Novel β–β-structures in lignins incorporating acylated monolignols

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ABSTRACT

Cross-coupling of γ-acylated sinapyl alcohols and sinapyl alcohol produces novel β–β-coupled products. Such structures are readily identified by HSQC NMR in isolated lignins (palm, kenaf and corn MWLs) containing γ-esters. This finding suggests that γ-acylated sinapyl alcohols are incorporated into lignins as monomers.

INTRODUCTION

The primary lignin monomers are the three monolignols, *p*-coumaryl, coniferyl and sinapyl alcohols. Polymerization of these monolignols by radical coupling catalyzed by plant peroxidases and/or laccases results in complex polymers (lignins) with various compositions and proportions of inter-unit linkages.[1] Lignin has been studied for more than a century but there is still a lot unknown about its structural and biological details.[2-4]

Lignins of many agriculturally important crops and woody plants have their sidechain primary alcohol (or γ-OH) acetylated, *p*-hydroxybenzoylated or *p*-coumaroylated.[3-5] The biochemistry of such acylation remains unresolved and the genes associated with the presumably involved transferase enzymes are unknown. As genes controlling various processes and the functions of such acylation are sought in order to improve the utilization of plant resources, it has become important to resolve whether monolignols are first acylated to produce ester conjugates that are then incorporated into lignin by free-radical coupling or cross-coupling reactions, or whether acylation occurs following the monolignol radical coupling reactions or on the lignin polymer itself.[6,7] We have provided preliminary evidence that the high level of γ-acetylation in Kenaf lignins arises, at least in significant part, from pre-acetylated sinapyl alcohol,[8] and have provided at least good circumstantial evidence for monolignol *p*-coumarates in grasses.[9,10] Here we report our observation of novel β–β-structures in isolated lignins from palm, kenaf and corn cell walls.

RESULTS AND DISCUSSION

Lignification in plant cells starts with dehydrogenative dimerization of monolignols and/or with cross-coupling between a monolignol and a ferulate.[4] The growth of a dehydrodimer continues primarily through cross-coupling with a monolignol radical, i.e. so-called "end-wise" polymerization.[2,11] Dehydrodimerization of coniferyl alcohol produces three dehydrodimers (β–O–4, β–5 and β–β) while only two dehydrodimers (β–O–4, β–β) are formed from dehydrodimerization of sinapyl alcohol. In addition to these three coupling modes (producing three types of inter-unit linkages), other inter-unit linkages found in lignin polymer include 5–5, 4–O–5, and β–1.

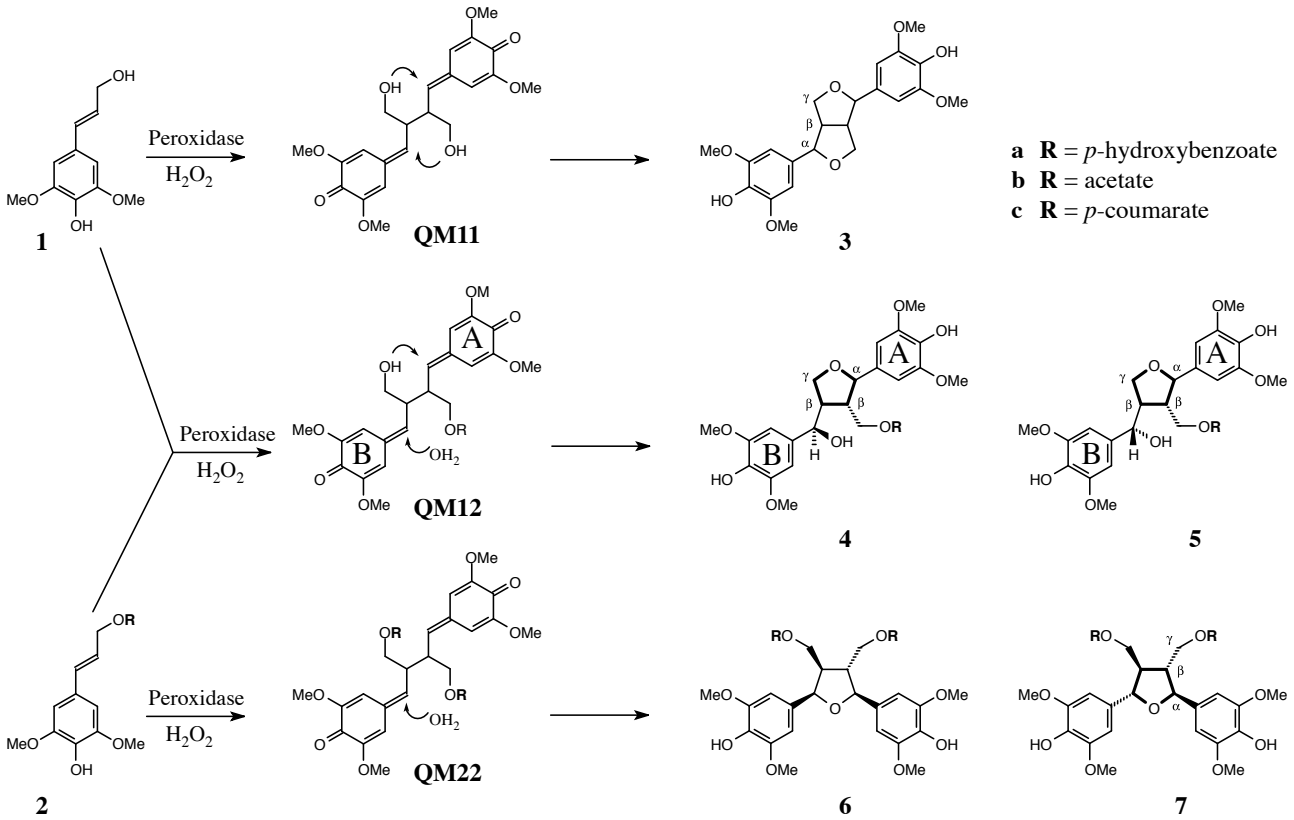

Figure 1. Radical coupling reactions of sinapyl alcohol **1** and γ-acylated sinapyl alcohols **2** produce β–β-coupled products **3-7**, suggesting that the structures could all be found in lignins incorporating γ-acylated monolignols. Compound **5a** has been found in actively lignifying xylem tissue in poplar, and here we provide NMR evidence for the other acylated sinapyl alcohol **2** products.

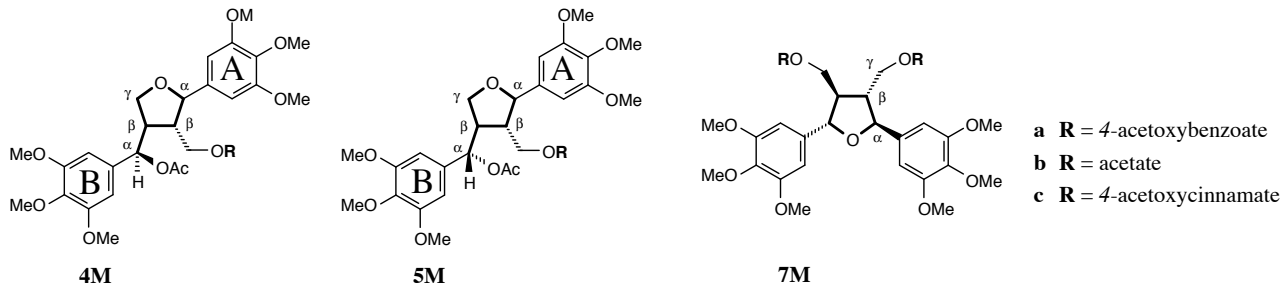

Figure 2. Model compounds **4M**, **5M** and **7M**

The β–β-coupling dehydrodimerization from coniferyl alcohol or sinapyl alcohol produces pinoresinol or syringaresinol. When the γ-OH of a monolignol is acylated, β–β-coupling can still presumably occur (the γ-OH is not required for the radical coupling step; the propenyl analog isoeugenol, for example, will also undergo β–β-coupling),[12] So the homo-dehydrogenative β–β-coupling of γ-acylated sinapyl alcohol forms an intermediate *bis*-quinone methide **QM22** (Figure 1), but the re-aromatization reactions following the radical coupling step can no longer be driven by the internal attack of the γ-OH on the quinone methide intermediate because the γ-acetylation prevents such a reaction. Consequently, one quinone methide rearomatizes by external water addition, as is typically seen following β–O–4-coupling, forming a α-OH that inter-molecularly adds to another quinone methide to form a tetrahydrofuran with isomer **6** as a major product. However, the cross-coupling reaction between sinapyl alcohol **1** and a γ-acylated sinapyl alcohol **2** produces quinone methide intermediate **QM12**. At this point there is an internal OH capable of trapping one quinone methide moiety, forming a γ–O–α-ether while the other (B-ring) quinone methide rearomatizes by external water addition to form the α-OH, producing isomeric products **4** and **5**.

Coupling reactions involving monolignols strongly favor homo-dehydrodimerization; cross-coupling reactions can be difficult to achieve.[13] However, peroxidase-H_2O_2 oxidation of equimolar amounts of sinapyl alcohol and γ-acylated sinapyl alcohol **2** produced a satisfactory 20 to 30% isolated yield of the cross-coupled products **4-5**. The ratio of products **3**:(**4**+**5**):(**6**+**7**) was essentially 1:2:1 suggesting that the coupling reactions were insensitive to the acylation of the γ-OH.

The novel β–β-cross- and homo-coupled products **4-7** from peroxidase-H_2O_2 oxidation of mixture of sinapyl alcohol **1** and γ-acylated sinapyl alcohol **2** imply that these structures may be present in lignins if γ-acylated

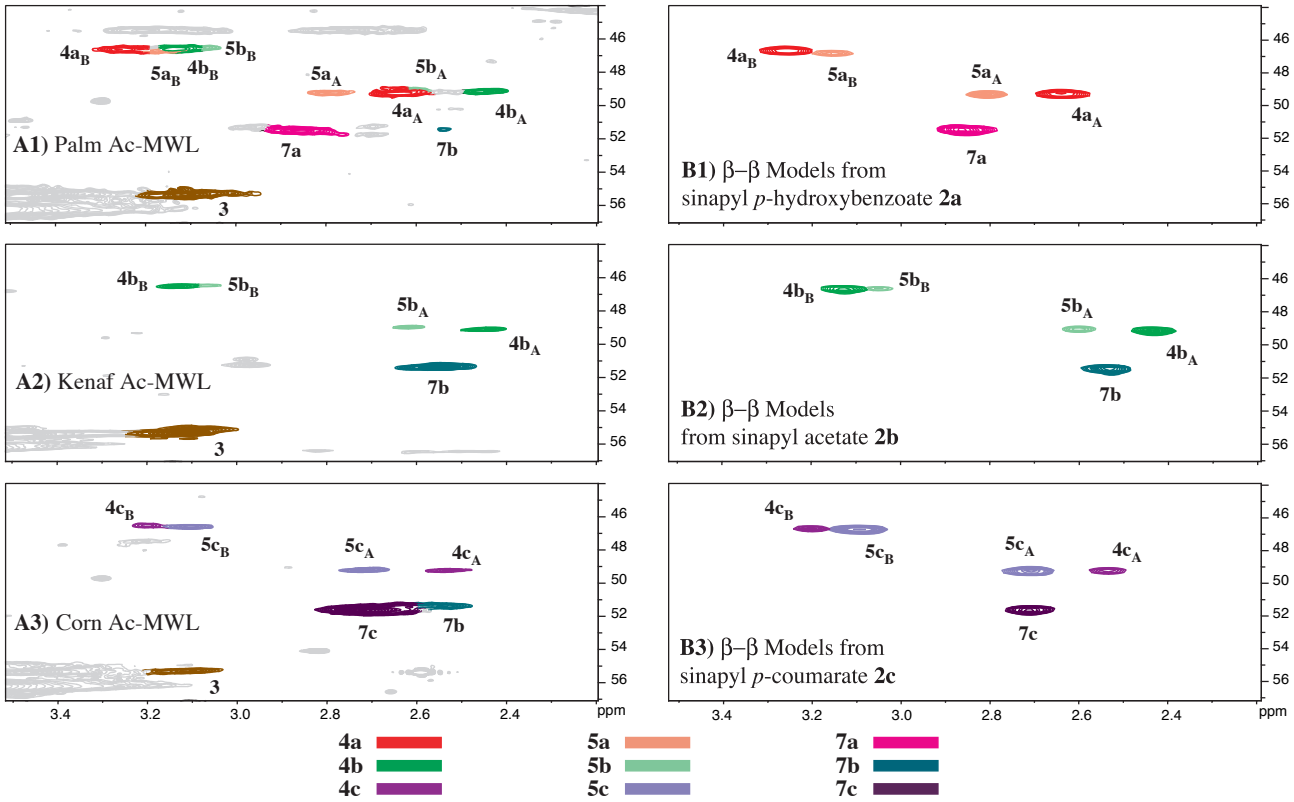

Figure 3. HSQC NMR showing the β ($^{13}C_\beta$–H_β) correlations of novel β–β-coupled structures produced from γ-acylated sinapyl alcohols in MWLs of palm (A1), kenaf (A2) and corn (A3). HSQC NMRs of methylated and acetylated model compounds (right column; individual spectra of compounds 4M, 5M and 7M are overlaid) are compared with MWLs (left column). [Note: the electronic version has this figure in color, which substantially helps clarify the information presented; see also http://www.dfrc.ars.usda.gov/DFRCWebPDFs/2005-Lu-ISWPC05.pdf].

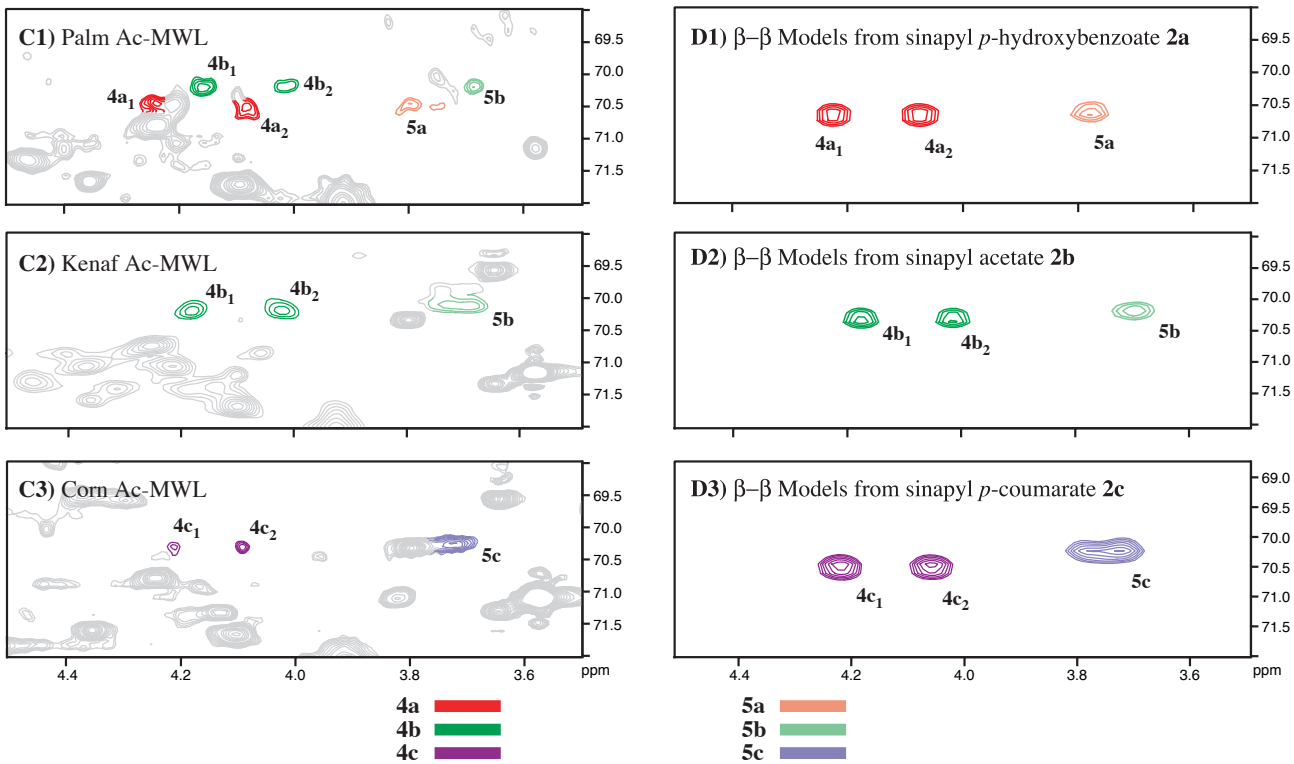

Figure 4. HSQC NMR showing the γ ($^{13}C_{\gamma}$–H_{γ}) correlations of novel β–β-coupled structures produced from γ-acylated sinapyl alcohols in MWLs of palm (C1), kenaf (C2) and corn (C3). HSQC NMRs of methylated and acetylated model compounds (right column; individual spectra of compounds **4M** and **5M** are overlaid) are compared with MWLs (left column). [Note: the electronic version has this figure also in color — see Fig. 3 caption].

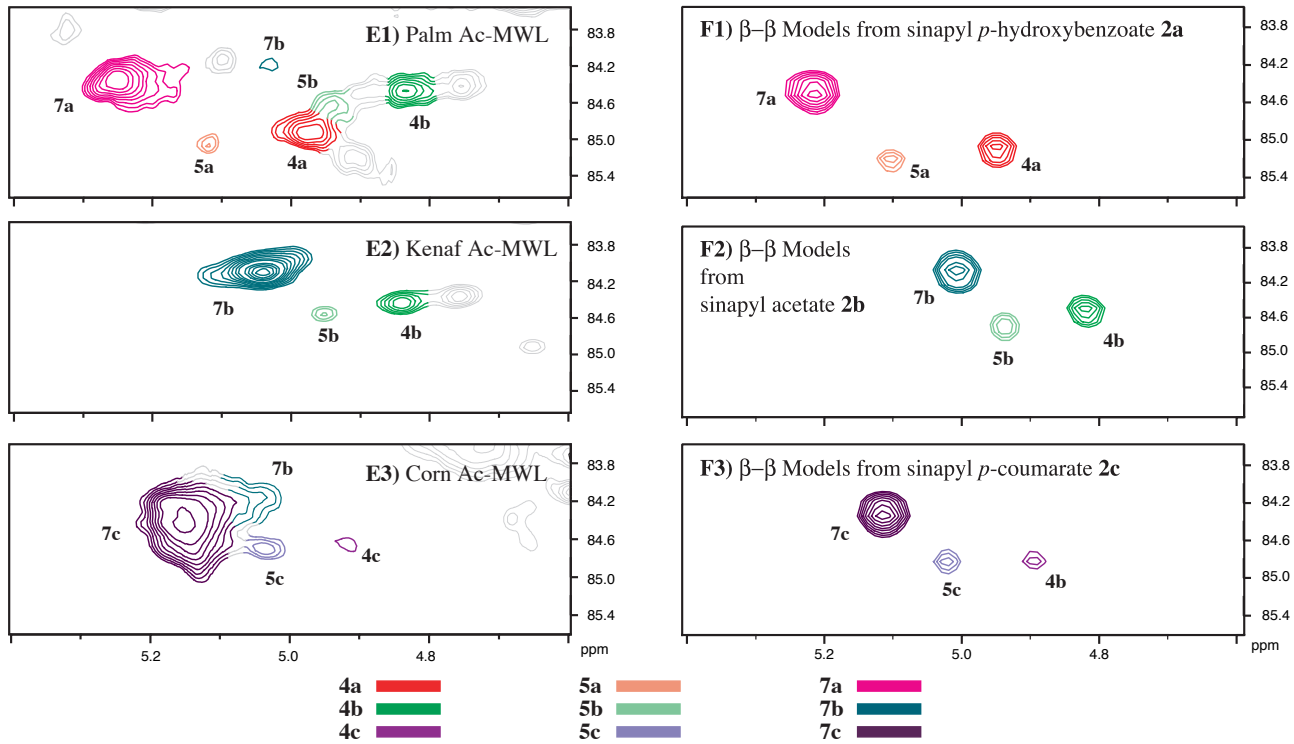

Figure 5. HSQC NMR showing the α ($^{13}C_{\alpha}$–H_{α}) correlations of novel β–β-coupled structures produced from γ-acylated sinapyl alcohols in MWLs of palm (E1), kenaf (E2) and corn (E3). HSQC NMRs of methylated and acetylated model compounds (right column; individual spectra of compounds **4M**, **5M** and **7M** are overlaid) are compared with MWLs (left column). [Note: the electronic version has this figure also in color — see Fig. 3 caption].

sinapyl alcohols **2** are incorporated as monomers during lignification. Cross-coupled product **5a** has been recently found in actively lignifying xylem tissue in poplar providing compelling evidence that sinapyl *p*-hydroxybenzoate must be a lignin monomer in the lignin (or lignan) pathway.[14,15] This finding encouraged us to carefully reexamine lignins in which esters have been found. Therefore three isolated lignins have been prepared from palm empty-fruit bunch fiber, kenaf bast fiber and corn stem cell walls according to published procedures.[9,16,17] In order to identify these novel β–β-structures potentially in lignin by NMR, β–β-coupled model compounds **4-7** were made from peroxidase-H_2O_2 oxidation of sinapyl alcohol **1** and γ-acylated sinapyl alcohols **2**. The structures of **4-7** were identified by the normal array of 1D and 2D (HSQC, HMBC, COSY) NMR experiments. Etherified and acetylated **4-7**, **4M-7M** (Figure 2) were synthesized to provide ideal models for identifying these novel structures in acetylated lignins (in which they are typically 4-O-etherified). Therefore HSQC NMR spectra of **4M-7M** were recorded at high resolution. The diagnostic sidechain regions of the HSQC spectra were compared with the corresponding regions of the three lignin samples from palm, kenaf and corn (Figure 3-5).

From the HSQC NMR spectra of the lignin samples in Fig. 3 (left column), it is obvious that all β ($^{13}C_\beta$–H_β) correlations corresponding to the novel β–β-structures are observed and identified by comparison with the synthesized model compounds **4M**, **5M** and **7M** (right column). Aspen lignin also contains structures **4a**, **5a** (spectra not shown here). One curious aspect however remains. Although peroxidase-H_2O_2 oxidative coupling of sinapyl alcohol **1** and γ-acylated sinapyl alcohol **2** produces compound **5** as the major isomer, palm and kenaf lignins contain slightly more of structure **5** than **4** while corn lignin has comparable amount of structures **4** and **5**. It was surprising to note that only structure **7**, which is a minor isomer produced by the peroxidase-H_2O_2 oxidative coupling reaction of γ-acylated sinapyl alcohol **2,** can be found in lignin samples. As we know so far, stereochemistry involved in lignin biosynthesis is under kinetic control and the isomer ratios of β–O–4 and β–β (resinol) in lignin are consistent with *in vitro* results.[4] The neo-olivil structure (guaiacyl analogue of compound **7**) was found in a spruce lignin preparation,[18] although in relatively small amounts compared to pinoresinol. It is difficult at present to understand why the neo-olivil structure is formed in spruce lignin since *in vitro* radical coupling reaction of coniferyl alcohol has never produced neo-olivil. Also why structure **6** cannot be detected in these lignin samples is still unknown. Whether there is a protein controlling the β–β-coupling reaction, or whether specific conditions produce only compound **7** from the β–β-coupling reaction of γ-acylated sinapyl alcohol awaits further investigation.

HSQC spectra in Figures 4 and 5 show the γ ($^{13}C_\gamma$–H_γ) and α ($^{13}C_\alpha$–H_α) correlations of these novel β–β-coupled structures found in palm, kenaf and corn lignins. The γ (C–H) correlations for structure **7** are buried under the overwhelming signals corresponding to β–O–4-ether structures and are not shown in Figure 4.

CONCLUSIONS

We have found and identified novel β–β-structures in palm, kenaf and corn lignins. These structures apparently derive from β–β-coupling involving γ-acylated sinapyl alcohols, providing compelling evidence that γ-acylated sinapyl alcohols are incorporated into lignin as such, and therefore must be regarded as authentic lignin monomers in these plants.

EXPERIMENTAL

Lignin NMR spectra were run on a Bruker DRX-600 equipped with a cryoprobe for enhanced sensitivity. Model compound spectra were run on a Bruker DRX-360. Conditions for HSQC experiments were standard.

Peroxidase-H_2O_2 oxidative coupling reactions of sinapyl alcohol or/and acylated sinapyl alcohols were performed according to previously described procedures.[8,15]

Synthesis of model compounds 4M, 5M and 7M

Compounds **4Mb, 5Mb** and **7Mb** were made from **4b**, **5b** and **7b** respectively by methylation (MeI-K_2CO_3, acetone) and acetylation (Ac_2O-Py). Compounds **4Ma**, **5Ma** and **7Ma** were synthesized from sinapyl 4-acetoxybenzoate and sinapyl alcohol by peroxidase-H_2O_2 oxidation, followed by methylation and acetylation. **4Mc**, **5Mc** and **7Mc** were obtained in the similar way starting from sinapyl 4-acetoxycinnamate.

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