* hydroxyl or a methoxyl group in the aromatic ring is essential for a good radical scavenging capacity, the catechol arrangement being the most effective. Compound **11** was totally inactive although it possesses phenolic hydroxyl groups. However, the *meta* position of the hydroxyl groups does not enable effective resonance stabilisation of a phenoxyl radical throughout the whole benzylic system, which makes this compound unable to effectively scavenge radicals by the proposed mechanism. The dimethylated derivatives **9** and **10** were also completely inactive. This indicates that the possible abstraction of benzylic hydrogen radicals is a secondary process, and that the donation of a benzylic radical by the lignan requires a free *para* phenolic hydroxyl group in the structure. This result also showed that the butanediol structure is not directly involved in the radical scavenging reactions. However, the structures including the diol are more effective than the corresponding butyrolactones, which indicates a synergistic effect by the diol structure.

This effect may be due to the possibility of the primary alcohol group attacking the oxidised benzylic position, resulting in regeneration of the phenolic system, as exemplified by the formation of **6** from **3** (Scheme 3). This intramolecular reaction should be favoured due to the noncyclic aliphatic part of the molecule, which allows a more flexible conformation. Oxomatairesinol (**7**) showed the lowest radical scavenging capacity of the guaiacyl lignans tested. This compound has a fully oxidised oxo-group at one of the benzylic positions, which will decrease the possibility for further oxidation of the molecule. Generally, a higher oxidation degree at the benzylic position will decrease the radical scavenging capacity of the lignans. This is clearly shown by the decreasing activity of compounds **12**, **5**, **13**, **14**, and **7**. According to these results, compound **14** should be a more effective radical scavenger than compound **5**. Also, compound **14** should be comparable to compound **6**. However, the results show that this not the case, and no definitive explanation for the poor scavenging capacity of **14** can be given.

The EC_{50} results show large differences in reaction stoichiometries of the lignans. For example, the number of reduced DPPH molecules per lignan molecule was 4.5 for **3** and 1.3 for **7**, respectively (Table 2). It should be emphasised that these numbers are the stoichiometric factors at the EC_{50} concentration, obtained after 90 min. A multiplication of this value by two should theoretically give the concentration able to reduce 100% of the initial DPPH concentration. However, the results show that the relationship between the percent of reduced radicals and the antioxidant concentration is non-linear (Fig. 5), and it would be erroneous to make this approximation. A concentration twice that of EC_{50}, resulted in 60–75% of scavenged radicals for most of the compounds tested. According to the mechanistic studies, the lignans may donate both phenolic and benzylic hydrogen radicals. Theoretically, this would result in the neutralisation of 4 equivalents of DPPH radicals for a compound bearing two phenolic hydroxyl groups and at least two benzylic hydrogen atoms (*i.e.* guaiacyl lignans). As seen for compound **3**, this compound is able to reduce over 4 equivalents of DPPH, which indicates additional hydrogen radical donating ability. Such reaction stoichiometry has been ascribed to the regeneration of the phenolic system by reactions such as dimerisation.[28] The lignans may be regenerated by several different reactions: 1) dimerisation and polymerisation; 2) combination to form adducts; 3) intramolecular rearrangements to form oxidised structures; 4) intermolecular disproportionation. In combination, these termination and regeneration reactions will lead to a scavenging of numerous radicals by one lignan molecule, as already discussed.

It should be emphasised that most lignans show a slow kinetic behaviour. Also, the activity of most lignans tested in this study can be compared to the activity of the well known synthetic antioxidant BHT. The slow kinetics of both BHT and the lignans is often related to compounds able to scavenge a large number of radicals and to act *via* mechanisms discussed above. Vit-E, which has been assigned an intermediate kinetic behaviour, showed similar behaviour to compound **2** (Fig. 6, Fig. 7 and Table 2).

This study has shown that the commonly found lignans **3**, **6**, **2**, and **1** have a high radical-scavenging capacity. Many of these plant lignans have been proposed to be cancer-preventing agents.[29] However, metabolic studies have shown that these lignans are effectively metabolised to the mammalin lignans enterolactone (**11**) and enterodiol.[30] Interestingly, **11** was completely inactive in this assay, which strongly indicated that if the anticancer effect is correlated to the antioxidant effects *in vivo*, the antioxidant effect is mediated *via* other mechanisms rather than the scavenging of free radicals. Previous studies comparing the antioxidant effects of the plant lignans and the mammalian lignans show that in the majority of the antioxidant assays, the mammalian lignans show weaker effects.[30,31] In accordance with this study, it has previously been indicated that the antitumour effect of mammalian lignans is not due to antioxidant effects.[32]

Experimental

Chemicals and lignans

DPPH (2,2-diphenyl-1-picrylhydrazyl, 97%), DPPHH (2,2-diphenyl-1-picrylhydrazine, 97%), ABAP (2,2′-azobis(2-methylpropionamidine) dihydrochloride, 97%), BHT (2,6-di-*tert*-butyl-4-methylphenol, 99%), L-ascorbic acid (Vit-C, 99%), and α-tocopherol (Vit-E, 97%) were purchased from Aldrich Chemical Co. **1** (Hydroxymatairesinol, 98%, mixture of 7-epimers), **3** ((−)-secoisolariciresinol, 97%), **4** ((−)-northachelogenin, 94%), **5** ((+)-pinoresinol, 95%) and **6** ((+)-lariciresinol, 97%) were isolated from knotwood as described by Willför *et al.*[10] **7** ((+)-oxomatairesinol, 93%), **11** ((−)-enterolactone, 97%), **12** (7-hydroxysecoisolariciresinol, 98%, mixture of 7-epimers), **13** ((−)-conidendrin, 98%) **14** (isohydroxymatairesinol, 96%) **15** ((+)-cyclolariciresinol, 98%) and **2** ((−)-matairesinol) were prepared from **1** as previously described.[24,33–35] Compound **8** ((−)-4,4′-*O*-didemethylmatairesinol, 96%) was prepared from **2** by demethylation with $AlCl_3$ in pyridine, as follows: **2** was dissolved in pyridine and 6 equivalents $AlCl_3$ was added. The reaction was stirred at 60 °C for 5 h, and then poured onto water. The mixture was acidified (pH ∼1) and then extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue was purified with column chromatography to yield **8** as a colourless powder ofter drying under vacuum. The spectroscopic and spectrometric data of **8** were as previously reported.[36] Compound **10** ((−)-dimethylmatairesinol) was obtained by methylation of **2** with MeI/K_2CO_3 in dry acetone.[37] Compound **9** ((−)-dimethylsecoisolariciresinol) was prepared from **10** by reduction with $LiAlH_4$ in THF, essentially as described by Eklund *et al.*[34] The purities of isolated lignans were determined by GC and NMR analyses.

Free radical scavenging by the DPPH method

A freshly prepared solution of DPPH (160 μM) in methanol (2 mL) was mixed with a methanol solution of the pure lignans **1–15** (2 mL) or the reference compounds (BHT, α-tocopherol (Vit-E), or ascorbic acid (Vit-C)) at different concentrations. The initial concentration of DPPH was 80 μM, whereas the concentrations of the antioxidants were 5, 10, 15, 20, 30, and 40 μM in the reaction medium. The mixture was allowed to stand at room temperature in the dark for 90 min and the absorbance at 515 nm was measured at 5, 10, 15, 30, 60, and 90 min with a UV-Vis spectrophotometer (Shimadzu model 160A). The measurements were conducted in triplicate. For determination of EC_{50} values, the values at 90 min were used. The remaining concentration of DPPH was determined from a calibration curve, determined by linear regression. Both DPPH and DPPHH contribute to the total absorbance of the mixture, and at higher concentrations the absorbance of DPPHH cannot be neglected. When combining the calibration curves obtained for pure DPPH and DPPHH, the actual calibration curve was obtained as follows:

$$x_1 = [\mathrm{DPPH}]$$

$$x_2 = [\mathrm{DPPHH}]$$

$$x_3 = x_1 + x_2 = 80 \text{ (total concentration, } \times 10^{-6} \text{ M, both DPPH and DPPHH)}$$

$$\mathrm{abs_{DPPH}} = 0.01\,x_1 + 0.0011$$

$$\mathrm{abs_{DPPHH}} = 0.0004\,x_2 - 0.0014$$

$$\mathrm{abs_{tot}} = \mathrm{abs_{DPPH}} + \mathrm{abs_{DPPHH}} = (0.01\,x_1 + 0.0011) + (0.0004\,x_2 - 0.0014)$$

The actual calibration curve for the DPPH concentration in the reaction mixture was calculated from:

$$\mathrm{abs_{tot}} = (0.01\,x_1 + 0.0011) + (0.0004\,x_2 - 0.0014)$$

which combined with $x_2 = 80 - x_1$ gives:

$$\mathrm{abs_{tot}} = 0.01\,x_1 + (0.0004 \times 80) - (0.0004\,x_1) - 0.0003$$

giving:

$$x_1 = (\mathrm{abs_{tot}} - 0.00317)/0.0096.$$

The percent-scavenged DPPH was first plotted against the concentration of the antioxidant, and the amount of antioxidant needed to scavenge 50% (EC_{50}) of the initial DPPH concentration was then graphically determined from the curve (Fig. 6). The EC_{50} values for the components were expressed in μM and mol mol^{-1} (DPPH/antioxidant).

For comparison of the rates of different antioxidants, the time taken to scavenege 50% DPPH ($T50\%$), when the initial concentration of DPPH was 80 μM and the antioxidant 30 μM, was used (Fig. 7).

Kinetic measurments of the reaction between 1, 2, 3, and DPPH

Solutions of the lignans (**1**, **2**, and **3**) and DPPH were prepared in methanol (deoxygenated by N_2). The solutions were then rapidly mixed and transferred to a cuvette. The initial ratios between AH and DPPH were 1 : 1, 1 : 2 and 1 : 3 (using the initial concentration of 100 μM of DPPH). The decreasing absorbances were spectrophotometrically measured at 515 nm with 20 s intervals. A kinetic model was mathematically fitted to the obtained experimental curves, as described above.

Reactions with DPPH and ABAP

Reactions with DPPH. **1**, **2**, or **3** was dissolved in anhydrous THF. 1–3 equivalents of DPPH was added to the solution and the mixture was stirred for 24 h. The solvent was removed under reduced pressure and the residue was submitted to chromatographic purification (see below).

Reactions with ABAP. The lignans (**1–3**) were dissolved in water–THF (1 : 1) and 1–3 equivalents ABAP was added. The mixture was stirred at 70 °C for 72 h. The reaction mixture was then extracted with EtOAc, the organic phase dried over Na_2SO_4, filtered, and the solvent removed under reduced pressure. The residue was submitted to chromatographic fractionation and purification.

Analyses, identification, isolation, and characterisation of products

Small samples of the crude reaction mixtures were silylated using HMDS and TMSCl in pyridine, and analysed by GC and GC–MS. For GC analyses of lignans, a HP-1 column (25 m × 0.20 mm) was used. For analyses of dimeric compounds, a short HP-1 column (6 m × 0.53 mm) was used. GC–MS (EI) analyses

were performed with an HP 6890–5973 GC–MSD instrument equipped with a HP-1 column (25 m × 0.20 mm). For GC–MS analyses of dimeric lignan structures, an MXT-65TG column (15 m × 0.25 mm) was used. Both retention times and mass spectra were compared with known isolated compounds when available. Underivatised samples of the reaction mixture, or fractions thereof, were also analysed by HPLC–ESI–MS using an Agilent 1100 series HPLC instrument equipped with an Agilent 1100 series LC/MSD Trap-SL detector with an electrospray source operating in the negative mode. The chromatographic conditions were the following: Zorbax Eclipse XDB-8 column (4.6 × 150 mm, 5-Micron), gradient elution (MeCN–H_2O (containing 0.1% acetic acid), 10–80%), flow rate 0.4 ml min^{-1}, and UV detection at 280 nm.

Fractionation and isolation of reaction products was performed by repeated column chromatography (Silica gel 60), using gradient elution with $CHCl_3$–MeOH (1–10% MeOH). The obtained fractions, containing either pure components or mixtures of products, were analysed as above, and by means of ^1H NMR (600.13 MHz), ^{13}C NMR (150.90 MHz), and correlation spectroscopy using a Bruker Avance 600 spectrometer.

Matairesinol dimers (16). HRMS (EI) *m/z* calculated for $C_{40}H_{42}O_{12}$ 714.2676, found 714.2680 (M^+). EIMS *m/z* 714 (M^+, 65%), 493 (15), 358 (8), 271 (17), 220 (6), 175 (9), 137 (100). ESI–MS^n (negative mode, the most abundant peak further fragmented): *Isomer I*: *m/z* 713 (100%) $[M - H]^-$, MS^2 of 713: 698 (40), 681 (89), 669 (38), 561 (100), MS^3 of 561: 517 (16), 477 (100), 465 (26). *Isomer II*: *m/z* 713 (100%) $[M - H]^-$, MS^2 of 713: 698 (36), 695 (100), 681 (50), 561 (31), MS^3 of 695: 545 (12), 543 (14), 517 (57), 477 (100), 465 (48). *Isomer III*: *m/z* 713 (100%) $[M - H]^-$, MS^2 of 713: 695 (100), 681 (10), 574 (7), 478 (8), MS^3 of 695: 651 (65), 619 (12), 574 (100), 559 (10). δ_H (*a mixture of isomers*, 600 MHz, $CDCl_3$, 25 °C) 2.36–2.61 (m, H-7′), 2.36–2.51 (m, H-8′), 2.45–2.60 (m, H-8), 2.77–2.92 (m, H-7), 3.65–3.84 (OMe × 8), 3.83–3.90 (m, H-9b), 4.13–4.18 (m, H-9a), 6.33–6.37 (d, *J* = 2.0 Hz, H-2′ × 4), 6.41–6.43 (dd, *J* = 2.0, 8.0 Hz, H-6′), 6.49 (d, *J* = 2.0 Hz, H-6′†), 6.52 (dd, *J* = 2.0, 8.0 Hz, H-6), 6.56–6.57 (d, *J* = 2.0 Hz, H-6†), 6.58–6.59 (m, H-2), 6.67 (d, *J* = 8.0 Hz, H-5′), 6.70–6.71 (d, *J* = 8.0 Hz, H-5). δ_C (151 MHz, $CDCl_3$, 25 °C) 34.55/34.58/34.59/34.63 (C-7), 38.24/38.28/38.30/38.32 (C-7′), 40.92 × 2/41.13/41.15 (C-8′), 46.54/46.57/46.69/46.70 (C-8), 55.82/55.85/55.95/55.96/56.16 × 2/56.19/56.21 (MeO/MeO′), 71.40/71.42/71.46/71.47 (C-9), 110.51/110.53/111.13/111.14/111.20/111.24/111.74 × 2 (C-2/2′), 114.27/114.29/114.52/114.56 (C-5/5′), 121.33/121.39/122.16/122.17 (C-6/6′), 123.48/123.56 (C-6′†), 123.83/124.04/124.10/124.22 (C-5/5′†), 124.32/124.34 (C-6†), 129.44/129.48/129.64/129.65/129.66/129.69/129.87 × 2 (C-1/1′), 141.58/141.59/141.60/141.62 (C-4/4′†). 144.46/144.47/144.61 × 2 (C-4/4′), 146.73/146.75/146.87 × 2 (C-3/3′), 147.44/147.48/147.51/147.56 (C-3/3′†), 178.93/178.98/178.99 × 2 (C-9).

Hydroxymatairesinol dimer (17). EIMS *m/z* 746 (M^+, detectable), 710 (65), 652 (10), 478 (15), 374 (26), 356 (40), 232 (28), 137 (100). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 745 (100%) $[M - H]^-$, MS^2 of 745: 727 (100), 709 (44), MS^3 of 727: 709 (100), 573 (33), 559 (38), 489 (16).

Secoisolariciresinol dimer (18). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 721 (100%) $[M - H]^-$, MS^2 of 721: 689 (53), 675 (100), 539 (40), 525 (95), 507 (58), 495 (96), 481 (59), MS^3 of 675: 657 (100), 645 (80), 505 (92), 494 (61), 449 (59).

Hydroxymatairesinol 7,8-dehydro dimer (21). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 743 (100%) $[M - H]^-$, MS^2 of 743: 725 (100), 707 (47), 681 (88), 575 (34), 557 (14), MS^3 of 725: 707 (68), 681 (100), 663 (29), 515 (7).

Hydroxymatairesinol 7-dehydro(oxo) dimer. (22). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 743 (100%) $[M - H]^-$, MS^2 of 743: 699 (2), 595 (100), 523 (16), MS^3 of 591: 573 (54), 529 (100), 507 (48), 497 (18), 423 (18).

Matairesinol 7,8-dehydro dimer (23). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 711 (100%) $[M - H]^-$, MS^2 of 711: 667 (100), 635 (18), 575 (14), 491 (46), MS^3 of 667: 652 (30), 635 (100), 491 (30), 357 (42), 355 (36), 311 (50).

7,8-Dehydromatairesinol (24). EIMS (silyl ether) *m/z* 500 (M^+, 3%), 364 (3), 291 (20), 209 (100). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 355 (100%) $[M - H]^-$, MS^2 of 355: 271 (7), 233 (23), 219 (95), 204 (20), 175 (41), 160 (100), MS^3 of 160: 147 (100).

Hydroximatairesinol 4′-*O*-(1-amidino-1-methylethyl) adduct (25). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 457 (100%) $[M - H]^-$, MS^2 of 457: 439 (39), 383 (21), 372 (100), 354 (43), 310 (14), MS^3 of 372: 354 (67), 328 (72), 310 (100), 236 (49).

Hydroxymatairesinol 5′-(1-cyano-1-methylethyl) adduct (26). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 440 (100%) $[M - H]^-$, MS^2 of 440: 422 (40), 413 (65), 395 (100), MS^3 of 395: 380 (100), 351 (79), 336 (34), 321 (32), 198 (32).

Matairesinol 4-*O*-(1-amidino-1-methylethyl) adduct (27). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 441 (100%) $[M - H]^-$, MS^2 of 441: 356 (100), 341(3), MS^3 of 356: 341 (100), 312 (12), 297 (52), 193 (8), 121 (14).

Matairesinol 5-(1-cyano-1-methylethyl) adduct (28). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 424 (100%) $[M - H]^-$, no further fragmentation.

Secoisolariciresinol 4-*O*-(1-amidino-1-methylethyl) adduct (29). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 445 (100%) $[M - H]^-$, MS^2 of 445: 360 (100), MS^3 of 360: 345 (97), 312 (38), 297 (59), 178 (37), 165 (100).

Secoisolariciresinol 5-(1-cyano-1-methylethyl) adduct (30). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 428 (100%) $[M - H]^-$, MS^2 of 428: 401 (100), MS^3 of 401: 386 (51), 371 (12), 353 (24), 219 (77), 205 (100), 187 (39), 162(58).

Secoisolariciresinol 5-furanone adduct (31). HRMS (EI) *m/z* calculated for $C_{24}H_{30}O_7$ 430.1991, found 430.1989 (M^+). EIMS (silyl ether) *m/z* 646 (M^+, 5%), 556 (12), 466 (16), 261 (52), 209 (100), 177 (36). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 429 (100%) $[M - H]^-$, MS^2 of 429: 414 (46), 399 (13), 381(100), 366 (60), 337 (18), 322 (19), 165 (29), MS^3 of 381: 366 (100), 245 (5), 121 (8). δ_H (600 MHz, acetone-d_6, 25 °C) 1.43 (3H, s, Me''_a), 1.43, (2H, s, Me''_b), 1.9–2.0 (2H, m, H-8, H-8′), 2.65 (1H, dd, *J* = 6.8, 13.7 Hz, H-7′b), 2.70 (1H, dd, *J* = 8.0, 13.7 Hz, H-7′a), 2.74 (1H, dd, *J* = 6.7, 13.6 Hz, H-7b), 2.80 (1 h, dd, *J* = 8.1, 13.7 Hz, H-7a) 3.56 (2H, m, H-9b, H-9′b), 3.68 (2H, m, H-9a, H-9′a), 3.75 (3H, s, MeO′), 3.84 (3H, s, MeO), 6.60 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 6.70 (1H, d, *J* = 8.0 Hz, H-5′), 6.73 (1 h, d, *J* = 2.0 Hz, H-2′), 6.77 (1H, d, *J* = 1.5 Hz, H-6), 6.81, (1H, *J* = 1.5 Hz, H-2); δ_C (151 MHz, acetone-d_6, 25 °C,) 25.5 (Me''_a, Me''_b), 36.0 (C-7′), 36.5 (C-7), 43.9 (C-1″) 44.7 (C-8′), 44.8 (C-8), 56.1 (MeO′), 56.4 (MeO), 61.1 (C-9′), 61.3 (C-9), 113.4 (C-2′), 113.9 (C-2), 115.5 (C-5′), 116.0 (C-6), 122.4 (C-6′), 133.5 (C-1′), 135,6 (C-5), 139.2

† Signals from the aromatic moieties containing the 5–5 coupling. More detailed NMR data will be published elsewhere.

(C-4), 139.9 (C-1), 144.5 (C-3), 145.5 (C-4′), 148.1 (C-3′). 181.3 (C-2″).

Hydroxymatairesinol 5′-furanone adduct (32a). EIMS (silyl ether) *m/z* 586 (M^+, 4%), 297 (100), 223 (4), 177 (12). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 441 (100%) $[M - H]^-$, MS^2 of 441: 423 (100), 408 (14), 379 (5), 364 (4), MS^3 of 423: 408 (100), 364 (10).

Hydroxymatairesinol 5-furanone adduct (32b). EIMS (silyl ether) *m/z* 586 (M^+, 24%), 293 (100), 265 (30), 249 (16), 209, (30) 179 (15). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 441 (100%) $[M - H]^-$, MS^2 of 441: 411 (11), 379 (15), 367 (55), 317 (17), 305 (100), 231 (33), MS^3 of 305: 275 (29), 246 (39), 230 (100), 205 (23).

Matairesinol 5-furanone adduct (33). EIMS (silyl ether) *m/z* 498 (M^+, 74%), 209(100), 193 (5), 179 (47). ESI–MS^n (negative mode, the most abundant peak further fragmented) *m/z* 425 (100%) $[M - H]^-$, MS^2 of 425: 410 (100), 381 (27), 366 (26), 349 (15), 214 (20), 205 (15), MS^3 of 410: 367 (100), 354 (46), 273 (45), 259 (35), 229 (52).

Conclusions

The results of this study show that different lignans have different radical–scavenging activity. The activity can be related to some structural features, which makes the tailoring of specific lignan-based antioxidants possible. The structure–activity relationship study clearly showed that the 3,4-dihydroxy derivatives were the most effective radical scavengers. However, these lignans appeared to be unstable upon storage in methanol solution and might therefore be unsuitable for utilisation. The 4-hydroxy-3-methoxy substitution pattern of the guaiacyl lignans also showed a good radical scavenging capacity and therefore **2**, **3**, **6**, and **15** seem to be the most promising antioxidants, mainly due to their natural origin and good stability. Compared to the known standards, Vit-E, Vit-C and BHT, the lignans showed equally good or even better radical-scavenging capacity. Equally as for BHT, most lignans showed a relatively slow kinetic profile, which may be beneficial in technical applications with long-lasting antioxidant effects.

It is also clearly shown from this study that if necessary, the lignans can scavenge numerous equivalents of free radicals.

Our results also indicated that it is most unlikely that lignans function as effective radical scavengers *in vivo*, due to their extensive metabolisation to the ineffective (in terms of radical scavenging properties) lignan enterolactone. The beneficial effects to health of lignans may, however, still be related to antioxidant effects through different mechanisms *i.e.* inhibition of enzymes. Also, other biological effects such as antiviral properties may be correlated to the radical-scavenging properties of lignans.

In order to further elucidate the antioxidant mechanisms of natural lignans, it would be interesting to study the bond dissociation energies of the phenolic O–H bond of the different lignans, and to study the stability of the phenoxyl radicals by ESR spectroscopy.

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