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Reactions of the natural lignan hydroxymatairesinol in basic and acidic nucleophilic media: formation and reactivity of a quinone methide intermediate

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The chemical properties and synthetic modifications of the natural lignan hydroxymatairesinol in basic and acidic nucleophilic media were studied. Hydroxymatairesinol presumably reacts *via* a quinone methide and a carbonium ion mechanism under basic and acidic conditions, respectively. In these conditions the benzylic hydroxyl group was displaced by nucleophiles yielding new 7-substituted butyrolactone lignans. Reactions in alcoholic basic solutions yielded the 7-alkoxy ethers diastereoselectively. Several previously known lignans as well as new lignans and lignan derivatives were synthesised. The transformations were monitored and the products identified by HPLC-MS and NMR.

Introduction

Plant lignans have attracted much interest over the years because of their wide occurrence in plants and their broad range of biological activity.[1] Consequently, a great deal of effort for the isolation of lignans from plant materials and for the total synthesis of these compounds has been undertaken. Until recently, flaxseeds were considered one of the richest sources of lignans, especially of the lignan glycoside secoisolarisiresinol diglucoside, but little attention was paid to other sources for the isolation of lignans in large-scale. Norway spruce (*Picea abies*) branch stubs (or knots) have, however, recently been shown to be extremely rich in lignans and up to 17% of their dry weight is constituted of the two isomers of hydroxymatairesinol (HMR refers to a mixture of both diastereomers of hydroxymatairesinol).[2] Methods for the separation of spruce knots and isolation of HMR have been developed, making it the most readily available lignan.[3]

HMR has been shown to metabolise to (−)-enterolactone by intestinal bacteria in rats[4] and humans[5] and to have chemopreventive effects on the development of DMBA induced mammary carcinoma in rats.[4] It has also been shown to have a chemopreventive effect in the Apc^{Min} mice model of human familial adenomatous polyopsis.[6] Furthermore, it has been shown to be a strong antioxidant and able to reduce the oxidation of LDL-particles *in vitro*.[5]

Previously, we have shown that HMR can be transformed into various valuable lignans by synthetic modification.[7–10] However, in our studies of the chemical properties of this lignan, we have discovered some limitations in the possibilities for chemical transformations under basic and acidic conditions. Unexpected side reactions have prompted us to perform a closer study of the chemical properties of each of the two naturally occurring diastereomers, namely (7*S*,8*R*,8′*R*)-(−)-7-hydroxymatairesinol (major isomer) (**1**) and (7*R*,8*R*,8′*R*)-(−)-*allo*-hydroxymatairesinol (minor isomer) (**2**) in basic and acidic conditions.

Already in 1957, when Freudenberg *et al*. did the pioneering work (isolation and identification) on HMR, they found that the major isomer selectively formed a K-acetate adduct upon addition of K-acetate in ethanol. They also showed that both isomers were hydrogenolysed to matairesinol with H_2 over Pd/C and that they formed α-conidendrin (**3**) when treated with formic acid.[11] Later, Kawamura *et al*. isolated HMR from western hemlock (*Tsuga heterophylla*) and studied the light-irradiation induced reactions of both isomers. They showed that both isomers formed 7-oxomatairesinol, α-conidendrin (**3**), (+)-*allo*-7-methoxymatairesinol (**4**), (−)-7-methoxymatairesinol and vanillin as well as coloured oligomers depending on the conditions used.[12] The amounts of products formed from (−)-hydroxymatairesinol (**1**), however, differed from those formed from (−)-*allo*-hydroxymatairesinol (**2**). Kawamura *et al*. presumed that the reactions proceeded *via* an initial formation of phenoxy radicals followed by formation of reactive quinone methide intermediates. In our study we have noticed similar reactions of HMR in basic media and we have previously shown that in strongly alkaline (6 M) aqueous NaOH solution HMR degrades quantitatively to (*E*)-4-(4-hydroxy-3-methoxyphenyl)-2-(4-hydroxy-3-methoxyphenylmethyl)but-3-enoic acid (**5**).[9]

However, this work showed that under milder basic conditions (pH 7–12) HMR reacted with nucleophiles at the benzylic alcohol group, providing a convenient route to the synthesis of new lignan derivatives. In alkaline solution HMR displayed a yellow colour, which upon acidification was lost. The colour changes and especially the reactions observed, indicated that HMR possibly formed a *para*-quinone methide (*p*-QM) intermediate *in situ.* Such intermediates have been shown to play an important part in lignin chemistry. They tend to undergo two types of reactions: nucleophilic addition and polymerisation.[13]

Quinone methides are also believed to be key intermediates in the action of several antitumour and antibiotic drugs.[14] Also, a large number of biologically active natural products, in particular polyphenolic antitumour agents, such as flavonoids, antocyanins and anthracyclines, possess quinone-type structures and they have been reported to undergo chemical transformations and reactions *via* the formation of quinone methide intermediates.[14,15]

In diluted aqueous alkaline solutions of HMR, the intramolecular nucleophilic addition to form α-conidendrin by far outweighed the attack by other nucleophiles. When the nucleophile served both as a solvent and as a nucleophile it was, however, possible to obtain good yields of the corresponding product. The substitution of the benzylic hydroxyl group was also possible under acidic conditions. Reaction in acidic alcoholic solution gave the alkyl ethers in moderate yields.

Results and discussion

To investigate the transformation of HMR induced under basic conditions we studied the reactions of HMR both in aqueous and non-aqueous, nucleophilic media at room temperature. Reactions in mildly alkaline non-buffered aqueous solution (pH 7–8.5) resulted mainly in epimerisation at the benzylic alcohol group. When the epimerisation in DMSO-d_6–D_2O was monitored by NMR, addition of 0.2 equivalents KOD–D_2O to separate samples of the pure isomers (**1** and **2**) (pH ~ 8) gave, after 24 h, almost identical NMR spectra showing a **1** to **2** ratio of 40 : 60. Also, small amounts of **3**, α-conidendric acid (**6**), β-conidendric acid (**7**) and a new isomer of HMR, here called isohydroxymatairesinol (**8**) (for the complete

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isolation and identification, see Eklund *et al*.[16]) and a new compound, tentatively identified as hydroxymatairesinolic acid (**9**) were detected in the spectrum (Scheme 1). The proposed quinone methide structure was however not detected during the experiment (1 scan), indicating a short lifetime or a low concentration of the intermediate.

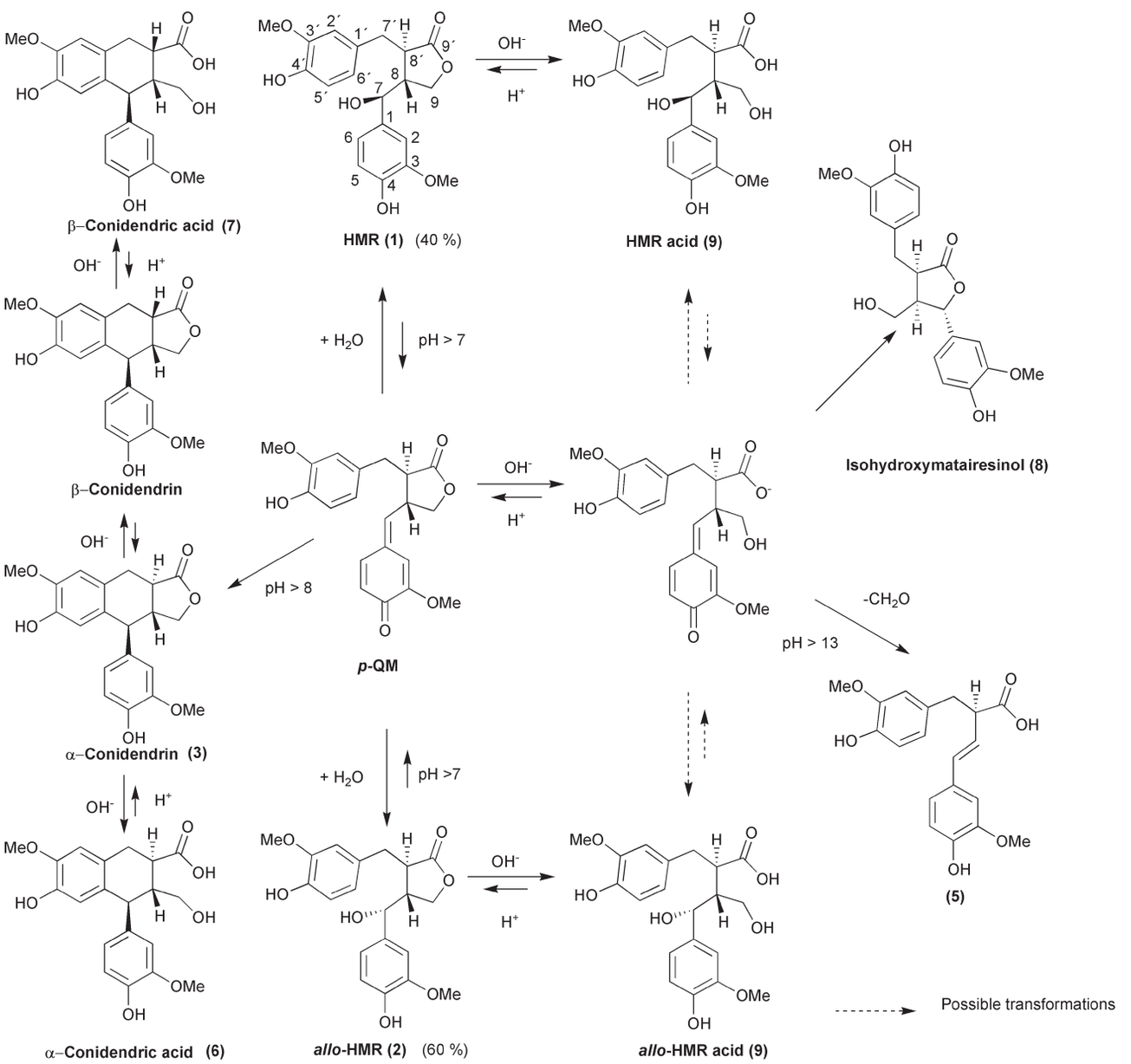
Reaction at pH 10 for 2 h yielded **6** and **7** as the main products. Also the epimerisation and the formation of **8** and **9** were detected by HPLC. In addition to **8** and **9**, diastereomeric forms (epimers at position 7) of these compounds and α- and β-conidendrin were detected in this experiment. Although these compounds were minor components in the alkaline aqueous transformations of HMR, they were detected in some of the experiments.

The studies above indicated that in alkaline aqueous solution, HMR, presumably, first forms a *p*-QM which undergoes nucleophilic addition resulting in epimerisation at the benzylic alcohol group. Subsequently HMR is partially transformed to compounds **8** and **9**, but eventually it forms α- and β-conidendric acids (**6** and **7**) *via* the irreversible intramolecular electron transfer route followed by the opening of the lactone ring (Scheme 1). The isomerisation at C-8 must precede the lactone ring opening as the isomerisation at the α-carbon of the carboxylate is not probable. The ring closure reaction leading to conidendrin formation, however, probably takes place prior to isomerisation at C-8. This is indicated by the α/β ratio of 65 : 35 of the conidendric acids and by the fact that no C-8 isomerisation can be detected for **1** and **2**. As previously proposed, the formation of the six-membered aliphatic ring in conidendrin probably increases the strain in the lactone ring which enhances the tendency for the α- to β-isomerisation (*via* enolisation).[17]

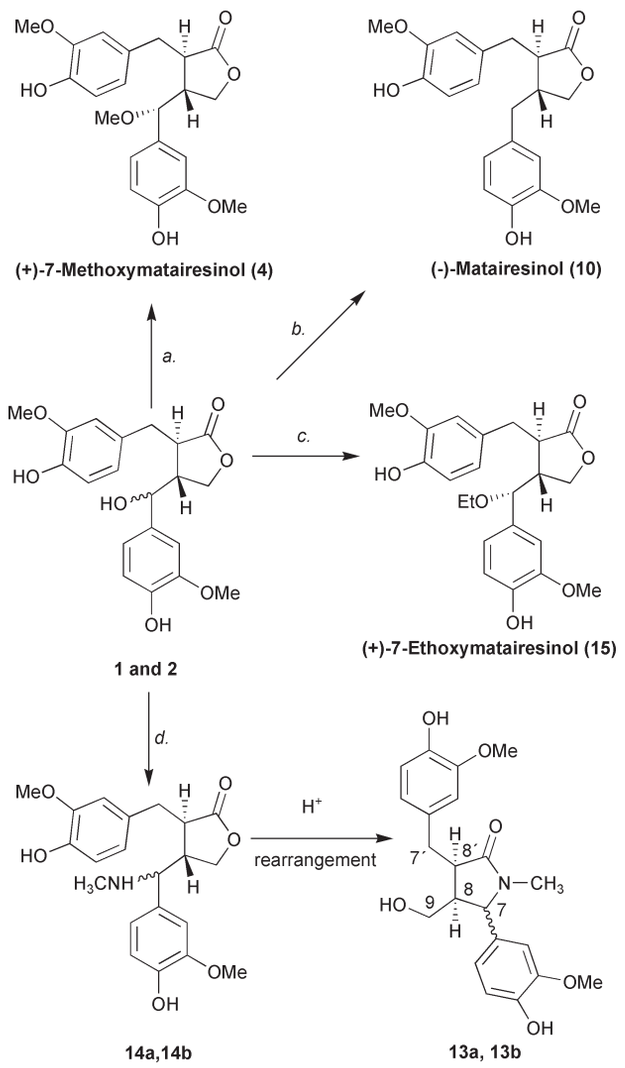
In the presence of an other nucleophile in addition to water, a competitive addition to the *p*-QM was observed. With $NaBH_4$ in aqueous solution, HMR reacted to (−)-matairesinol (**10**) in excellent yield (Scheme 2). Interestingly, the two diastereomers reacted at different rates. The major isomer (**1**) (7*S*) was almost quantitatively transformed to (−)-matairesinol, whereas the minor isomer (**2**) (7*R*) showed only *ca*. 15% conversion at the same conditions.

Reaction in 20% methanolic buffered water solution at pH ~ 9.5 resulted in epimerisation of **1** and **2** and formation of **6** and **7**, but as expected, a few percent of 7-methoxymatairesinol (**4**) and isomers of 7-methoxymatairesinolic acid (**11**, **12**) were also detected. Although the amounts of the 7-methoxymatairesinol derivatives were very low, together with the reaction of the hydride, they clearly indicated that a competing reaction between the nucleophiles and water (OH^-) occurred.

The transformation of **1** and **2** in both 20% and 80% methanolic buffered solution (pH ~ 9.5) was monitored with HPLC. In 20% methanol both isomers were almost completely transformed into **6**, **7** and **8** within 24 h. As indicated in Fig. 1, **2** showed a different kinetic behaviour and reacted more slowly than **1** also in this experiment. Already after 30 min **1** was transformed into several products



Scheme 1 Transformation of HMR in alkaline aqueous solution.



Scheme 2 Reactions of HMR with various nucleophiles in basic and acidic media. *Reagents and conditions*: a, MeONa in MeOH, 3 h, 25 °C or MeOH–H_2SO_4, 48 h, 25 °C; *b*, $NaBH_4$ in H_2O–THF, 4 h, 0 °C; *c*, EtONa in ethanol, 7 h, 25 °C or EtOH–H_2SO_4, 24 h, 25 °C; *d*, $MeNH_2$ in H_2O–THF, 4 h, 0 °C.

as shown in Fig. 2. Comparison of the NMR experiment at pH 7–8.5 and the experiment at pH 9.5 showed that formation of the aryltetralin structure is much faster at pH 9.5 and that the epimerisation of **1** and **2** can not be monitored correctly because of the competing reaction to form **6** and **7**. At pH 7–8.5 the formation of conidendric acids was insignificant on the time scale of epimerisation, which was easily observed in the NMR experiment. In 80% methanolic solution the epimerisation and formation of **4** was immediately detected. However, only small amounts of **6** and **7** were formed after 24 h (data not shown). It seems that the formation of the aryltetralin structure is significantly hindered in alcoholic solution. Thus, the transformations in alkaline solutions may be strongly dependent on the structural conformation and therefore solvent dependent.

When methylamine was used as the nucleophile, three products were detected by GC and GC-MS after acidification and neutralisation of the reaction mixture. When the reaction mixture was extracted at pH 1, two diastereomers of a *N*-methyllactam derivative, **13a** and **13b** (Scheme 2) were obtained in a ratio of 95 : 5. Under neutral conditions one diastereomer of 7-*N*-methylaminomatairesinol was obtained (**14b**). Compound **14b** rearranged partially and slowly to **13b** (detected by GC and GC-MS) upon prolonged extraction and storage in the liquid phase. We therefore assumed that both diastereomers **14a** and **14b** were formed in the reaction, but the rearrangement to the lactam derivative was considerably more favourable for one of the diastereomers and that particular diastereomer could only be isolated as the lactam (**13a**). The other diastereomer could be isolated (**14b**) but was not easily obtained as

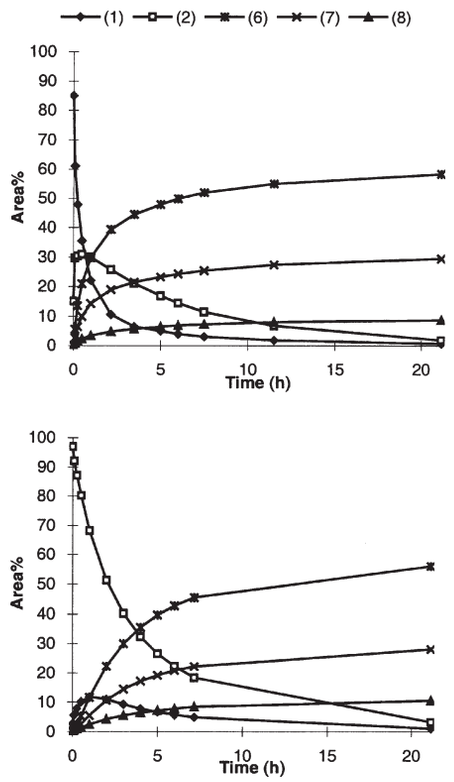


Fig. 1 Degradation of HMR and formation of products in 20% methanolic aqueous solution at pH 9.5. *Upper*: (−)-hydroxymatairesinol (**1**); *Lower*: (−)-*allo*-hydroxymatairesinol (**2**).

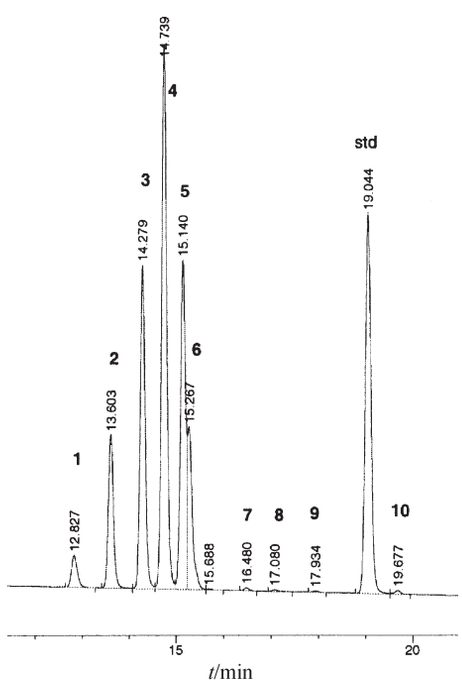


Fig. 2 HPLC chromatogram showing the distribution of products in the transformation of **1** in alkaline 20% methanol solution at pH ~ 9.5 after 30 min. 1: hydroxymatairesinolic acid (**9**); 2: β-conidendric acid (**7**); 3: α-conidendric acid (**6**); 4: hydroxymatairesinol (**1**); 5: *allo*-hydroxymatairesinol (**2**); 6: isohydroxymatairesinol (**8**); 7 and 8: 7-methoxymatairesinolic acids; 9: α-conidendrin (**3**); 10: 7-methoxymatairesinol (**4**).

the lactam (**13b**). NMR spectroscopic analyses of **13a** confirmed the structure and strongly indicated the stereochemistry to be 7*S*,8*S*,8′*R*. In the proton NMR spectrum (DMSO-d_6) H-7 showed a signal at 4.32 ppm as a doublet with a coupling constant of 2.1 Hz ($J_{7–8}$). This signal was correlated to a multiplet at 2.06 ppm (H-8) in the ^{1}H–^{1}H COSY spectrum. The H-8 signal was further correlated to

a multiplet at 2.96 ppm (H-8′). Comparison of the coupling constants and dihedral angles by molecular modelling confirmed the *cis* relationship of protons H-8 and H-8′ ($J_{8-8'}$ = 8.2 Hz) and a *trans* relationship of the protons H-8 and H-7. Also, the lactam formation with the 7*S* configuration should be energetically favoured by the *trans* relationship of the bulky substituents (**13a** *vs*. **13b**). The structure of **14b** was confirmed by NMR analyses but the stereochemistry could not be deduced from NMR data alone (non-cyclic structure). The coupling constant (8.2 Hz) of H-7 indicated the 7*R* configuration (comparison of the coupling constant for compound **2**), however, the rearrangement to the diastereomeric lactam derivative **13b** definitely confirmed the assumed stereochemistry to be 7*R*,8*R*,8′*R*.

Reaction with dimethylamine in H_2O–THF gave only detectable amounts (GC-MS) of two isomeric products, tentatively identified by GC-MS as diastereomers of 7-(*N*,*N*-dimethylamino)matairesinol. Reaction with aqueous KCN gave no substitution products and only formation of **6** and **7** was detected.

When HMR was reacted in a non-aqueous medium in MeONa–MeOH at room temperature, both isomers were completely transformed exclusively to compound **4** in excellent yield. The same reaction occurred with EtONa–EtOH, which yielded (+)-7-ethoxymatairesinol (**15**).

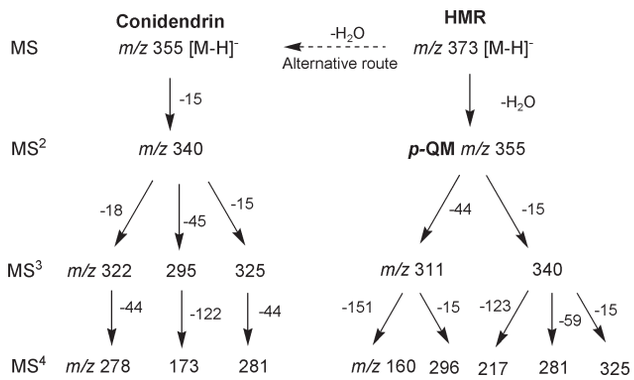
Under acidic conditions both isomers of HMR were easily transformed to α-conidendrin. This Friedel–Crafts-like cyclisation has been extensively studied and employed in the synthesis of aryltetralin type lignans.[7,11,18] However, the generated benzylic cation could also be trapped by other nucleophiles. In acidic (H_2SO_4) non-aqueous methanol and ethanol HMR reacted in moderate yields to give the corresponding ethers **4** and **15** at position 7 (Scheme 2). Both **1** and **2** gave the same results and predominantly one diastereomer (7*R*) of the ethers were formed. Inevitably, the strongly competitive reaction to form α-conidendrin was a prominent side reaction in these reactions. Also, transesterification of the lactone ring to form the corresponding alkyl esters was observed. It is of note that in the acidic conditions the reactions proceeded without any colour changes, whereas in alkaline conditions the reaction mixture was clearly yellow.

Quinone methides have previously been reported to act as bioreactive alkylating agents and to react with biomolecules forming adducts.[13,19] To investigate if HMR reacts with biomolecules *via* the QM mechanism we reacted HMR with glucose, adenosine and glycine in an aqueous THF solution at pH 8. Even if reaction times up to 2 weeks were applied, no adducts were observed by HPLC-MS analyses.

Quinone methide formation and stereoselective addition of nucleophiles

Quinone methides have been widely studied as reference compounds in the chemistry of lignin intermediates. They have been prepared in a stable form by oxidizing the corresponding phenols or by treating the benzyl halides with bases such as triethylamine among other methods.[13,15,20] The structure of QMs and the addition of nuleophiles both under aqueous and non-aqueous conditions have been reported.[13,20] Also, base and acid catalysed formation of *p*-QMs followed by isomerisation and rearrangement reactions for oligoflavonoids have been reported.[15] However, the combination of the alkaline induced formation of *p*-QMs and the simultaneous addition of nucleophiles in alkaline conditions has, to our knowledge, not been reported.

In order to detect the formation of the *p*-QM in alkaline aqueous solution, we recorded both NMR and UV spectra of HMR under alkaline conditions. In both attempts, however, we failed to detect the *p*-QM, but electrospray mass spectrometry, which has previously been used as evidence for QM formation,[21] indicated that the *p*-QM structure was easily generated from HMR. When a sample of HMR was analysed using the direct infusion technique with an ESI-MS ion trap detection in negative mode, it generated a primary fragment peak at *m*/*z* 355 at MS^2, which was explained by the loss of water from the parent molecule. In fact, *m*/*z* 355 was detected already in MS^1. In principle two routes for the loss of water from HMR are possible, formation of conidendrin or formation of the *p*-QM structure. Trapping the fragment *m*/*z* 355 for both conidendrin $(M - H)^-$ and HMR $(M - H) - H_2O$, followed by further fragmentation (MS^n) and comparison of the fragmentation patterns, allowed the assignment of *m*/*z* 355 generated from HMR to the *p*-QM structure (Scheme 3). Although, the mass-spectrometric analysis is not an evidence of the QM as an intermediate in the reactions, it is an indication that HMR easily loses the benzylic OH-group.



Scheme 3 Comparison of the fragmentation pattern in LC-MS^n analyses of α-conidendrin and the *p*-QM structure formed from HMR.

In Scheme 1, the transformations of HMR in alkaline aqueous solution are outlined. The epimerisation and the formation of conidendrin derivatives would require an addition to the benzylic carbocation or the QM or possibly a substitution of the OH-group. In the alkaline conditions neither a substitution nor the presence of a benzylic cation seem probable. Therefore we suggest that an addition to the postulated QM occurs. In alkaline conditions the phenol is deprotonated, which promotes the elimination of the benzylic hydroxy group leading to the QM. This remarkable property seems to be unique for HMR. Also, the butyrolactone ring seems to be crucial for the formation of the QM. It is possible that the benzylic hydroxy group is intramolecularly hydrogen bonded to the carbonyl group leading to an activated benzylic position, which facilitates the QM formation with OH as a leaving group. When the diol analog of the butyrolactone, 7-hydroxysecoisolariciresinol, obtained by LAH reduction of HMR[7] was reacted with $NaBH_4$, in alkaline aqueous solution at pH 8 and pH 14 and in methanol with MeONa as above, no reduction, isomerisation, degradation or substitution of the benzylic hydroxyl group occurred. Also, no colour changes upon addition of base were observed. Under acidic conditions (MeOH–H_2SO_4), (+)-lariciresinol and (+)-cyclolariciresinol were obtained, as we reported previously.[7]

The formation of the QM seems to precede the lactone ring opening because the formation of β-conidendrin and β-conidendric acid requires an α-isomerisation of the lactone ring in α-conidendrin as already discussed above.

In strongly alkaline conditions the formation of **5** indicates a retro-Aldol reaction of the QM. Also, in the non-aqueous alcoholic conditions the formation of the benzylic ethers indicate an (1,6-conjugate) addition which supports the presence of the QM intermediate. The formation of **8** presumably proceeds *via* the open form of the QM intermediate (Scheme 1), but a direct transesterification of the lactone can not be excluded. In fact, when 4,4′-O-methylated HMR was reacted in MeONa–MeOH it was slowly transesterified to the analog of **8** without any colour changes. Under the same conditions HMR formed a yellow reaction mixture and yielded 7-methoxymatairesinol (**4**), exclusively. Therefore both mechanisms for the formation of **8** seem equally possible.

In the experiments performed under aqueous alkaline conditions, **1** was shown to react more readily than **2**. Since both isomers are believed to form the same *p*-QM, the rate-determining step appeared to be the formation of the intermediate. Even if no definite explanation for the difference can be given it seems probable that the difference is due to conformation and stereoelectronic effects. Compound **1** may adopt a conformational structure more favoured

for orbital overlapping (possible hyperconjugation) during the formation of the *p*-QM. Also, the possible hydrogen bonding of the benzylic hydroxy group could greatly be influenced by conformational differences between the two diastereomers.

Comparison of the reaction products formed in aqueous and non-aqueous conditions showed that the addition of the nucleophiles was highly stereoselective in the non-aqueous conditions, whereas aqueous conditions yielded almost a 1 : 1 ratio of diastereomers. Compound **4**, obtained in methanol treated with sodium methoxide was formed at a diastereomeric ratio of 99 : 1 (by integration of the proton NMR signals of 7-OMe). In acidic methanolic solution the ratio was 87 : 13 (by NMR), indicating a less stereoselective reaction. These results are in accordance with previously reported studies of the stereoselective addition of nucleophiles to QMs and benzylic carbonium ions.[18] However, the stereoselective addition of nucleophiles to the *p*-QMs seems to be determined mainly by sterical and/or solvation effects and are therefore much dependent on the structure of the QM.

The absolute configuration of **4** was determined by comparison of chromatographic and spectrometric data of permethylated **1** and 4,4′-O-dimethylated **4**. These two derivatives showed different chromatographic behaviour resulting in different retention times when analysed by GC and GC-MS (Δt = 0.4 min), which allowed us to conclude that the formed methoxymatairesinol is the analog of compound **2** with the 7*R* configuration. Also, NMR analyses showed that the chemical shifts of the 7-OMe proton signals were different for the two derivatives, *i.e.* 3.09 *vs*. 3.21 ppm. The coupling constants of H-7 could not be measured due to severe overlapping, however, the coupling constant of 7.9 Hz for compounds **4** and **15** was similar to that of compound **2** (8.1 Hz). Also, all spectroscopic and spectrometric data of **4** were in agreement with the previously published data on the analog of compound **2**, (+)-*allo*-7-methoxymatairesinol.[12] The 7*R* configuration of **4** indicated an attack of the nucleophile from the *Re* side in the alcoholic conditions. However, in the aqueous conditions the epimerisation equilibrium indicated an attack at a 40 : 60 ratio (*Si* : *Re*).

Conclusions

The chemical properties of HMR, and its transformations in acidic and basic media have been thoroughly investigated. Furthermore, alternative methods for the derivatisation of the benzylic alcohol group and methods for preparation of lignan derivatives have been developed. Based on the observations above and the fact that the benzylic hydroxyl group was easily replaced by nucleophiles, we concluded that HMR easily loses water to form the corresponding *p*-QM under alkaline conditions.

Under acidic conditions HMR reacted at position 7 without any colour changes, which indicated the formation of a carbonium ion intermediate. In alcoholic solution the addition of nucleophiles was stereoselective resulting almost exclusively in the 7*R* derivatives, whereas aqueous conditions yielded mixtures of diastereomers.

The spontaneous formation of the *p*-QMs at pH > 7 seems to be a unique property of HMR, but similar properties may also exist in other natural phenolic compounds having an electron donating group at the *para* position and a leaving group at the benzylic position. Such properties may be important both for the chemical and biological properties of natural compounds.

Experimental

All commercially available chemicals were purchased and used as supplied by the manufacturers. Hydroxymatairesinol was isolated from Norway spruce knots by the methods described in ref. 2. Shortly, knots of Norway spruce were separated, ground, and freeze-dried prior to extraction in a Soxhlet apparatus. The raw extract obtained with acetone–water after the removal of lipophilic extractives with hexane, was purified using flash chromatography to yield hydroxymatairesinol.

GC-MS analyses were performed on a HP-5890 Series II gas chromatograph equipped with a 5971A mass selective detector and a HP-1 column. The samples were silylated using hexamethyldisilazane/chlorotrimethylsilane in pyridine, prior to analyses. Elemental composition and accurate mass were determined with a Fisons ZAB-Spec high-resolution MS EI instrument.

HPLC-DAD analyses were performed using an Agilent 1100 series instrument equipped with a Zorbax Eclipse XDB-8, column (4.6 × 150 mm, 5-Micron) using gradient elution (MeOH–0.1% HAc, 20–66%), 0.4 mL min^{-1}, and UV-detection at 230 nm. HPLC-MS analyses were performed with an Agilent 1100 series HPLC instrument equipped with (1) an Agilent 1100 series LC/MSD Trap-SL detector with an electrospray source and (2) a Micromass Quattro Micro tiple-quadrupole mass detector (ESI). The same HPLC conditions as in HPLC-DAD were used and negative ionisation mode was applied.

The identities of the conidendric and hydroxymatairesinolic acid peaks were confirmed using HPLC–ESI-MS, by their specific fragmentation patterns. Compound **6** and **7** showed the molecular ion *m*/*z* 373 $[M - H]^-$ at MS and *m*/*z* 314 at MS^2. The fragment ion peak may be formed by cleavage of CO_2 and CH_3 from the deprotonised molecular ion. Compound **9** showed the molecular ion *m*/*z* 391 and a fragment ion at *m*/*z* 373 at MS^2 indicating elimination of H_2O from the deprotonised molecular ion. The fragment *m*/*z* 373 followed the fragmentation pattern of HMR in MS^n. The identities of **6** and **7** were also confirmed by comparing with peaks formed after storing of conidendrin in alkaline aqueous solution (pH 10).† The identity of methoxymatairesinolic acids were based on LC-MS by the molecular ion peak *m*/*z* 405 followed by the transitions *m*/*z* 343 → 299. Compounds **1**, **2**, **3**, **4** and **8** were identified by comparison with authentic pure substances.

^1H and ^{13}C NMR spectra were recorded with a JEOL JNM-A500 instrument at 500 and 125 MHz, respectively. 2D experiments (COSY, HMQC, HMBC, COLOC) were recorded using standard pulse sequences, and chemical shifts are reported downfield from TMS.

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

Synthesis of (+)-7*R*-methoxymatairesinol (4)

Procedure A. A mixture of **1** (94%) and **2** (6%) (0.515 g) was dissolved in methanol (50 mL). To the solution, MeONa (0.385 g, 5 equiv.) was added portionwise. The mixture immediately turned yellow and was stirred at room temperature for 3 h, poured onto saturated NaCl-solution (100 mL) and acidified with HCl-solution (10%). The mixture was then extracted with CH_2Cl_2 (5 × 50 mL) and the combined extracts were dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified using normal phase silica column chromatography using $CHCl_3$–MeOH (98 : 2 v/v) as eluent, to yield a colourless powder of (+)-7*R*-methoxymatairesinol (0.446 g, 83%) after drying under vacuum. Purity >95% by GC and GC-MS.

Reaction with **2** (purity >95%) in similar conditions gave the same results (yield 0.472 g, 90%).

$[\alpha]^{21}_D$ = +33.4 (*c* 0.004 g mL^{-1}, EtOH), lit.[12] $[\alpha]^{25}_D$ = +32.1 (*c* 1.53 in MeOH); δ_H (500 MHz, $CDCl_3$, 30 °C) 2.40 (1H, dddd, *J* = 7.9, 7.8, 5.9, 5.9 Hz, H-8), 2.53 (1H, ddd, *J* = 8.2, 6.1, 4.9 Hz, H-8′), 2.59 (1H, dd, *J* = 13.7, 8.2 Hz, H-7a′), 2.67 (1H, dd, *J* = 13.7, 4.9 Hz, H-7b′), 3.03 (3H, s, 7-OMe), 3.69 (3H, s, OMe′), 3.70 (1H, d, *J* = 7.9 Hz, H-7), 3.71 (3H, s, OMe), 4.09 (1H, dd, *J* = 9.4, 7.6 Hz, H-9a), 4.29 (1H, dd, *J* = 9.4, 5.6 Hz, H-9b), 6.35 (1H, d, *J* = 2.0 Hz, H-2′), 6.38 (1H, d, *J* = 2.0 Hz, H-2), 6.40 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.53 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 6.69 (1H, d, *J* = 8.0 Hz, H-5′), 6.77 (1H, d, *J* = 8.0 Hz, H-5); δ_C (125 MHz, $CDCl_3$, 30 °C) 35.02 (C-7′), 43.18 (C-8′), 45.83 (C-8), 55.64 (MeO′), 55.66 (OMe), 56.69 (7-OMe), 68.71 (C-9), 83.64 (C-7), 108.09 (C-2), 111.05 (C-2′), 113.87 (C-5′), 113.98 (C-5), 120.46 (C-6), 121.99 (C-6′), 129.33 (C-1′), 130.43 (C-1), 144.44 (C-4′), 145.66 (C-4), 146.65

† Compounds **6**, **7** and **9** are detected only in alkaline aqueous solution and they undergo ring closure of the open lactone ring upon storage in solid form.

(C-3′), 147.01 (C-3), 178.90 (C-9′); EI-MS: *m*/*z* 388 (M^+, 22%), 356 (20), 241 (4), 177 (4), 167 (100), 152 (7), 137 (17), 122 (3); HRMS (EI): *m*/*z* calc. for $C_{21}H_{24}O_7$ (M^+) 388.1532, found 388.1522.

Procedure B. A mixture of **1** (94%) and **2** (6%) (99 mg) was dissolved in methanol (20 mL) and one drop of concentrated sulfuric acid was added. The mixture was stirred at room temperature for 48 h and then poured onto water (60 mL) and extracted with CH_2Cl_2 (2 × 50 mL). The organic phase was dried over Na_2SO_4 and the solvent removed under reduced pressure. Analyses by GC-MS showed a 60% formation of **4**. Reaction with **2** in the same conditions gave essentially the same results.

Synthesis of (+)-7*R*-ethoxymatairesinol (15)

A mixture of **1** (94%) and **2** (6%) (0.410 g) was dissolved in ethanol (40 mL). To the mixture stirred at room temperature was added EtONa (0.370 g, 5 equiv.) as small portions. The mixture was stirred at room temperature for 7 h and then poured onto saturated NaCl-solution and was acidified with a 10% HCl solution to pH 2. The product was then extracted with CH_2Cl_2 (2 × 50 mL) and the combined organic phases were dried over Na_2SO_4. The solvent was removed under reduced pressure to afford a yellow oil which was purified by normal phase column chromatography using $CHCl_3$–MeOH (98 : 2 v/v) as eluent to yield (+)-7-ethoxymatairesinol (0.371 g, 84%) as a colourless gum after drying under vacuum. Purity >95% by GC and GC-MS.

$[\alpha]^{21}_D$ = +23.7 (*c* 0.01 g mL^{-1}, EtOH); δ_H (500 MHz, $CDCl_3$, 30 °C) 1.10 (3H, t, *J* = 7.0 Hz, CH_2CH_3), 2.47 (1H, dddd, *J* = 7.9, 7.6, 5.9, 5.6 Hz, H-8), 2.60 (1H, ddd, *J* = 8.3, 5.9, 4.9 Hz, H-8′), 2.67 (1H, dd, *J* = 13.8, 8.3 Hz, H-7′a), 2.75 (1H, dd, *J* = 13.8, 4.9 Hz, H-7′b), 3.15 (1H, dq, *J* = 9.4, 7.0 Hz, CH_2CH_3), 3.31 (1H, dq, *J* = 9.4, 7.0 Hz, CH_2CH_3), 3.75 (3H, s, OMe′), 3.78 (3H, s, OMe), 3.88 (1H, d, *J* = 7.9 Hz, H-7), 4.16 (1H, dd, *J* = 9.4, 7.6 Hz, H-9′a), 4.38 (1H, dd, *J* = 9.4, 5.6 Hz, H-9′b), 5.48 (1H, s, OH′), 5.48 (1H, s, OH), 6.41 (1H, d, *J* = 2.0 Hz, H-2′), 6.43 (1H, d, *J* = 2.0 Hz, H-2), 6.60 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 6.78 (1H, d, *J* = 8.0 Hz, H-5′), 6.81 (1H, d, *J* = 8.0 Hz, H-5); δ_C (125 MHz, $CDCl_3$, 30 °C) 15.08 (CH_2CH_3), 35.07 (C-7′), 43.85 (C-8′), 45.78 (C-8), 55.65 (2 × C, OMe, OMe′), 64.27 (CH_2CH_3), 68.81 (C-9), 81.69 (C-7), 108.08 (C-2), 111.04 (C-2′), 113.85 (C-5′), 113.88 (C-5), 120.28 (C-6), 122.03 (C-6′), 129.38 (C-1′), 131.19 (C-1), 144.43 (C-4′), 145.54 (C-4), 146.65 (C-3′), 146.95 (C-3), 179.00 (C-9′); EI-MS: *m*/*z* 402 (M^+, 57%), 356 (14), 181 (100), 177 (7), 153 (18), 137 (20), 122 (4), 93 (9); HRMS (EI): *m*/*z* calc. for $C_{22}H_{26}O_7$ (M^+) 402.1679, found 402.1689.

Synthesis of (−)-matairesinol (10)

A mixture of **1** (94%) and **5** (6%) (0.767 g) was dissolved in THF (20 mL) and water (20 mL). To the mixture stirred at 0 °C was added $NaBH_4$ (0.388 g, 5 equiv.) portionwise. The mixture was stirred for 4 h and then acidified with a 10% HCl solution to pH 5 and extracted with CH_2Cl_2 (2 × 30 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was chromatographed on a silica column using $CHCl_3$–MeOH (98 : 2 v/v) to yield (−)-matairesinol (0.568 g, 78.5%) as a colourless powder after drying in vacuum. Purity >95% by GC and GC-MS.

$[\alpha]^{20}_D$ = −40.8 (*c* 0.01 g mL^{-1}, acetone), lit.[11] $[\alpha]^{25}_D$ = −45.0 (*c* 4.2, acetone); δ_H (500 MHz, $CDCl_3$, 30 °C) 2.39 (1H, m, H-8′), 2.43 (1H, dd, overlapping, H-7′a) 2.49 (1H, m, H-8), 2.52 (1H, dd, *J* = 13.5, 6.6 Hz, H-7′b), 2.80 (1H, dd, *J* = 14.1, 7.1 Hz, H-7a), 2.88 (1H, dd, *J* = 14.1, 5.2 Hz, H-7b), 3.73 (3H, s, OMe′), 3.74 (3H, s, OMe), 3.81 (1H, dd, *J* = 9.2, 7.2 Hz, H-9′a), 4.07 (1H, dd, *J* = 9.2, 7.3 Hz, H-9′b), 5.55 (1H, s, OH′), 5.56 (1H, s, OH), 6.34 (1H, d, *J* = 2.0 Hz, H-2′), 6.44 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 6.53 (1H, dd, *J* = 2.0, 7.8 Hz, H-6), 6.55 (1H, d, *J* = 2.0 Hz, H-2), 6.73 (1H, d, *J* = 8.0 Hz, H-5′), 6.75 (1H, d, *J* = 7.8 Hz, H-5); δ_C (125 MHz, $CDCl_3$, 30 °C) δ 34.62 (C-7), 38.32 (C-7′), 41.04 (C-8′), 46.59 (C-8), 55.85 (2 × OMe), 71.37 (C-9′), 111.07 (C-2′), 111.61 (C-2), 114.17 (C-5′), 114.47 (C-5), 121.35 (C-6′), 122.11 (C-6), 129.59 (C-1′), 129.83 (C-1), 144.46 (C-4′), 145.59 (C-4), 146.68 (C-3′), 146.78 (C-3), 178.85 (C-9); EI-MS: *m*/*z* 358 (62%, M^+), 221 (7), 164 (6), 137 (100), 122 (8); HRMS (EI): *m*/*z* calc. for $C_{20}H_{22}O_6$ (M^+) 358.1417, found 358.1419.

The reaction of HMR with methylamine

A mixture of **1** (94%) and **2** (6%) (0.487 g) was dissolved in THF (60 mL) and water (30 mL). To the mixture stirred at 0 °C $MeNH_2$ (8 mL, 10% in H_2O) was added dropwise over 15 min. The reaction was allowed to warm to room temperature over night and then poured onto water (30 mL). The mixture was then acidified with a 10% HCl solution to pH 1 and extracted with (2 × 40 mL) ethyl acetate. The combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was chromatographed on a silica column using $CHCl_3$–MeOH (98 : 2 v/v) to yield compound **13a** (0.141 g, 28%). Purity >95% by GC and GC-MS.

The aqueous phase was neutralised to pH 7 and again extracted with ethyl acetate (2 × 50 mL) followed by the same workup procedure as described above to yield compound **14b** (0.140 g, 27%). Purity >95% by GC and GC-MS.

Compound 13a. $[\alpha]^{21}_D$ = +38.5 (*c* 0.01 g mL^{-1}, EtOH); δ_H (500 MHz, $CDCl_3$, 30 °C) 2.37 (1H, dddd, *J* = 8.0, 7.9, 6.1, 3.7 Hz, H-8), 2.66 (1H, dd, *J* = 15.1, 10.8 Hz, H-7′a), 2.78 (3H, s, NCH_3), 3.11 (1H, ddd, *J* = 10.8, 8.0, 4.0 Hz, H-8′), 3.25 (1H, dd, *J* = 15.1, 4.0 Hz, H-7′b), 3.58 (1H, dd, *J* = 10.7, 7.9 Hz, H-9a), 3.86 (1H, dd, *J* = 10.7, 6.1Hz, H-9b), 3.86 (3H, s, OMe′), 3.87 (3H, s, OMe), 4.35 (1H, d, *J* = 3.7 Hz, H-7), 5.57 (1H, s, OH′), 5.69 (1H, s, OH), 6.63 (1H, d, *J* = 2.0 Hz, H-2), 6.66 (1H, dd, *J* = 2.0, 8.1 Hz, H-6), 6.73 (1H, dd, *J* = 2.0, 8.1 Hz, H-6′), 6.78 (1H, d, *J* = 2.0 Hz, H-2′), 6.81 (1H, d, *J* = 8.1 Hz, H-5′), 6.89 (1H, d, *J* = 8.1 Hz, H-5). δ_C (125 MHz, $CDCl_3$, 30 °C) δ 28.59 (NCH_3), 31.54 (C-7′), 43.45 (C-8′), 47.67 (C-8), 56.06 (OMe′), 56.14 (OMe), 61.11 (H-9), 65.21 (C-7), 108.41 (C-2), 111.20 (C-2′), 114.47 (C-5′), 114.78 (C-5), 119.25 (C-6), 120.85 (C-6′), 131.50 (C-1′), 131.54 (C-1), 144.28 (C-4), 145.54 (C-4′), 146.74 (C-3), 147.27 (C-3′), 175.95 (C-9′); EI-MS: *m*/*z* 387 (M^+, 100%), 356 (21), 250 (63), 232 (24), 220 (96), 206 (36), 180 (23), 137 (94); HRMS (EI): *m*/*z* calc. for $C_{21}H_{25}NO_6$ (M^+) 387.1682, found 387.1682.

7*R*-(*N*-Methylamino)matairesinol (14b). $[\alpha]^{21}_D$ = +38.1 (*c* 0.01 g mL^{-1}, EtOH); δ_H (500 MHz, DMSO-d_6, 30 °C) 1.98 (1H, s, NCH_3), 2.14 (1H, dd, *J* = 7.0, 5.9 Hz, H-7′a), 2.33 (1H, m, H-8), 2.57 (1H, dd, *J* = 7.0, 5.7 Hz, H-7′b), 2.74 (1H, dt, *J* = 8.1, 5.9, 5.7 Hz, H-8′), 3.15 (1H, d, *J* = 8.2 Hz, H-7), 3.68 (3H, s, OMe′), 3.72 (3H, s, OMe), 4.07–4.18 (2H, m, H-9′a, H-9′b) 6.36 (1H, dd, *J* = 1.9, 8.0 Hz, H-6′), 6.47 (1H, d, *J* = 1.9 Hz, H-2′), 6.61 (1H, d, *J* = 8.0 Hz, H-5′), 6.64 (1H, dd, *J* = 1.7, 7.9 Hz, H-6), 6.72 (1H, d, *J* = 7.9 Hz, H-5), 6.80 (1H, d, *J* = 1.7 Hz, H-2). δ_C (125 MHz, $CDCl_3$, 30 °C) δ 33.58 (C-7′), 33.70 (NCH_3), 43.73 (C-8′), 44.64 (C-8), 55.42 (OMe′), 55.52 (OMe), 66.05 (C-7), 69.34 (C-9), 111.03 (C-2), 113.46 (C-2′), 115.08 (C-5′), 115.13 (C-5), 119.67 (C-6), 121.60 (C-6′), 128.55 (C-1′), 132.78 (C-1), 145.01 (C-4′), 145.45 (C-4), 147.19 (C-3′), 147.69 (C-3), 178.80 (C-9′); EI-MS: *m*/*z* 387 (M^+, 7%), 356 (5), 220 (6), 177 (7), 166 (100), 149 (6), 137 (17); HRMS (EI): *m*/*z* calc. for $C_{21}H_{25}NO_6$ (M^+) 387.1682, found 387.1680.

Reaction of HMR in buffered methanolic (20 and 80%) solution at pH 9.5

HMR was dissolved (each isomer **1** and **2**, individually, in separate experiments) in a small volume of methanol (6 mL) and 24 mL of a 0.1 M $NaCO_3$ buffer was added (pH = 9.6). The solution was stirred for 24 h and small samples (0.250 mL) were withdrawn during the experiment for analyses by HPLC. The samples were diluted with a 1 mL standard solution of 0.1 M benzoic acid in 10% MeOH, 0.1% HAc (the final pH value of approximately 5, stopped the reaction). The samples were then immediately analysed by HPLC by the method described above.

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