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Oxidative transformation of the natural lignan hydroxymatairesinol with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone

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Abstract—The oxidative transformation of the two isomers of the natural lignan hydroxymatairesinol from Norway Spruce (*Picea abies*) by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ), has been studied. Significant differences in the outcome of the reactions were observed when the pure isomers of hydroxymatairesinol were reacted with DDQ under the same conditions. The different stereoelectronic effects in the two isomers as well as their conformational structures seem to determine the site of reaction, which results in different reaction products. Several products were identified by GC–MS and NMR spectroscopy. Oxomatairesinol was obtained in a yield of 25%.

1. Introduction

Many lignans of the α,β-dibenzylbutyrolactone type, having significant biological effects, can be isolated from different wood species.[1,2] Hydroxymatairesinol (HMR), the major lignan in Norway spruce (*Picea abies*), was isolated as a ca. 1:3 mixture of two isomers by Freudenberg and Knof in 1957.[3] The two isomers were separated, characterised and designated by Freudenberg et al. as (−)-*allo*-HMR and (−)-HMR, respectively. We separated the two isomers by chromatography and showed that (−)-*allo*-HMR is the minor and (−)-HMR is the major isomer in Norway spruce wood, here referred to as **HMR-I** and **HMR-II**. For both isomers, the *R* and *R* configurations of the lactone ring carbons H-8 and H-8′, respectively, have been established by comparison of the stereochemistry of stereoselectively synthesised α-conidendrin[4] with that of conidendrin prepared by acidic cyclisation of a natural mixture of HMR isomers from spruce.[3,5] We recently published a paper in which we showed that the configurations at C-7 in **HMR-I** and **HMR-II** are *R* and *S*, respectively.[5] The complete IUPAC name of the minor isomer of hydroxymatairesinol from Norway spruce is then (−)-(7*R*,8*R*,8′*R*)-4,4′,7-trihydroxy-3,3′-dimethoxylignano-9,9′-lactone (**HMR-I**, Fig. 1) and the major isomer is (−)-(7*S*,8*R*,8′*R*)-4,4′7-trihydroxy-3,3′-dimethoxylignano-9,9′-lactone (**HMR-II**, Fig. 2). In the following, the natural mixture of HMR in Norway spruce (**HMR-I/HMR-II**∼1:3)[6] is referred to, unless otherwise stated.

HMR has been shown to metabolise to the known mammalian lignan enterolactone, which has antitumourigenic properties for hormone dependent cancer forms.[7] HMR has also been identified as a powerful antioxidant and is able to decrease the oxidation of LDL-particles.[8] The high concentration of HMR, especially in the knots and in the heartwood of branches of Norway spruce, makes HMR available in large scale.[9] Due to its good availability and its biological properties HMR has been proposed as a chemopreventive agent against cancers, hormone dependent diseases and cardiovascular diseases.

During metabolisation, HMR undergoes demethylation and dehydroxylation by intestinal bacteria. Enterolactone, one of the main metabolites of HMR, has been widely studied and its physiological effects have been evaluated.[10–13] Jacobs et al.[14] have shown that enterolactone undergoes further oxidative metabolisation by hepatic microsomes, resulting in hydroxylated products by modifications in the aliphatic as well as the aromatic parts of the molecule. However, very little is known about the biotransformation of HMR and the biological effects of its metabolites.

HMR also undergoes oxidative transformations upon exposure to light. Kawamura et al.[15] reported that the two isomeric forms can equilibrate and that both are oxidised to oxomatairesinol, and that they finally form coloured oligomers when exposed to irradiation by light.

Keywords: hydroxymatairesinol; lignan; oxidative transformation; DDQ; 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; benzylic hydroxylation; dehydrogenation.

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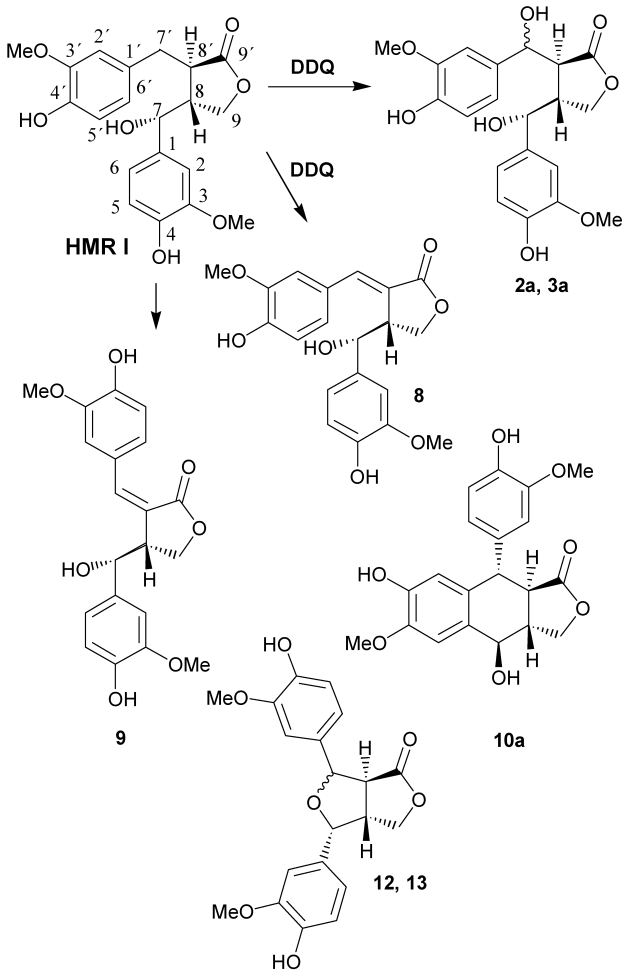


Figure 1. Reaction products formed in the reaction between **HMR-I** and DDQ.

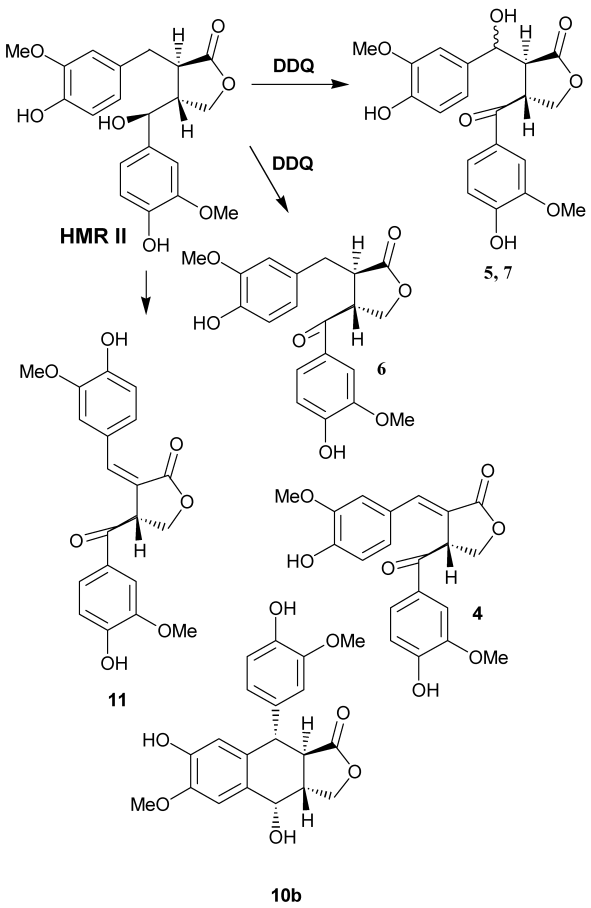


Figure 2. Reaction products formed in the reaction between **HMR-II** and DDQ.

In our studies of oxidative transformations of HMR, the use of a conventional oxidising agent such as PCC lead to decomposition of the starting material and thereby failed to oxidise the benzylic alcohol group to the corresponding ketone. High potential quinones such as DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) are known to dehydrogenate benzylic alcohols to ketones and to functionalize benzylic positions under mild conditions.[16–19] DDQ has previously been shown to react with lignans of the butyrolactone, aryltetralin and dibenzocyclo-octadiene series.[20–28] However, reactions between DDQ and lignans containing a benzylic alcohol group have not been reported previously.

In this study we performed reactions between HMR and DDQ in order to obtain oxomatairesinol and other oxygenated products (possible metabolites) of HMR. We also aimed at gaining more knowledge of the chemical properties of HMR. The outcome of the reactions as well as mechanistic aspects are presented in this paper.

2. Results and discussion

When HMR was reacted with 1.3 equiv. of DDQ in 1,4-dioxane at room temperature for 6 h, a mixture of altogether 16 products was detected by GC and GC–MS. However, when pure isomers (isomeric purity >95%) of **HMR-I** and **II**, respectively, were reacted at the same conditions, the outcome of the reactions showed great differences (Fig. 3).

Analysis by GC-FID and GC–MS of the reaction mixtures (TMS–ether derivatives) showed that **HMR-II** yielded oxomatairesinol (**6**) as the major product. **HMR-I** yielded two isomers of the benzylidene lignan 7′,8′-dehydro-7-hydroxymatairesinol (**8**, **9**) as the major products and surprisingly only a few percent of **6** was formed (Figs. 1–3). When HMR was treated with 2 equiv. of DDQ for 16 h the main product was 7′,8′-dehydro-7-oxomatairesinol (**11**) (Fig. 2).

The products formed in the reactions above, support an initial formation of benzylic cations by abstraction of hydride ion by DDQ. The cationic intermediates then deprotonate to form the dehydrogenated final products. The difference in the extent of oxidation of the benzylic alcohol group (formation of **6**) of **HMR-I** (~1%) and **HMR-II** (~15%) could be due to different stereoelectronic effects in the two stereoisomers. As the benzylic C–H bond approaches an angle of 90° to the plane of the aromatic ring, the rate of dehydrogenation has been shown to increase, due to the progressive improvement in overlap of the empty orbital of the developing benzylic carbonium ion with the orbitals of the aromatic π-system.[17] In our case the carbonium ion can be further stabilised by the resonance

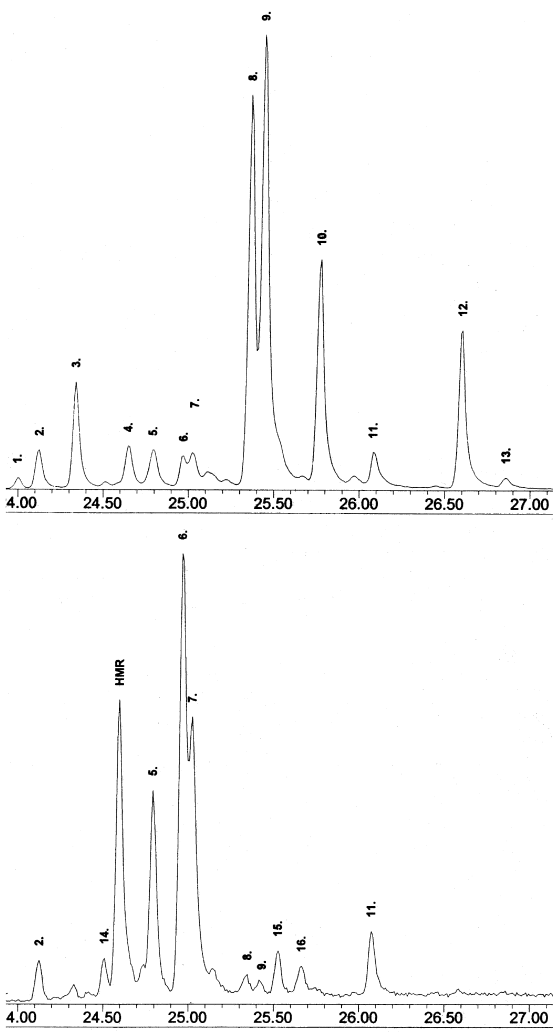


Figure 3. GC–MS chromatograms of the reaction mixtures (TMS ether derivatives) of **HMR-I** (upper) and **HMR-II** (lower).

effect of the *para* hydroxyl group in the aromatic ring of HMR.

It thus seems, that **HMR-II** most likely takes a conformation having a nearly orthogonal relationship between the C–H bond at C-7 and the aromatic plane under the present conditions. **HMR-I** would then adopt a structure with a more parallel relationship, unfavourable for orbital overlapping, leading to less dehydrogenation of the alcohol group.

Dehydrogenation of benzylic alcohols by quinones, has been proposed to occur via the formation of charge-transfer complexes.[18] In the present case the guaiacyl group with its high electron density would be a very efficient donor to the electron deficient aromatic ring of DDQ. In fact, on addition of DDQ to HMR, the solution turned dark green and then the colour slowly faded to light yellow. Such transitory colours observed in reactions with DDQ have been ascribed to $\pi-\pi$ charge-transfer complexes. The structural differences in the two diastereomers of HMR, could affect the formation of such complexes. In addition to the stereoelectronic effects discussed above, this would also affect the rate of the oxidation of the benzylic alcohol group in **HMR-I** and **HMR-II**, respectively.

The dehydrogenation at the benzylic alcohol group (C-7), preferred to dehydrogenation at the benzylic methylene group (C-7′) in **HMR-II** (note the reverse order in **HMR-I**), may be due to a more stabilised carbonium ion formed in the hydride abstraction step. Besides charge delocalisation to the aromatic ring, which is possible in each case, some charge delocalisation to the hydroxyl group oxygen is possible in the former case. In conclusion, if the conformational structure and the stereoelectronic arrangement are favourable, DDQ dehydrogenates the alcohol group prior to the methylene group, by forming a highly stabilised intermediate in the rate determining hydride abstraction step.

The low yield of **6** from **HMR-I** shows that the benzylic position C-7 is relatively unaffected by DDQ. In the reactions with **HMR-I** the formation of benzylic cations therefore mainly takes place at the other benzylic position (C-7′), as shown by the formation of **8**, **9**, **10a**, **12**, and **13** (Fig. 1).

In addition to dehydrogenation, the benzylic cations may undergo inter- or intramolecular nucleophilic attack in the presence of a suitable nucleophile (e.g. the solvent). Functionalization of the benzylic position of *para* substituted aromatic systems by DDQ, has been studied by Ramdayal et al.[19] A free OH group at the *para* position was found to enhance oxygenation occurring at the benzylic methylene carbon. Lignan derivatives bearing guaiacyl groups should therefore be suitable substrates for such functionalisation reactions by DDQ.

The mass spectra of the peaks 1, 2, 3, 5 and 7 showed molecular ion peaks indicating the presence of one additional benzylic OH group in the molecule. Formation of these products confirmed the attack by water on the benzylic cation.

In a closer examination of the mass spectra of the reaction mixtures, peaks 2 and 3 were identified as the isomeric compounds **2a** and **3a** in the reaction of **HMR-I** and **2b** and **3b** in the reaction of **HMR-II**, by their molecular ion peak at *m/z* 678 and by the characteristic fragment *m/z* 297 and also by the absence of *m/z* 209 (Fig. 4). The structures of **2b** and **3b** were confirmed by NMR spectroscopy. Mass spectra of peaks 5 and 7 (also isomers) had molecular ion peaks at *m/z* 604 and showed the characteristic fragments *m/z* 297 and *m/z* 223, which indicated one benzylic position containing an alcohol and the other one a ketone function, i.e. compounds **5** and **7**.

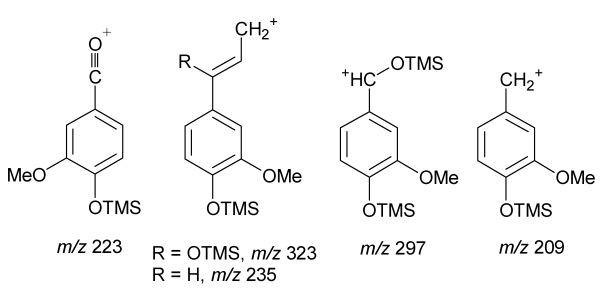


Figure 4. The most common characteristic fragment ion peaks in masspectra of TMS-derivatives of bytyrolactone type lignans derived from HMR.

Peaks 8 and 9 in the reaction mixture of **HMR-I**, gave identical mass spectra and showed molecular ion peaks at *m/z* 588 together with a strong fragment ion peak at *m/z* 297 and were assigned to compounds **8** and **9**, which was in accordance with NMR spectroscopic data. The corresponding compounds, albeit epimers at position 7 were detected as minor components in the reaction mixture of **HMR-II**. Peaks 4 and 11 showed a molecular ion peak at *m/z* 514 and a strong fragment peak at *m/z* 223 and were assigned to the isomers **4** and **11**, also in accordance with NMR spectroscopic data.

The additional peaks 10, 12 and 13 detected only in the reaction mixture of **HMR-I**, showed mass spectra somewhat different from those of the butyrolactone type lignans. Peak 10 gave a strong molecular ion at *m/z* 588 and a weak fragmentation pattern, which indicated the podophyllotoxin-like structure **10a**. Peaks 12 and 13 gave fragmentation patterns related to furo-furan type lignan derivatives (cf. pinoresinol) and a molecular ion peak at *m/z* 516. This indicated the presence of the isomeric lignan derivatives **12** and **13** (Fig. 1).

Formation of **12** and **13** can be explained by an intramolecular nucleophilic attack by the hydroxyl group at position C-7 on the benzylic cation at position C-7′ (Scheme 1, route b). Compound **10** could be formed via a similar attack from C-6 forming the intermediate (**i**) as shown in route a of Scheme 1. Alternatively, **10**, **12** and **13** can be formed by an intramolecular cyclisation of compounds **2** and **3**. Peaks 1, 14, 15 and 16 have not been identified.

Treatment of HMR with 1.2 equiv. DDQ in anhydrous THF under Ar for 6 h at room temperature gave a lower yield of hydroxylated products and a higher yield of **6**. To explore the yields of **6** formed from **HMR-II**, we performed reactions in dioxane containing different amounts of water. The presence of water in the reaction medium affected the formation of hydroxylated products as well as the formation of benzylidene lignans. Clearly, more anhydrous conditions (freshly vacuum dried **HMR-II**, anhydrous 1,4-dioxane, flame dried glasware and Ar-gas atmosphere) yielded more of the benzylidene lignan **11** and less of the hydroxylated products **5** and **7**. The same result was obtained when longer reaction times were applied. It is therefore likely that product **11** is formed from **6**. In aqueous conditions hydroxylation occurred instead of dehydrogenation. Oxomatairesinol formation was only slightly affected by the presence of water. However, the highest yield (25%) of **6** was obtained in 3% aqueous dioxane, and due to the absence of benzylidene lignans in aqueous conditions, **6** was easily isolated by chromatography. In a similar reaction (3% aqueous dioxane), **HMR-I** was partly unreacted and formed mainly the dihydroxy compounds **2a**, and **3a**, but only detectable amounts of **6** and **10a** were formed.

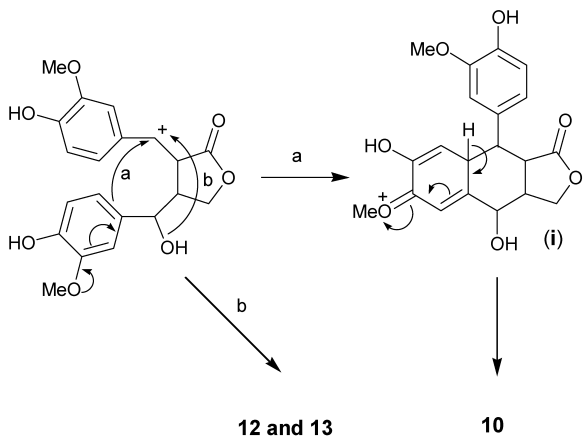
In the photo-oxidation of HMR reported by Kawamura et al.[15] isomerisation between **HMR-I** and **HMR-II** and formation of oxomatairesinol and conidendrin was explained by light induced radical reactions forming a quinone methide intermediate. Kawamura et al. did not report any reactions at the unsubstituted benzylic position.

In our reactions, no isomerisation of HMR and no formation of conidendrin was detected. The reaction therefore most likely proceeded via the direct hydride abstraction mechanism, forming the same quinone methide intermediate (**ii**) as reported by Kawamura et al. (Scheme 2). Reactions at C-7′ also support this mechanism.

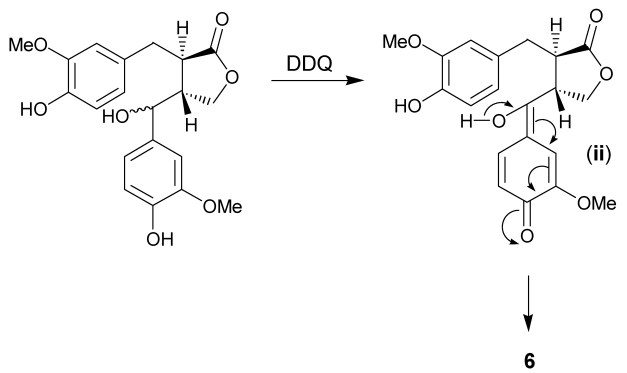
When the reaction mixtures were analysed by GC-FID, using a short capillary column (HP-5, 6 m) oligolignans were detected.[9,29] The presence of oligolignans confirmed the presence of polymerisation reactions, probably also taking place via polar mechanisms. The total recovery of monolignan derivatives from the reaction mixtures was about 60%.

As shown above, HMR undergoes both dehydrogenation, hydroxylation, cyclisation and polymerisation reactions when treated with DDQ. Structural differences in **HMR-I** and **HMR-II** direct the formation of benzylic cations to the most favourable position, yielding different products from the two isomers. However, the reactions are competitive and no reaction conditions yielding a single product could be found.

HMR-II favours the formation of oxomatairesinol by oxidation of the 7-OH group but hydroxylation at C-7′ is a competing reaction in aqueous media while dehydrogenation at C-7′ competes in anhydrous media. **HMR-I** favours reactions at C-7′ yielding **8**, **9**, **10a** and **12** at anhydrous conditions and the hydroxylated products **2a** and **3a** together



Scheme 1.



Scheme 2.

with **10a** in aqueous conditions. The formation of several products as well as polymeric structures, inevitably lead to poor yields of single products in the reactions. Only oxomatairesinol could be isolated in moderate yield.

Dibenzylbutyrolactone lignans have been reported to form aryl–aryl coupled dibenzocyclo-octadiene lignans by the action of DDQ in TFA.[28] The possibility to obtain dibenzocyclo-octadienes in the reactions between HMR and DDQ by reaction in TFA, is restricted by the formation of α-conidendrin from HMR in acidic media.[3] Treatment of HMR with TFA resulted in the instantaneous formation of α-conidendrin as shown by GC–MS and NMR spectroscopy (unpublished results).

Compounds **4**, **8**, **9**, **10**, and **11** are to the best of our knowledge new compounds and hitherto unreported. Oxomatairesinol (**6**), the monoepoxylignanolid (**12**) and lignans **2**, **3** have been reported as naturally occurring lignans.[3,30,31] Though, the relative stereochemistry of the reported lignans could differ from our obtained products.

2.1. Identification of compounds in the reaction mixtures

From the reaction mixtures, fractions containing minor products were isolated by repeated column chromatography and analysed by NMR spectroscopy (Tables 1 and 2). The analysed fractions were not chromatographically pure and comprised several components. Hence, no yields of minor products are given. GC analyses showed that the yields of the minor products were from a few percent up to 10%.

Compounds **2b** and **3b** were isolated as a mixture from the reaction of **HMR-II** in aqueous dioxane and identified as isomers of 7-7′-dihydroxymatairesinol, differing in the stereochemistry at position 7′. NMR data of **2b** and **3b** showed the absence of benzylic methylene group signals. Instead, two signals from the benzylic alcohol group protons appeared as doublets. The signals from H-7′ were observed approximately 0.8 ppm downfield from those of H–7, due to the anisotropy effect of the carbonyl group. Both stereoisomers can adopt a conformation with bond angles resulting in coupling constants near the observed values. Based on couplig constants alone, the stereochemistry at 7′ of **2b** and **3b** can therefore not be determined. The complete ^{1}H NMR signal assignments were based on COSY spectra. Additional analyses by HPLC-MS gave molecular ion peaks at *m/z* 390, in accordance with the proposed structures. It should be observed that compounds **2b** and **3b** in the reactions of **HMR-II** differ in stereochemistry from **2a** and **3a** obtained from **HMR-I**. The stereoisomers show small differences in retention times in GC and GC/MS analyses, but identical mass spectra. Attempts to isolate **2a** and **3a** from the reaction mixture of **HMR-I** were unsuccesfull and due to the small amounts and the impurity of the fractions, NMR data of these compounds could not be obtained.

Compounds **8** and **9** were isolated from the reaction of **HMR-I** and identified as the *E* and *Z* isomers of 7′,8′-dehydro-7-hydroxymatairesinol. Compound **8** was assigned the *E* configuration, based on the following argument. In the *E* isomer the vinylic proton H-7′ is positioned in the deshielding area of the carbonyl group and the signal is expected to appear downfield (δ=7.45 ppm) from that of the corresponding *Z* isomer (δ=6.51 ppm). Consequently, the H-2′ proton in the *Z* isomer is situated closely to the carbonyl group and shifted downfield (δ=8.11 ppm) compared to the *E* isomer (δ=7.29 ppm). Complete ^{1}H NMR signal assignments were based on COSY spectra.

Compounds **4** and **11** were isolated as a mixture. Similar effects on the chemical shifts for the H-7′and H-2′signals in **4** and **11** as in **8** and **9** were observed. Compound **4** was assigned the *Z* configuration and compound **11** the *E* configuration, respectively. COSY spectra allowed the complete ^{1}H NMR assignment of **4** and **11** (see Section 4 for NMR data). A fraction containing compound **10** together with isomers of 7,7′-dihydroxy matairesinol was isolated from the reaction mixture of **HMR-I**. The magnitude of the coupling constant, $J_{7',8'}$=11.5 Hz, pointed to a *trans* (aa) relation between H-7′ and H-8′ and an equatorial position of the guaiacyl group. Likewise, the coupling between H-7 and H-8 of similar magnitude, $J_{7,8}$=9.9 Hz, supported a *trans* position of H-7 and H-8 with an equatorial 7-OH group.

Table 1. ^{1}H NMR data of compounds **2b**, **3b**, **8**, **9**, **10a** and **10b**

Proton[a]	Compound, peak position, multiplicity and coupling constants (*J* values/Hz)					
	2b	**3b**	**8** (*E*)	**9** (*Z*)	**10a**	**10b**
H-2	6.45 d (2.0)	6.37 d (2.0)	7.05 d (1.8)	7.04 d (1.9)	7.21 s	6.92 s
H-5	6.73 d (8.1)	6.70 d (8.0)	6.80 d (8.0)	6.81 d (8.0)	6.25 s	6.31 s
H-6	6.59 dd (2.0, 8.1)	6.46 dd (2.0, 8.0)	6.91dd (1.8, 8.0)	6.86 dd (1.9, 8.0)	–	–
H-2′	6.54 d (1.8)	6.45 d (1.8)	7.29 d (1.8)	8.11 d (2.0)	6.83 d (2.0)	6.87 d (2.0)
H-5′	6.77 d (8.1)	6.81 d (8.1)	6.94 d (8.2)	6.78 d (8.2)	6.75 d (8.0)	6.77 d (8.0)
H-6′	6.74 dd (1.8, 8.1)	6.78 dd (1.8, 8.1)	7.26 dd (1.8, 8.2)	7.10 dd (2.0, 8.2)	6.64 dd (2.0, 8.0)	6.71 dd (2.0, 8.0)
H-7	4.64 d (5.2)	4.16 d (7.9)	5.11 d (2.7)	4.80 d (6.7)	4.96 d (9.9)	4.85 bs (2.9)
H-7′	5.15 d (3.8)	5.21 d (2.9)	7.45 d (2.1)	6.50 d (1.5)	4.01 d (11.5)	3.91 d (11.5)
H-8	2.75 m	2.73 m	4.10 m	3.36 m	2.60 m	2.72 m
H-8′	2.92 dd (4.4, 3.8)	2.65 dd (4.4, 2.9)	–	–	2.92 dd (11.5, 14.7)	3.22 dd (11.5, 13.9)
H-9a[b]	4.18 dd (8.9, 4.3)	4.52 dd (8.9, 3.9)	4.51 dd (8.2, 3.2)	4.52 dd (9.1, 2.9)	4.56 dd (6.7, 8.6)	4.40 dd (7.2, 8.2)
H-9b	4.30 dd (8.9, 8.4)	4.33 dd (8.9, 7.6)	4.14 dd (8.2, 8.0)	4.29 dd (9.1, 7.6)	4.16 dd (8.6, 10.8)	4.30 dd (8.2, 11.0)
OMe	3.77 s	3.72 s	3.86 s	3.81 s	3.77 s	3.78 s
OMe′	3.78 s	3.75 s	3.92 s	3.85 s	3.81 s	3.82 s

Compounds **2b**, **3b**, **8**, **9** and **10a** dissolved in $CDCl_3$–$(CD_3)_2CO$ and compound **10b** dissolved in $(CD_3)_2CO$.
[a] Numbering according to HMR.
[b] H-8–H-9 *cis*.

Table 2. ^{13}C NMR data of compounds **2b**, **3b**, **8**, **9**, **10a** and **10b**

Carbon[a]	Compound					
	2b	**3b**	**8** (*E*)	**9** (*Z*)	**10a**	**10b**
1	{131.67–133.21	{131.67–133.21	134.65	135.55	133.73	{133.85–136.25
1′			127.11	127.23	131.11	
2	{107.18–107.55	{107.18–107.55	110.03	111.02	107.35	113.90
2′			114.23	115.00	111.46	114.07
3	{144.25–148.80	{144.25–148.80	148.25	{147.25–147.80	{143.60–145.62	{146.16–148.70
3′			148.05			
4	{144.25–148.80	{144.25–148.80	146.35	146.60	{143.60–145.62	{146.16–148.70
4′			149.10	149.00		
5	{117.02–117.29	{116.75–117.90	116.07	115.37	114.20	116.60
5′			114.97	115.16	113.12	115.40
6	{113.00–113.70	{113.00–113.70	118.90	120.06	q	131.11
6′			124.80	126.99	120.52	123.00
7	73.33	72.63	71.68	75.90	69.57	66.84
7′	71.76	70.87	137.30	142.35	44.50	46.68
8	41.73	41.59	45.67	50.93	44.28	46.05
8′	48.05	49.84	121.72	123.90	46.06	41.62
9	70.06	68.21	66.27	67.30	68.63	68.75
9′	177.98	177.57	173.00	170.88	173.53	q
OMe	{54.40–54.90	{54.40–54.90	{56.10–56.40	56.07	{53.70–54.20	{56.10–56.40
OMe′				56.40		

Compounds **2b**, **3b**, **8**, **9** and **10a** dissolved in $CDCl_3$–$(CD_3)_2CO$ and compound **10b** dissolved in $(CD_3)_2CO$.
[a] Numbering according to HMR.

Formation of a ring with an equatorial guaiacyl group should be favoured on stereochemical grounds. An equatorial 7-OH group confirms the expected *R* configuration (as in **HMR-I**) at C-7. This particular isomer of 4,4′,7-trihydroxy-3,3′-dimethoxy-6,7′-cyclolignano-9,9′-lactone was designated **10a**.

From the reaction mixture of **HMR-II** in aqueous dioxane (See Section 4) a corresponding fraction containing compound **10** together with isomers of 7, 7′-dihydroxy matairesinol (**2b** and **3b**) was isolated. The masspectrum of the compound designated **10b** was identical with that of **10a**. Their NMR spectra, however, were different. In addition to the shift differences in ^1H and ^{13}C NMR spectra, the H-7, H-8 and H-7′, H-8′ couplings were most informative. The large coupling constant, $J_{7',8'}$=11.5 Hz, again indicated the expected *trans* relationship of H-7′ and H-8′ and an equatorial guajacyl group. However, in this case $J_{7,8}$ was 2.9 Hz indicating a *cis* (*ae*) relation and the expected *S* configuration at C-7 (as in **HMR-II**). This strongly supports our previous results which showed that the configuration at C-7 is *S* in **HMR-II** and *R* in **HMR-I**.[5] The identification of **10a** and **10b** in fractions containing dihydroxymatairesinol, strongly indicate a formation of these by an intramolecular cyclisation reaction of compounds **2** and **3**.

^{13}C NMR signals were assigned by ^1H–^{13}C-correlation spectroscopy (HMQC, HMBC) and by chemical shifts. Due to the complexity of the mixtures of products resulting in overlapping and poor quality HMBC spectra, the unambiguous assignments of quaternary carbons could not be made. Therefore, their chemical shifts are reported as intervals and unresolved quaternary carbon signals are denoted by a q in Table 2.

Compounds **6** and **11** were isolated as pure substances, the spectroscopic and spectrometric data are reported in Section 4. The identification of compounds **5**, **7**, **12**, and **13** was based solely on interpretation of mass spectra.

In addition to the compounds above, we also identified two diastereomers of 7′-methoxy-7-oxo-matairesinol coeluting with oxomatairesinol. HPLC–MS analyses gave a molecular ion peak at *m*/*z* 401.2 and GC–MS analyses (TMS–ether-derivatives) gave a molecular ion peak at *m*/*z* 546 and the fragments *m*/*z* 223 and 239. NMR spectroscopic analyses confirmed the structures (see Section 4).

The formation of the methoxy derivatives may be explained by reactions of residual methanol (originating from the purification procedure) in the starting material, with reactive intermediates in the DDQ reaction. Alternatively, the products were formed when the reaction mixture was dissolved in methanolic chloroform prior to column chromatorgaphy. According to this, it seems possible that various compounds with nucleophilic character may add to the carbonium ion intermediaties, forming new compounds. The formation of the hydroxy and methoxy derivatives in the above reactions, also indicate that hydroxymatairesinol effectively binds water and/or methanol (small amounts of these products were detected, even if hydroxymatairesinol was dried under high vacuum and the reactions were performed under anhydrous conditions).

3. Conlusions

We have shown that DDQ reacts with the benzylic positions in HMR by a hydride abstraction mechanism. We also showed that the reaction of DDQ has regioselective properties, which probably depend on stereoelectronic effects as well as on the conformational structure of the substrate. This was demonstrated by the different reaction modes of the two known diastereomers of natural hydroxymatairesinol from Norway spruce. Although a definitive

explanation to the differences in the reactions could not be given, it seems resonable to suppose that the two isomers can behave differently in mammalian metabolism. According to our results, regioselective functionalisation of benzylic positions in non-rigid phenolic compounds such as lignans may be accomplished by DDQ.

4. Experimental

2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Fluka purum ~97%), 1,4-dioxane (LAB-SCAN, max. water 0.05%) and 1,4-dioxane (Aldrich, anhydrous 99.8%) were purchased from the manufacturers and used as received. THF (LAB SCAN) was freshly distilled from sodium benzophenone ketyl. All other commercially available chemicals were used as supplied by the manufacturers.

All experiments were monitored by thin layer chromatography using pre-coated aluminium based sheets (Merck, 60 F_{254}).

The reaction mixtures were silylated using BSTFA (bis-(trimethylsilyl)-trifluoroacetamide) and TMCS (trimethylchlorosilane) in pyridine and quantitatively analysed by gas chromatography using a HP-5 capillary column (25m, Temp. progr. 150°C 0.5 min, 7°C/min→230°C, 10°C/min→290°C 10 min) and betulinol as an internal standard. Mass spectra were recorded using a HP-5 capillary column GC-system equipped with a HP-5973 mass detector. HRMS were recorded on a ZabSpecETOF system. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-A500 spectrometer at 500 and 125 MHz, respectively. TMS was used as an internal standard and 2D experiments were recorded using standad JEOL pulse sequences. The ^{1}H and ^{13}C NMR spectra of the compounds were assigned using homonuclear and heteronuclear direct and long-range chemical shift-correlation spectroscopy (COSY, HMQC, HMBC). Chemical shifts are reported in ppm (δ) downfield from TMS.

Optical rotations were measured with a Perkin Elmer 241 digital polarimeter, using a 1 dm−1 mL cell.

Preparative chromatography was performed on silica gel columns (Merk, Kiselgel 60). Hydroxymatairesinol was isolated from Norway spruce as described by Ekman.[5]

4.1. Reactions with pure isomers of HMR-I and II

To a stirred solution of HMR (160 mg, 0.43 mmol of isomer I or II) in dioxane (10 mL) a solution of DDQ (127 mg, 0.56 mmol) in dioxane (10 mL) was added dropwise. On addition of DDQ, the reaction mixture turned dark green, then slowly turned to yellow. The mixture was allowed to react at room temperature for 6 h. The reaction was then quenched by adding 25 mL CH_2Cl_2 and filtering trough a sintered glass filter to remove precipitated $DDQH_2$. The mixture was extracted with 10 mL CH_2Cl_2 and 30 mL 10% $NaHSO_3$. The CH_2Cl_2 phase was washed with 20 mL saturated NaCl-solution and dried with $NaSO_4$. Evaporation of the solvent gave a light brown-yellow residue.

4.2. Reaction of HMR in anhydrous THF

All glassware was flame dried under argon before use.

To a solution of freshly distilled THF (20 mL) and HMR (1.5 g, 4.01 mmol) under Ar, was added dropwise DDQ (1.09 g, 4.81 mmol) dissolved in 20 mL THF (the mixture turned dark green). The reaction mixture was stirred for 6 h at room temperature and then quenched by pouring it into 30 mL of 10% $NaHSO_3$. The mixture was extracted with CH_2Cl_2 (50+30 mL) and dried with $NaSO_4$. The organic phase was evaporated to dryness. The residue was then re-dissolved in CH_2Cl_2 and filtered trough a sintered glass filter to remove undissolved $DDQH_2$. Evaporation of the solvent gave a light yellow residue.

2.5 g of a reaction mixture obtained as above was chromatographed on a silica column using EtOAc–Hexane (1:1 v/v) yielding a fraction containing (**6**). The fraction was then re-chromatographed using CH_2Cl_2 containing 2% methanol yielding (410 mg, 16.5%) **6** as a pale yellow powder; mp 68–70°C ($CHCl_3$).

4.2.1. Oxomatairesinol (6). $[\alpha]_D^{23}$=+44.3 (*c* 0.01 g/mL in THF), lit. $[\alpha]_D^{25}$=+42.6 (*c* 4.0 in THF).[3] δ_H (500 MHz, $CDCl_3$, 30°C) 2.90 (1H, dd, *J*=7.0, 14.3 Hz, H-7′a), 2.96 (1H, dd, *J*=5.5, 14.3 Hz, H-7′b), 3.44 (1H, m, H-8′), 3.67 (3H, s, OMe′), 3.86 (3H, s, OMe), 4.00 (1H, m, H-8), 4.04 (1H, t, *J*=8.1 Hz, H-9′a), 4.30 (1H, t, *J*=8.1 Hz, H-9′b), 5.43 (1H, s, OH′), 6.13 (1H, s, OH), 6.48 (1H, dd, *J*=2.0, 7.9 Hz, H-6′), 6.54 (1H, d, *J*=2.0 Hz, H-2′), 6.65 (1H, d, *J*=7.9 Hz, H-5′), 6.81 (1H, d, *J*=8.3 Hz, H-5), 7.13 (1H, dd, *J*=2.0, 8.3 Hz, H-6), 7.27 (1H, d, *J*=2.0 Hz, H-2); δ_C (125 MHz, $CDCl_3$, 30°C) 34.51 (C-7′), 44.98 (C-8′), 46.58 (C-8), 55.85 (OMe′), 56.22 (OMe), 69.40 (C-9), 110.12 (C-2), 111.81 (C-2′), 113.97 (C-5), 114.42 (C-5′), 122.35 (C-6′), 123.61 (C-6), 128.73 (C-1), 128.98 (C-1′), 144.71 (C-4′), 146.71 (C-3′), 147.12 (C-3), 151.49 (C-4), 177.38 (C-9′), 195.06 (C-7); *m/z* (EIMS) 372 (M^+, 90%), 221 (17), 194 (100), 177 (20), 151 (70), 137 (55), 123 (13), 77 (8); HRMS: found 372.1211 (M^+). $C_{20}H_{20}O_7$ requires 372.1209.

4.2.2. 7′-Methoxy-7-oxo-matairesinol. (isomer I) δ_H (500 MHz, $CDCl_3$, 30°C) 3.28 (3H, s, 7′-MeO), 3.41 (1H, dd, *J*=2.9, 7.5 Hz, H-8′), 3.59 (3H, s, OMe′), 3.82 (3H, s, OMe),4.00–4.10 (1H, dd, overlapping, H-9b), 4.44 (1H, m, H-8), 4.50 (1H, dd, *J*=8.9.17.0 Hz, H-9b), 4.76 (1H, d, *J*=2.9 Hz, H-7′), 6.56 (1H, d, *J*=1.8 Hz, H-2′), 6.65 (1H, dd, *J*=1.8, 8.0 Hz, H-6′), 6.69 (1H, d, *J*=8.0 Hz, H-5′), 6.79 (1H, d, *J*=8.3 Hz, H-5), 7.07 (1H, dd, *J*=2.0, 8.3 Hz, H-6), 7.16 (1H, d, *J*=2.0 Hz, H-2); δ_C (125 MHz, $CDCl_3$, 30°C) 42.09 (C-8), 51.51 (C-8′), 55.70 (MeO′), 56.20 (MeO), 57.99 (7′-MeO), 69.65 (C-9), 80.33 (C-7′), 108.18 (C-2′), 110.10 (C-2), 113.79 (C-5), 114.64 (C-5′), 118.89 (C-6′), 123.43 (C-6), 128.57 (C-1), 130.40 (C-1′), 145.32 (C-4′), 146.70-147.10 (C-3, C-3′), 151.18 (C-4), 176.17 (C-9′), 195.18 (C-7); (isomer II) δ_H (500 MHz, $CDCl_3$, 30°C) 3.25 (3H, s, 7′-MeO), 3.70 (1H, dd, *J*=4.3, 6.7 Hz, H-8′), 3.77 (3H, s, MeO′), 3.85 (1H, dd, overlapping, H-9b), 4.00–4.10 (1H, dd, overlapping, H-9a), 3.90 (3H, s, MeO), 4.12 (1H, m, H-8), 4.68 (1H, d, *J*=4.3 Hz, H-7′), 6.75 (1H, d, *J*=1.8 Hz, H-2′), 6.78 (1H, dd, *J*=1.8, 7.5 Hz, H-6′), 6.84 (1H, d, *J*=7.5 Hz, H-5′), 6.89 (1H, d, *J*=8.3 Hz, H-5), 7.37

(1H, dd, J=2.0, 8.3 Hz, H-6), 7.58 (1H, d, J=2.0 Hz, H-2); δ_C (125 MHz, $CDCl_3$, 30°C) 43.34 (C-8), 49.52 (C-8′), 56.06 (MeO′), 56.20 (MeO), 57.30 (7′-MeO), 68.78 (C-9), 81.40 (C-7′), 109.70 (C-2′), 110.56 (C-2), 114.04 (C-5), 114.46 (C-5′), 119.72 (C-6′), 123.98 (C-6), 128.28 (C-1), 129.14 (C-1′), 145.80 (C-4′), 146.70–147.10 (C-3, C-3′), 151.41 (C-4), 176.17 (C-9′), 195.35 (C-7′).

4.3. Reaction of HMR with 2 equiv. of DDQ in 1,4-dioxane

HMR (2 g, 5.35 mmol) was dissolved in 50 mL dioxane and DDQ (2.43 g, 10.7 mmol) was added to the solution in small portions. The reaction mixture was stirred at room temperature for 16 h and then passed trough a sintered glass filter to remove precipitated $DDQH_2$. The mixture was washed with 130 mL 10% $NaHSO_3$ and extracted with 100 mL CH_2Cl_2. The CH_2Cl_2 phase was dried with $NaSO_4$ and evaporated under reduced pressure to leave a yellow residue. The recovery of lignans from the residue was very poor, which showed that higher molar ratios of DDQ result in more polymerized material.

Compound **11** (150 mg, 7.6%) was isolated by column chromatography using $CHCl_3$–MeOH (99:1 v/v) as eluent; mp>150°C, decomp. ($CHCl_3$). NMR analysis showed one pure product. GC and GC–MS showed two peaks 4 and 11 indicating thermal isomerisation of the E and Z isomers. Thermal isomerisation of closely related benzylidene-lactone lignans has been reported earlier.[24] Small amounts of the Z isomer (**4**), were detected in remaining fractions from the above purification and the compound was identified by NMR.

4.3.1. (7*E*′)-7′,8′-Dehydro-7-oxomatairesinol (11). $[\alpha]_D^{23}$=+252.3 (*c* 0.01 g/mL in THF). δ_H (500 MHz, $CDCl_3$, 30°C) 3.51 (3H, s, OMe′), 3.88 (3H, s, OMe), 4.31 (1H, dd, J=3.8, 9.3 Hz, H-9a), 4.65 (1H, t, J=9.3 Hz, H-9b), 5.08 (1H, ddd J, 3.8, 9.3, 2.3 Hz, H-8), 5.77 (1H, s, OH′), 6.15 (1H, s, OH), 6.73 (1H, d, J=2.0 Hz, H-2′), 6.75 (1H, d, J=8.3 Hz, H-5′), 6.86 (1H, dd, J=2.0, 8.3 Hz, H-6′), 6.94 (1H, d, J=8.3 Hz, H-5), 7.44 (1H, dd, J=2.0, 8.3 Hz, H-6), 7.46 (1H, d, J=2.0 Hz, H-2), 7.67 (1H, d, J=2.3 Hz, C-7′); δ_C (125 MHz, $CDCl_3$, 30°C) 46.76 (C-8), 55.55 (OMe′), 56.20 (OMe), 67.69 (C-9), 110.50 (C-2), 111.56 (C-2′), 114.20 (C-5), 114.86 (C-5′), 119.83 (C-8′), 123.39 (C-6), 125.20 (C-6′), 126.30 (C-1′), 127.84 (C-1), 140.20 (C-7′), 146.59 (C-3′), 147.31 (C-3), 147.92 (C-4′), 151.52 (C-4), 171.20 (C-9′), 194.19 (C-7); *m/z* (EIMS) 370 (M^+, 29%), 335 (4), 219 (5), 151 (100), 137 (10), 123 (15), 108 (10), 68 (10); HRMS: found 370.1050 (M^+). $C_{20}H_{18}O_7$ requires 370.1052.

4.3.2. (7*Z*′)-7′,8′-Dehydro-7-oxomatairesinol (4). δ_H (500 MHz, $CDCl_3$, 30°C) 3.86 (3H, s, OMe′), 3.91 (3H, s, OMe), 4.52 (1H, dd, J=8.9, 5.0 Hz, H-9a), 4.65 (1H, dd, J=8.9, 8.9 Hz, H-9b), 4.93 (1H, m, H-8), 6.71 (1H, d, J=2.0 Hz, H-7′), 6.75 (1H, d, J=8.2 Hz, H-5′), 6.89 (1H, dd, J=2.0, 8.2 Hz, H-6′), 6.97 (1H, d, J=8.3 Hz, H-5), 7.48 (1H, dd, J=2.0, 8.3 Hz, H-6), 7.53 (1H, d, J=2.0 Hz, H-2), 8.18 (1H, d, J=2.0 Hz, H-2′); δ_C (125 MHz, $CDCl_3$, 30°C) 49.43 (C-8), 56.26 (OMe), 56.48 (OMe′), 68.22 (C-9), 112.20 (C-2), 112.51 (C-2′), 115.38 (C-5′), 115.85 (C-5), 122.28 (C-8′), 125.32 (C-6), 127.10 (C-1), 127.70 (C-6′), 128.99 (C-1′), 143.01 (C-7′), 147.74 (C-3), 148.90 (C-3′), 149.71 (C-4′), 153.23 (C-4), 169.28 (C-9′), 196.63 (C-7). HRMS: found 370.1050 (M^+). $C_{20}H_{18}O_7$ requires 370.1052.

4.4. Reaction of HMR-II in aqueous 1,4-dioxane

HMR-II (1 g, 2.67 mmol) was dissolved in 30 mL aqueous dioxane (5%). DDQ (0.789 g, 3.48 mmol) was dissolved in 20 mL dioxane and added dropwise to the solution above. After 8 h the reaction was quenched by adding 10% $NaHSO_3$ (50 mL). The mixture was then extracted with CH_2Cl_2 (50 mL) and EtOAc (50 mL). The organic phases were combined, dried with $NaSO_4$ and the solvents were removed under reduced pressure. The residue was chromatographed on a Biotage 40i flash chromatographic system using $CHCl_3$:MeOH (99:1, v/v) as eluent, yielding **6** (248 mg, 25%) as a pale yellow powder.

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