Microbial production of natural and non-natural monolignols with *Escherichia coli*

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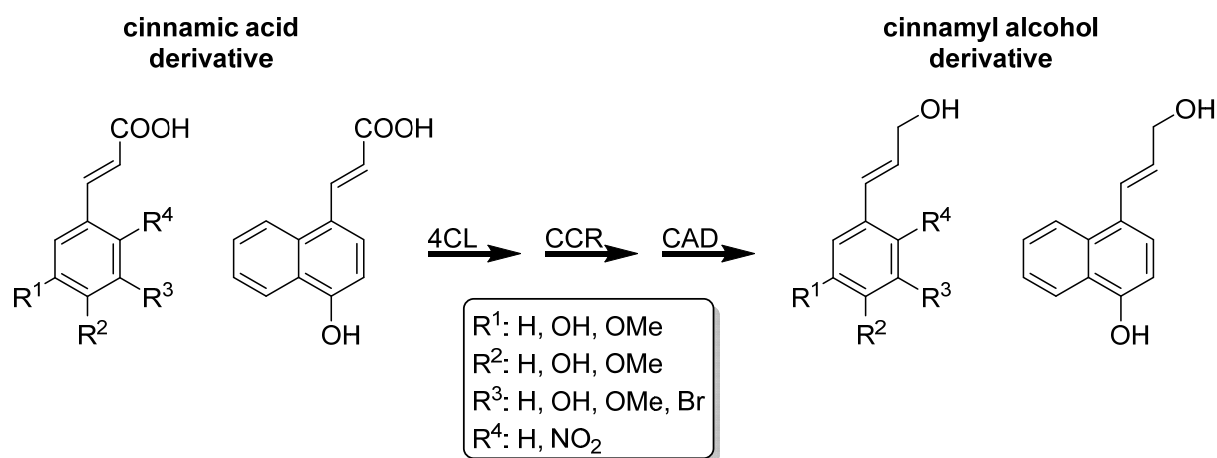
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24 **Table of contents:**

cinnamic acid derivative → 4CL → CCR → CAD → cinnamyl alcohol derivative

R^1: H, OH, OMe
R^2: H, OH, OMe
R^3: H, OH, OMe, Br
R^4: H, NO$_2$

25

26 **One for all.** The promiscuity of three enzymes constituting a synthetic

27 phenylpropanoid pathway in *Escherichia coli* was exploited for the stepwise reduction

28 of six naturally occuring as well as four non-natural phenylpropenoic acids to their

29 corresponding monolignols.

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Abstract

Phenylpropanoids and phenylpropanoid-derived plant polyphenols find numerous applications in food and pharmaceutical industries. In recent years, several microbial platform organisms were engineered towards producing such compounds. However, for the most part, microbial (poly)phenol production is inspired by nature, and thus predominantly naturally occurring compounds have been produced to this date.

Here, we took advantage of the promiscuity of enzymes involved in phenylpropanoid synthesis and exploited the versatility of an engineered *E. coli* strain harboring a synthetic monolignol pathway to convert supplemented natural and non-natural phenylpropenoic acids to their corresponding monolignols. Performed biotransformations showed that this strain is able to catalyze the stepwise reduction of chemically interesting non-natural phenylpropenoic acids such as 3,4,5-trimethoxycinnamic acid, 5-bromoferulic acid, 2-nitroferulic acid, and a 'bicyclic' *p*-coumaric acid derivative in addition to six naturally occurring phenylpropenoic acids.

Introduction

Many plant polyphenols such as flavonoids, stilbenes or lignans are important compounds for the food and pharmaceutical industries.[1] Here they find an application, e.g., as flavors, colorants, therapeutic agents or antibiotics. General precursor molecules of these valuable compounds are phenylpropanoids, which in turn are derived from the aromatic amino acids L-phenylalanine or L-tyrosine (**1**). Phenylpropanoid synthesis starts with the non-oxidative deamination of the aromatic amino acid yielding the typical phenylpropanoid core structure: a phenyl group attached to a propene tail (Figure 1).[1–3] This decisive reaction is catalyzed by ammonia lyases, either phenylalanine ammonia lyases (PAL) or tyrosine ammonia lyases (TAL).
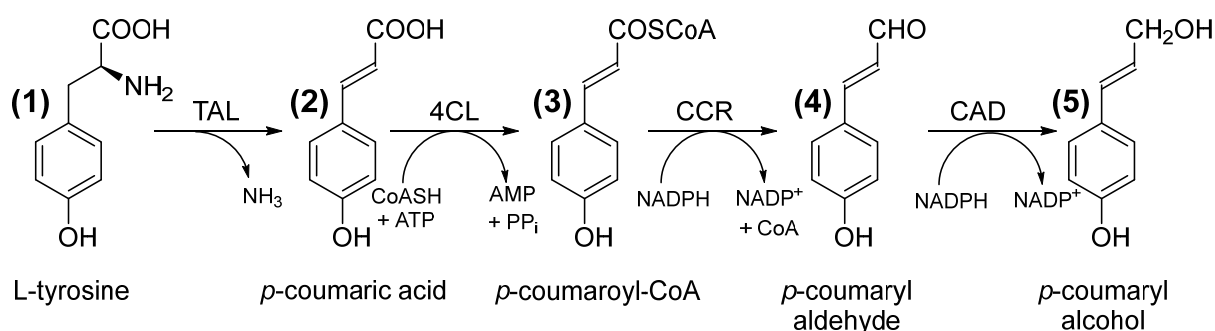

Figure 1. Biosynthetic pathway for p-coumaryl alcohol synthesis from L-tyrosine. TAL, tyrosine ammonia-lyase; 4CL, 4-coumaroyl-CoA ligase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase.

In case of L-tyrosine, the resulting phenylpropenoic acid p-coumaric acid (**2**) is subsequently activated by 4-coumarate-CoA ligases (4CL) yielding 4-coumaroyl-CoA. This CoA-activated compound **3** can subsequently serve as precursor molecule for the synthesis of flavonoids and stilbenes. Alternatively, p-coumaroyl-CoA can be stepwise reduced to the respective alcohol **5**, which is also referred to as monolignol. The required two reduction steps are catalyzed by cinnamoyl-CoA reductases (CCR) and

83   cinnamyl alcohol dehydrogenases (CAD), respectively. In plants the resulting

84   monolignols represent key building blocks for the synthesis of lignin, but are also

85   necessary for the synthesis of the pharmaceutically interesting group of lignans.[4,5]

86   In principle, phenylpropanoids and phenylpropanoid-derived polyphenols can be

87   isolated from plants as their natural producers, but polyphenol concentrations in the

88   plant usually account for less than one percent of the plant dry weight only.[6]

89   Furthermore, plant extraction is also limited by slow plant growth as well as

90   environmental and regional factors affecting overall product yields.[7,8] Total chemical

91   synthesis represents an interesting alternative, but depending on the complexity of the

92   target compound the synthesis route comprises a number of individual steps with

93   intermediate purifications.[9–11] Microbial phenylpropanoid production offers a

94   promising alternative to the uneconomic isolation from plant material as modern

95   molecular tools allow for the functional implementation of plant biosynthetic pathways

96   into the microbial metabolism.[1] Following this strategy, many microbial strains for plant

97   phenol synthesis were developed in recent years, especially for the production

98   phenylpropanoid-derived flavonoids and stilbenes.[12,13]

99   In this context, an *Escherichia coli* strain has been engineered to accumulate up to

100   52 mg/L *p*-coumaryl alcohol (**5**) without supplementation of any precursor

101   molecules.[14] The strain harbors a full synthetic phenylpropanoid pathway, which is

102   plasmid-encoded by a tetracistronic operon. Interestingly, all four enzymes

103   participating in monolignol biosynthesis have been previously described to be

104   promiscuous with regard to their substrate specificities.[15] This finding could enable

105   biosynthesis of other natural, and possibly also non-natural monolignols with

106   interesting applications from supplemented precursor molecules.[15,16] However,

107   practicability of this concept has been only demonstrated for the microbial production

108   of cinnamyl alcohol (**6**), caffeoyl alcohol (**7**) and coniferyl alcohol (**8**) from

109   supplemented natural cinnamic acid derivatives.[17–19] In addition, individual enzymes

110   of the monolignol pathway were successfully used for the microbial synthesis of

111   different non-natural flavanones and stilbenes from various precursors.[20–25] Here,

112   more detailed studies exploring the catalytic promiscuity of the enzymes of the

113   monolignol pathway will not only help to gain a deeper understanding of the enzymes

114   involved, but might also provide access to new compounds with interesting chemical

115   or pharmaceutical properties.[26–30]

116   In this study, we set out to explore the catalytic versatility of a synthetic monolignol

117   pathway in *E. coli* by supplementing naturally and non-natural occurring cinnamic acid

118   derivatives.

119

Results and Discussion

Microbial synthesis of naturally occurring monolignols with *E. coli*

122   Recently, *E. coli* BL21-Gold (DE3) *lacI*[Q1] pALXtreme-*tal-4cl-ccr-cad* was designed and

123   constructed, which can synthesize the monolignol *p*-coumaryl alcohol (**5**).[14] This strain

124   harbors a synthetic monolignol pathway composed of a tyrosine ammonia lyase from

125   *Rhodobacter sphaeroides* (TAL$_{Rs}$, GenBank: ABA81174.1), a 4-coumarate: CoA ligase

126   from *Petroselinum crispum* (4CL$_{Pc}$, GenBank: X13324.1), a cinnamoyl-CoA reductase

127   from *Zea mays* (CCR$_{Zm}$, GenBank: Y15069.1) and a cinnamyl-alcohol dehydrogenase

128   from *Z. mays*. All four genes, organized as synthetic operon under control of the IPTG-

129   inducible T7 promoter are plasmid-encoded. Initially, it was tested, if this monolignol

130   pathway is also capable of reducing cinnamic acid (**9**), caffeic acid (**10**), ferulic acid

131 (**11**), hydroxyferulic acid (**12**) and sinapic acid (**13**) as the most abundant naturally
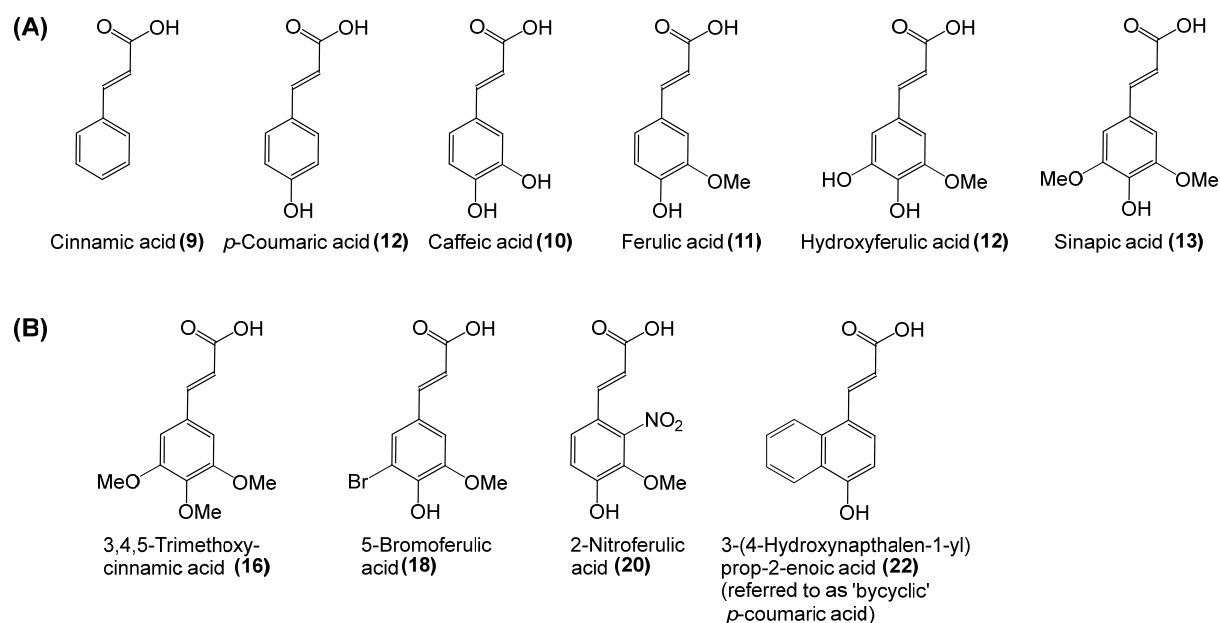
132 occurring cinnamic acid derivatives (Figure 2).

(A)

Cinnamic acid (**9**)   *p*-Coumaric acid (**12**)   Caffeic acid (**10**)   Ferulic acid (**11**)   Hydroxyferulic acid (**12**)   Sinapic acid (**13**)

(B)

3,4,5-Trimethoxy-cinnamic acid (**16**)   5-Bromoferulic acid (**18**)   2-Nitroferulic acid (**20**)   3-(4-Hydroxynapthalen-1-yl)prop-2-enoic acid (**22**) (referred to as 'bycyclic' *p*-coumaric acid)

133

134 **Figure 2. (A)** Naturally occurring and **(B)** non-natural cinnamic acid derivatives used in this

135 study.

136 For this purpose, all phenylpropenoic acids were individually supplemented to cultures

137 of growing *E. coli* cells at a concentration of 2.5 mM right at the start of the cultivation.

138 After 17 hours of cultivation, the concentrations of supplemented acid precursor

139 molecules as well as their corresponding monolignols in the supernatant were

140 determined by HPLC.

141 As a result of this systematic approach, it could be confirmed that the synthetic

142 pathway, although comprised of enzymes originating from three different organisms,

143 is indeed capable to reduce all supplemented natural cinnamic acid derivatives to their

144 corresponding monolignols in *E. coli* (Table 1). In case of cinnamic acid (**9**) as

145 chemically "most simple" precursor without any additional substituent on the aryl ring,

146 a product titer of 195 mg/L (1.46 mM) cinnamyl alcohol (**6**) could be determined (Table

147 1). In the past, cinnamyl alcohol (**6**) was produced in *E. coli* with a different set of

148 enzymes yielding 300 mg/L (2.24 mM) after 24 h.[17] However, biotransformations in

this study were performed using TB media containing a glycerol/glucose mixture (1 g/L), which served as carbon and energy source as this turned out to be the most suitable medium for monolignol synthesis with *E. coli* in previous studies.[14]

Table 1. Monolignol titers obtained through biotransformations with *E. coli* BL21-Gold (DE3) *lacI^Q1* pALXtreme-*tal-4cl-ccr-cad* from supplemented phenylpropenoic acids. For production, *E. coli* cells were cultivated in 50 mL LB medium and 2.5 mM of the respective cinnamic acid derivatives were individually supplemented. All biotransformations were performed at 25 °C for 17 h. Data represents average values and standard deviations from three biological replicates.

	Monolignol concentration	
	[mg/L]	[mM]
Natural monolignols		
Cinnamyl alcohol	195 ± 62	1.46
p-Coumaryl alcohol	121 ± 5	0.81
Caffeoyl alcohol	5 ± 1	0.03
Coniferyl alcohol	327 ± 10	1.82
Hydroxyconiferyl alcohol	102 ± 30	0.52
Sinapyl alcohol	30 ± 3	0.14
Non-natural monolignols		
3,4,5-Trimethoxycinnamyl alcohol	4 ± 1	0.02
5-Bromoconiferyl alcohol	462 ± 40	1.78
2-Nitroconiferyl alcohol	74 ± 15	0.33
'Bicyclic' *p*-coumaryl alcohol	25 ± 44	0.13

In addition to cinnamyl alcohol (**6**), the already described capability of this strain to produce *p*-coumaryl alcohol (**5**) could be confirmed as a concentration 121 mg/L (0.81 mM) of this monolignol could be determined in culture supernatants under the cultivation conditions described. Caffeic acid (**10**), characterized by an additional *O*-

164 methyl group on the aryl-ring in comparison to *p*-coumaric acid, was the least favored

165 substrate for the synthetic pathway as only 5 mg/L (0.03 mM) caffeoyl alcohol (**7**)

166 accumulated in the supernatant. In previous studies, the microbial production of

167 caffeoyl alcohol (**7**) with *E. coli* was achieved by using immobilized cells.[18] The

168 engineered strain equipped with a different set of enzymes produced up to 39 mg/L

169 (0.24 mM) caffeoyl alcohol (**7**) in LB medium within eight hours. In another recent

170 study, 534 mg/L (3.22 mM) caffeoyl alcohol (**7**) could be produced with an engineered

171 *E. coli* strain, but in total 4 mM caffeic acid (**10**) were supplemented at several time

172 points during 22 hours of cultivation using an optimized cultivation protocol and M9-

173 medium with yeast extract supplementation.[19] Interestingly, ferulic acid (**11**) turned

174 out to be the preferred natural substrate in this study since a product titer of 327 mg/L

175 (1.82 mM) of the corresponding coniferyl alcohol (**8**) could be determined in our

176 experiments. Extraction of coniferyl alcohol (**8**) from one liter culture supernatant

177 yielded 280 mg (1.55 mmol) of the pure compound.

178 For the first time the microbial production of hydroxyconiferyl alcohol (**14**) and sinapyl

179 alcohol (**15**) from supplemented hydroxyferulic acid (**12**) and sinapic acid (**13**),

180 respectively, could be demonstrated *in vivo*. After 17 h of cultivation, monolignol

181 concentrations of 102 mg/L (0.52 mM) and 30 mg/L (0.14 mM), respectively could be

182 determined (Table 1).

183 Noteworthy, not converted phenylpropenoic acids were not degraded and could be

184 detected in the supernatants of the *E. coli* cultures (data not shown).

185

186 **Microbial synthesis of non-natural monolignols with *E. coli***

187 Hitherto, only the microbial synthesis of naturally occurring monolignols has been

188 described. This is somewhat surprising, as access to non-natural monolignols would

189   also enable the synthesis pharmaceutically interesting lignans with novel properties.

190   With the aim to explore the catalytic flexibility of the established synthetic pathway for

191   the synthesis of such compounds, we attempted the conversion of four structurally very

192   different non-natural phenylpropenoic acids in order to probe the scope of the

193   approach. In particular, 5-bromoferulic acid (**18**) and 2-nitroferulic acid (**20**) were

194   chosen based on their potential for further diversification, e.g., through palladium-

195   catalyzed cross-couplings or after reduction to the corresponding aniline derivative.

196   Among these, 3,4,5-trimethoxycinnamic acid (**16**) and 5-bromoferulic acid (**18**) were

197   commercially available, but the substrates 2-nitroferulic acid (**20**) and 3-(4-

198   hydroxynaphthalen-1yl)prop-2enoic acid (**22**) needed to be synthesized (see

199   Supporting Information). In addition, the corresponding monolignols of all four non-

200   natural substrates tested were chemically synthesized to serve as reference

201   compounds for qualitative and quantitative analyses (see Supporting Information).

202   First experiments with 3,4,5-trimethoxycinnamic acid (**16**), a compound closely related

203   to sinapic acid (**13**) revealed that only a small fraction of 0.02 mM (4 mg/L) of this

204   substrate could be efficiently reduced to 3,4,5-trimethoxycinnamyl alcohol (**17**) (Table

205   1). In contrast, 5-bromoferulic acid (**18**) was rapidly reduced by the synthetic

206   monolignol pathway and a final product titer of 462 mg/L (1.78 mM) 5-bromoconiferyl

207   alcohol (**19**) could be determined in the supernatant (Table 1). Interestingly, under the

208   conditions tested, 5-bromoferulic acid (**18**) proved to be a much better substrate

209   compared to any of the naturally occurring phenylpropenoic acids used in this study.

210   The engineered *E. coli* strain also successfully reduced 2-nitroferulic acid (**20**) to 2-

211   nitroconiferyl alcohol (**21**). After 17 hours of biotransformation 74 mg/L (0.33 mM) 2-

212   nitroconiferyl alcohol (**21**) accumulated in the supernatant (Table 1). Motivated by

213   these results, the conversion of 'bicyclic' *p*-coumaric acid (**22**) as sterically most

214   challenging substrate was also attempted (Figure 2). This naphthalene derivative also

215   proved to be a suitable substrate as 25 mg/L (0.13 mM) of the corresponding

216   monolignol 'bicyclic' *p*-coumaryl alcohol **23** could be detected in culture supernatants

217   (Figure 1). Noteworthy, qualitative NMR experiments revealed that the pathway

218   intermediate 'bicyclic' *p*-coumaryl aldehyde accumulated in the supernatants of the

219   *E. coli* cultures. This indicates that this aldehyde is not a favored substrate for the CAD,

220   which catalyzes the last reduction step of the synthetic monolignol pathway.

221

222   **Optimization of the microbial 5-bromoconiferyl alcohol production**

223   Subsequently, the 5-bromoconiferyl alcohol (**19**) production with the engineered *E. coli*

224   strain was further optimized. Until this point substrate concentrations of 2.5 mM were

225   used in all biotransformations since natural cinnamic acid derivatives are known to

226   have an inhibitory effect on microbial growth.[31,32] With the aim to balance microbial

227   growth and product yield, biotransformations with different 5-bromoferulic acid (**18**)

228   concentrations were performed in 48-well microtiter plates in a microbioreactor system.

229   Unfortunately, 5-bromoferulic acid (**18**) concentrations exceeding 4 mM led to

230   substrate precipitation, which rendered determination of the culture backscatter over

231   time impossible (data not shown). This in turn impeded the evaluation of the impact of

232   elevated substrate concentrations on microbial growth. However, performed cultivation

233   experiments with substrate concentrations ranging from 0 mM and 2.5 mM already

234   revealed, that presence of 5-bromoferulic acid (**18**) has a growth-inhibiting effect

235   similar to the naturally phenylpropenoic acids tested here and in other studies (Figure

236   3A).[31,32] With regard to the maximum achievable product titer when considering the

237   cytotoxic effects of this compound for the cells, substrate concentrations between 2.5

238   mM and 3 mM turned out to most suitable as up to 0.9 mM 5-bromoferulic acid (**18**)

239   could be efficiently converted 5-bromoconiferyl alcohol (**19**) (Figure 3B). Higher

240   substrate concentrations of up to 6 mM 5-bromoferulic acid (**18**) resulted in a reduced

241   product formation, most likely due to the (probably) even more pronounced growth-

242   inhibitory effect of substrate concentrations exceeding 2.5 mM. The observed

243   substrate toxicity could be circumvented by stepwise addition of 5-bromoferulic acid

244   (**18**) during the biotransformation as it was also previously demonstrated for the

245   microbial production of *p*-coumaryl alcohol (**5**) and caffeoyl alcohol (**7**).[19] For microbial

246   monolignol production at reactor-scale, fed-batch fermentations are a suitable

247   option.[33] For future experiments at smaller scale, a slow-release technique could be

248   used to avoid growth inhibiting effects of elevated phenylpropenoic acid

249   concentrations. This technique is based on a diffusion-driven substrate release and

250   requires a feed reservoir filled with a concentrated substrate solution.[34,35] Here, a

251   dialysis membrane separating the reservoir from the *E. coli* cells, enables the diffusion

252   of the substrate into the culture medium.[34,35] In principle, this approach could be also

253   used for biotransformations at microtiter plate-scale.[36]
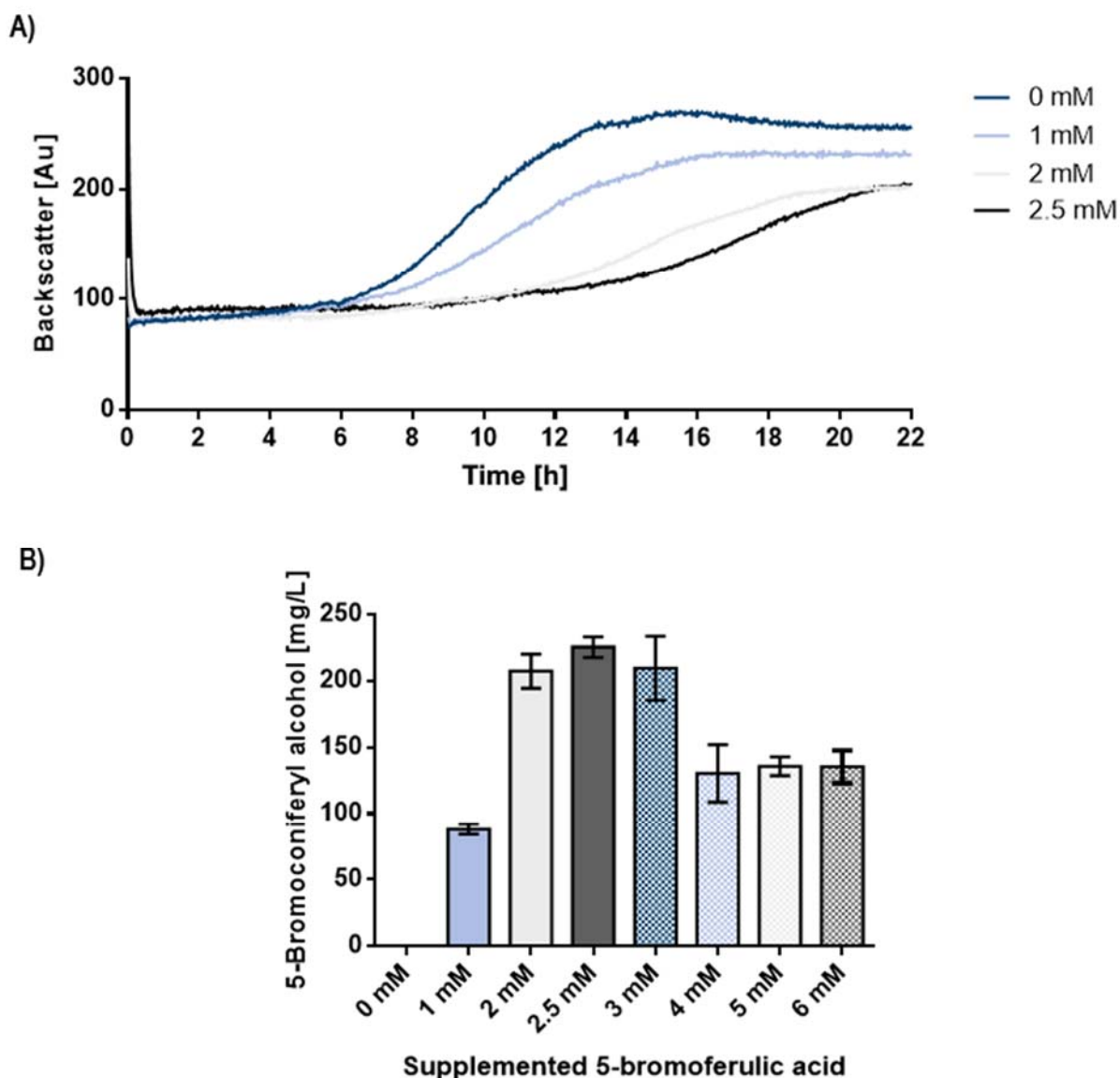
Figure 3. Impact of different 5-bromoferulic acid (**18**) concentrations on cell growth and 5-bromoconiferyl alcohol (**19**) production. A) Growth of the engineered *E. coli* strain in the presence of 5-bromoferulic acid (**18**) concentrations ranging from 0 mM to 2,5 mM B) Obtained 5-bromoconiferyl alcohol (**19**) concentrations in the presence of varying 5-bromoferulic acid (**18**) concentrations. *E. coli* BL21-Gold (DE3) *lacI^{Q1}* pALXtreme-*tal-4cl-ccr-cad* was cultivated in 900 μL LB medium with different 5-bromoferulic acid (**18**) concentrations in 48-well microtiter plates at 25 °C and 900 rpm. Heterologous gene expression was induced with 1 mM IPTG at the time point of inoculation. 5-Bromoconiferyl alcohol (**19**) concentrations were determined by HPLC. Data represents average values and standard deviations from three biological replicates.

Conclusions

In this study, *E. coli* BL21-Gold (DE3) *lacI^{Q1}* pALXtreme-*tal-4cl-ccr-cad* was characterized with regard to the biosynthetic versatility of the heterologous monolignol pathway. In this context, it could be shown that this strain represents a suitable catalyst for the production of six naturally and four non-natural occurring monolignols. Key to the success was the relaxed substrate specificity of the enzymes within this synthetic pathway, which accept a broad range of phenylpropanoid-like compounds as substrate.

In the context of this study, microbial synthesis of the naturally occurring hydroxyconiferyl alcohol (**14**) and sinapyl alcohol (**15**) could be demonstrated for the first time. In addition, the chemically interesting monolignols 3,4,5-trimethoxycinnamyl alcohol (**17**), 5-bromoconiferyl alcohol (**19**), 2-nitroconiferyl alcohol (**21**) and the 'bicyclic' *p*-coumaryl alcohol **23** could be synthesized by this *E. coli* strain. These compounds represent interesting starting points for the synthesis of more complex plant-inspired active agents.

Experimental Section

Bacterial strains, plasmids, media and growth conditions

E. coli BL21-Gold (DE3) *lacI^{Q1}* pALXtreme-*tal-4cl-ccr-cad* was used for monolignol production.[37] The pALXtreme vector backbone was constructed from a pET-28a(+) standard vector (Merck KGaA, Darmstadt, Germany) by removing 63 % of its sequence[38]. The resulting smaller vector was originally designed to improve the transformation efficiency in the context of screening campaigns, in which the efficient cloning and transformation of large and genetically diverse libraries is required.

291 Redesign of this plasmid required the genomic integration of the *lacI*^{Q1} gene from pET-

292 vector system yielding *E. coli* BL21-Gold (DE3) *lacI*^{Q1}. Hence, pALXtreme can be only

293 used in combination with this strain.[38] *E. coli* was cultivated aerobically in Luria Bertani

294 (LB) medium on a rotary shaker (130 rpm) or on LB plates (LB medium with 1.5 %

295 agar) at 37°C.[39] Where appropriate, kanamycin (50 µg/mL) was added to the medium.

296 Growth was determined by following the optical density at 600 nm (OD_{600}).

Chemical synthesis of phenylpropenoic acids and monolignols

299 Cinnamic acid derivatives and cinnamyl alcohol derivatives were either commercially

300 available or synthesized (see Supporting Information). The compounds were

301 supplemented during microbial monolignol synthesis and used standards for HPLC-

302 analyses.

Microbial monolignol production with *E. coli*

305 For monolignol production in 500 mL baffled shake flasks, 50 mL LB medium

306 containing 2.5 mM of the respective phenylpropenoic acid substrate was inoculated

307 with an *E. coli* over-night culture to an OD_{600} of 0.1. The culture was incubated at 37 °C

308 and 120 rpm until an OD_{600} of 0.2 was reached. Subsequently, the cultivation

309 temperature was decreased to 25 °C and heterologous gene expression was induced

310 with 1 mM IPTG when an OD_{600} of 0.6 was reached. Samples were taken 17 h after

311 IPTG addition for substrate/product analyses. All cultivations were performed in

312 biological triplicates.

313 For the microbial production of monolignols at microtiter plate-scale, *E. coli* cells were

314 cultivated using a BioLector device (m2p-labs GmbH, Germany). For this purpose,

315 cultivations were performed in 900 µL LB medium using 48-well flower plates. These

316 plates were incubated at 900 rpm and 25 °C, a humidity of 85% and a throw of ø 3

317 mm. When using this cultivation format, heterologous gene expression was induced

318 with 1 mM IPTG at the time point of inoculation. All cultivations were performed in

319 biological triplicates.

320

Quantification of phenylpropenoic acids and monolignols

322 Concentrations of phenylpropenoic acids and monolignols in cell free culture

323 supernatants were determined by HPLC using an Agilent 1260 infinity LC device

324 (Santa Clara, CA, USA) coupled with a DAD detector. For analyses, a mixture of water

325 with 2 % (v/v) acetic acid (buffer A) and acetonitrile with 2 % (v/v) acetic acid (buffer B)

326 as the mobile phases was used. LC separation was carried out using a ZORBAX

327 Eclipse AAA (3.5 µm, 4.6 × 75 mm) column with a guard cartridge (4.6 × 12.5 mm) at

328 50°C. For an efficient separation, 85 % buffer A and 15 % buffer B were used for a

329 maximum of 35 min with one additional minute as post time. Substrates and products

330 were detected by monitoring the absorbance at a defined single wavelength (**Fehler!**

331 **Verweisquelle konnte nicht gefunden werden.**2). Benzoic acid (final concentration

332 100 mg/L, 0.82 mM) was used as internal standard. Authentic metabolite standards

333 were either purchased from Sigma-Aldrich (Schnelldorf, Germany) or chemically

334 synthesized in-house. Six different concentrations of each standard dissolved in

335 acetonitrile were measured for each calibration curve. Calibration curves were

336 calculated based on analyte/internal standard ratios for the obtained area values.

Coniferyl alcohol extraction from culture supernatant

338 Culture supernatants were carefully acidified to pH 6.0 using 1 M hydrochloric acid.

339 Subsequently, the coniferyl alcohol was extracted three times with 450 mL ethyl

340 acetate. The combined organic layers were dried with $MgSO_4$, filtrated and the solvent

341   was removed under reduced pressure. The resulting product was purified via column

342   chromatography (*n*-pentane: ethyl acetate 60:40).

343

344

345   **Acknowledgements**

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List of contents

General syntheses

General: All reagents were used as purchased from commercial suppliers without further purification. Petroleum ether, *n*-pentane and ethyl acetate for column chromatography were distilled before usage. Brine refers to a saturated solution of NaCl in deionized water. Microwave reactions were performed in a CEM Discover system (SN: DU8708), equipped with an CEM Intelligent explorer (SN: NX2069). Flash column chromatography was performed on silica gel 60, particle size 40–63 µm (230–240 mesh). Absorbance measurements were conducted using an UV-160 spectrophotometer. ^{1}H and ^{13}C-NMR spectra were recorded on an Advance/DRX 600 nuclear magnetic resonance spectrometer (Bruker) at ambient temperature in CDCl$_3$ or DMSO-*d$_6$* at 600 and 151 MHz, respectively. The chemical shifts are given in ppm relative to the solvent signal [^{1}H: δ (CHCl$_3$) = 7.26 ppm], [^{13}C: δ (CDCl$_3$) = 77.2 ppm], [^{1}H: δ (DMSO-*d$_6$*) = 2.50 ppm], [^{13}C: δ (DMSO-*d$_6$*) = 39.5 ppm], [^{1}H: δ (Acetone-*d$_6$*) = 2.05], [^{13}C: δ (Acetone-*d$_6$*) = 29.8 ppm]. NMR signals were assigned by means of H-COSY-, HSQC- and HMBC-experiments and coupling constants J are given in Hz. Chiral HPLC measurements were performed on a Dionex system equipped with a pump with a gradient mixer and devolatilizer included a WPS-3000TSL autosampler and a DAD-3000 UV-detector. Chiralpak IA column (250 mm×4.6 mm, Daicel) and a mixture of *n*-heptane/2-propanol (70:30) as solvent was used applying a flow rate of 0.5 mL/min^{-1} at r.t. Samples were dissolved in degassed *n*-heptane: 2-propanol 2:1.

Synthesis of reference alcohols

Method A

R^1 = H, OH, OMe
R^2 = OH, OMe
R^3 = H, OMe, Br
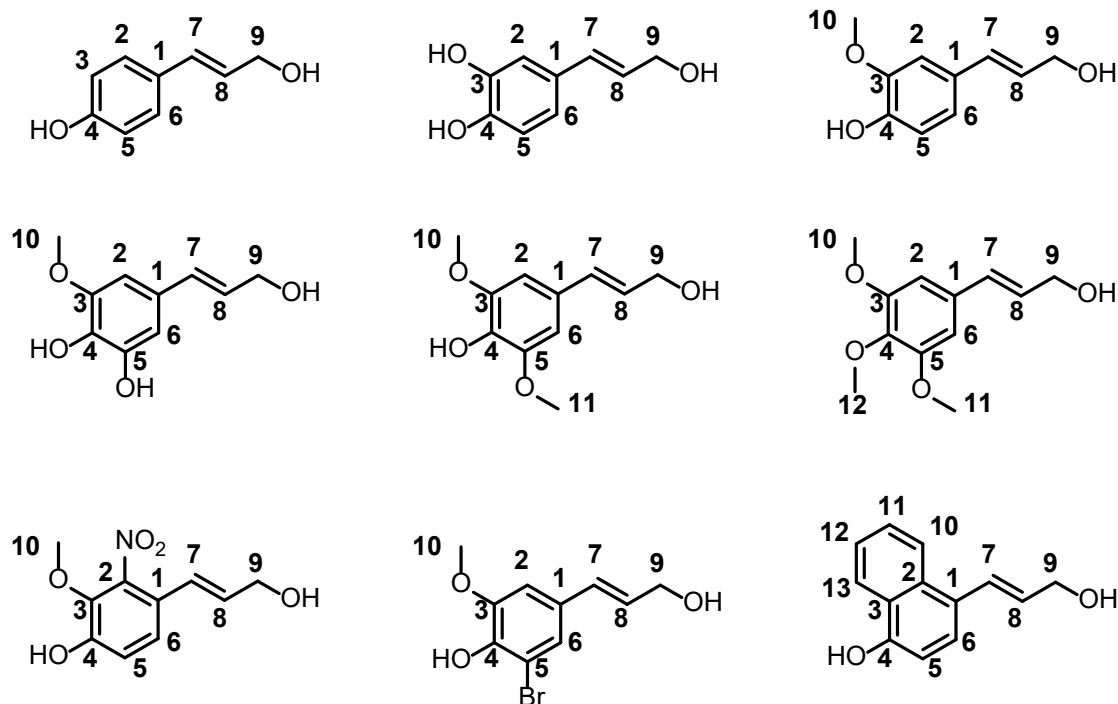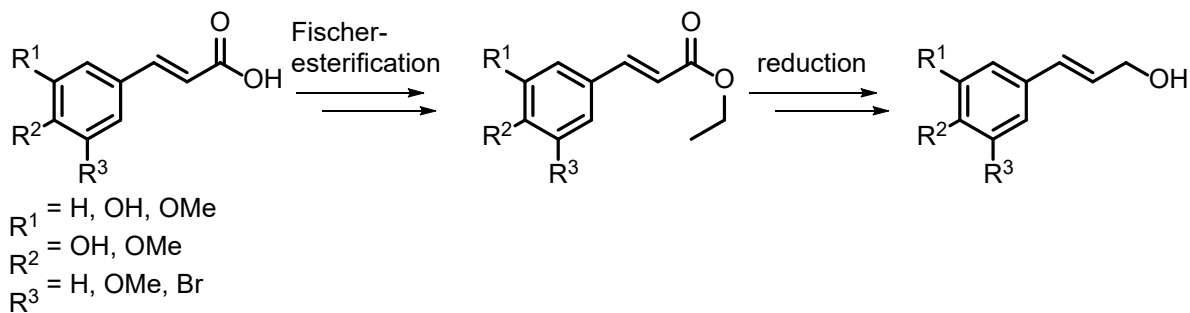
Fischer esterification

The acid (1 mmol) was solved in 2.7 mL of ethanol inside a microwave reaction tube. One drop of conc. sulfuric acid was added. The solution was then heated to 95 °C using a microwave for 30-90 min. The reaction was monitored using TLC (thin layer chromatography). After complete conversion the solution was diluted with ethyl acetate and then washed with water, followed by washing with brine. The organic phase was dried with MgSO₄, filtered and concentrated to

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give the crude product. If necessary, the product was purified using column chromatography (petroleum ether:ethyl acetate).

TBS-Protection of ethyl (*E*)-3-(3,4-dihydroxyphenyl)acrylate (24)

The **ethyl (*E*)-3-(3,4-dihydroxyphenyl)acrylate** (24) (1.1 mmol) was solved in 7 mL dichloromethane. *tert*-Butyldimethylchlorosilane (2.5 eq., TBS-Cl) and *N,N*-ethyldiisopropylamine (3.5 eq.) were added to the stirring solution. The solution was stirred for 22 h. After complete conversion, observed by TLC, 3 mL of dichloromethane were added. The resulting solution was added to 5 mL of water. The two-phase system was washed with 10 mL brine. After phase separation the organic phase was dried with $MgSO_4$, filtered and concentrated to give pure product.[1]

Ester reduction using DiBAl-H

Diisobutylaluminium hydride (DiBAl-H, 2.5 eq., 1 additional equivalent was added per hydroxyl group) solution (1 M in dichloromethane) was diluted with 2.3 mL tetrahydrofuran. The solution was stirred and cooled to -20 °C. The ester (3 mmol) was solved in 2.3 mL tetrahydrofuran and added dropwise over 30 min. The reaction was stirred for 1 h at -20 °C and monitored using TLC. After complete conversion, the excess DiBAl-H was quenched using ethyl acetate at 0 °C. Half-saturated NaK-tartrate solution was added. After phase separation, the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried with MgSO$_4$ and subsequently filtered. Pure product could be isolated after removal of the solvent under reduced pressure.[2]

Deprotection of (*E*)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy)phenyl)-prop-2-en-1-ol

(*E*)-3-(3,4-bis((*tert*-butyldimethylsilyl)oxy)phenyl)-prop-2-en-1-ol (0.5 mmol) was solved in 30 mL tetrahydrofuran and 1.1 mL acetic acid. The solution was cooled to 0 °C and *tetra*-butylammonium fluoride solution (2.5 eq., 1 mol/L in tetrahydrofuran) was added. Afterwards the reaction was stirred for 2 h at 0 °C. After completion the volume was reduced to 50%, which resulted in precipitation of a yellow solid. This solid was collected and washed with chloroform, until the yellow colour disappeared. Pure (*E*)-3-(3,4-dihydroxy)phenyl)-prop-2-en-1-ol (7) could be isolated after drying.[3]

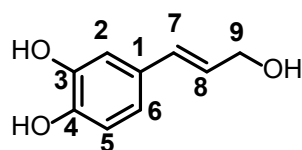## (*E*)-3-(4-Hydroxy)phenyl)-prop-2-en-1-ol (*p*-coumaryl alcohol) (5)
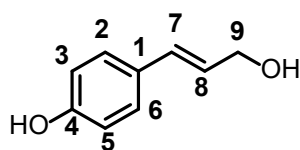
Yield: 35%

¹H-NMR (CDCl₃, 600 MHz) δ [ppm]: 4.16-4.20 (m, 2 H, 9-H); 6.20 (dt, $^3J_{8,7}$ = 15.8 Hz, $^3J_{8,9}$ = 5.6 Hz, 1 H, 8-H); 6.51 (d, $^3J_{7,8}$ = 15.9 Hz, 1 H, 7-H); 6.79 (d, $^3J_{3/5,2/6}$ = 8.2 Hz, 2 H, 3-H and 5-H); 7.27 (d, $^3J_{2/6,3/5}$ = 8.2 Hz, 2 H, 2-H and 6-H)

¹³C-NMR (CDCl₃, 151 MHz) δ [ppm]: 62.6 (s, C-9); 115.3 (s, C-3 and C-5); 127.0 (s, C-8); 127.5 (s, C-2 and C-6); 128.9 (s, C-1); 129.2 (s, C-7); 156.9 (s, C-4)

**(*E*)-3-(3,4-Dihydroxy)phenyl)-prop-2-en-1-ol (caffeoyl alcohol) (7)**

Yield: 43%

¹H-NMR (CDCl₃, 600 MHz) δ [ppm]: 4.04 (dd, $^3J_{9,9\text{-}OH}$ = 5.4 Hz, $^4J_{9,7}$ = 1.7 Hz, 2 H, 9-H); 4.73 (d, $^3J_{9\text{-}OH,9}$ = 5.5 Hz, 1 H, 9-O*H*); 6.03 (dt, $^3J_{7,8}$ = 15.9 Hz, $^3J_{9,8}$ = 5.5 Hz, 1 H, 8-H); 6.03 (d, $^3J_{7,8}$ = 15.8 Hz, 1 H, 7-H); 6.67-6.64 (m, 2 H, 6-H and 5-H); 6.80 (d, $^3J_{2,6}$ = 1.4 Hz, 1 H, 2-H); 8.85 (s, 1 H, 3-OH); 8.92 (s, 1 H, 4-O*H*)

¹³C-NMR (CDCl₃, 151 MHz) δ [ppm]: 61.7 (s, C-9); 113.0 (s, C-2); 115.6 (s, C-5); 117.9 (s, C-6); 127.0 (s, C-8); 128.4 (s, C-1); 129.0 (s, C-7); 145.0 (s, C-4); 145.3 (s, C-3)

**(*E*)-3-(4-Hydroxy-3-methoxy)phenyl)-prop-2-en-1-ol (Coniferyl alcohol) (8)**
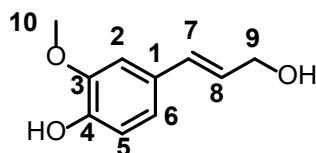
Yield: 90%

¹H-NMR (CDCl₃, 600 MHz) δ [ppm]: 3.82 (s, 1 H, 9-OH); 3.87 (s, 3 H, 10-H); 4.19 (dd, $^3J_{9,8}$ = 5.9 Hz, $^4J_{9,7}$ = 1.7 Hz, 2 H, 9-H); 5.63 (s, 1 H, 4-OH); 6.23 (dt, $^3J_{8,7}$ = 15.8 Hz, $^3J_{8,9}$ = 6.0 Hz, 1 H, 8-H); 6.54 (dt, $^3J_{7,8}$ = 15.8 Hz, $^4J_{7,9}$ =1.6 Hz,

1 H, 7-H); 6.77 (d, $^3J_{5,6}$ = 8.1 Hz, 1 H, 5-H); 6.86 (dd, $^3J_{6,5}$ = 8.1 Hz, $^4J_{6,2}$ = 2.0 Hz, 1 H, 6-H); 7.06 (d, $^4J_{2,6}$ = 2.0 Hz, 1 H, 2-H)

^{13}C-NMR (CDCl$_3$, 151 MHz) δ [ppm]: 56.0 (s, C-10); 63.9 (s, C-9); 108.5 (s, C-2); 114.6 (s, C-5); 120.4 (s, C-6); 126.3 (s, C-8); 129.3 (s, C-1); 131.5 (s, C-7); 145.7 (s, C-3 or C-4); 146.77 (s, C-4 or C-3)

## (*E*)-3-(3,4,5-Trimethoxy)phenyl)-prop-2-en-1-ol(3,4,5-trimethoxycinnamoyl alcohol) (17)
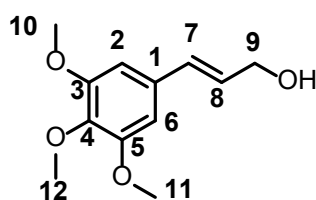
Yield: 46%

^{1}H-NMR (CDCl$_3$, 600 MHz) δ [ppm]: 3.84 (s, 3 H, 12-H); 3.87 (s, 6 H, 10-H and 11-H); 4.32 (m, 2 H, 9-H); 6.29 (dt, $^3J_{8,7}$ = 15.8 Hz, $^3J_{8,9}$ = 7.1 Hz, 1 H, 8-H); 6.54 (d, $^3J_{7,8}$ = 15.8 Hz, 1 H, 7-H); 6.61 (s, 2 H, 2-H and 6-H)

^{13}C-NMR (CDCl$_3$, 151 MHz) δ [ppm]: 56.2 (s, C-10 and C-11); 61.1 (s, C-9); 63.8 (s, C-12); 103.7 (s, C-2 and C-6); 128.2 (s, C-8); 131.3 (s, C-7); 132.6 (s, C-1); 138.0 (s, C-4); 153.4 (s, C-3 and C-5)

(*E*)-3-(5-Bromo-4-hydroxy-3-methoxy)phenyl)-prop-2-en-1-ol (5-bromo-coniferyl alcohol) (19)
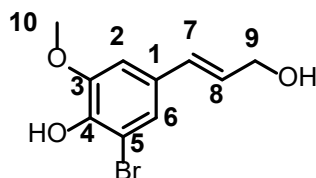
Yield: 80%

^{1}H-NMR (CDCl$_3$, 600 MHz) δ [ppm]: 3.92 (s, 3 H, 10-H); 4.31 (d, $^3J_{9,8}$ = 5.7 Hz, 2 H, 9-H); 5.30 (s, 1 H, 9-O*H*); 5.92 (s, 1 H, 4-O*H*); 6.23 (dt, $^3J_{8,7}$ = 15.8 Hz, $^3J_{8,9}$ = 5.8 Hz, 1

H, 8-H); 6.47 (d, $^3J_{7,8}$ = 15.8 Hz, 1 H, 7-H); 6.85 (d, $^4J_{6,2}$= 1.8 Hz, 1 H, 6-H); 7.13 (d, $^4J_{2,6}$= 1.8 Hz, 1 H, 2-H)
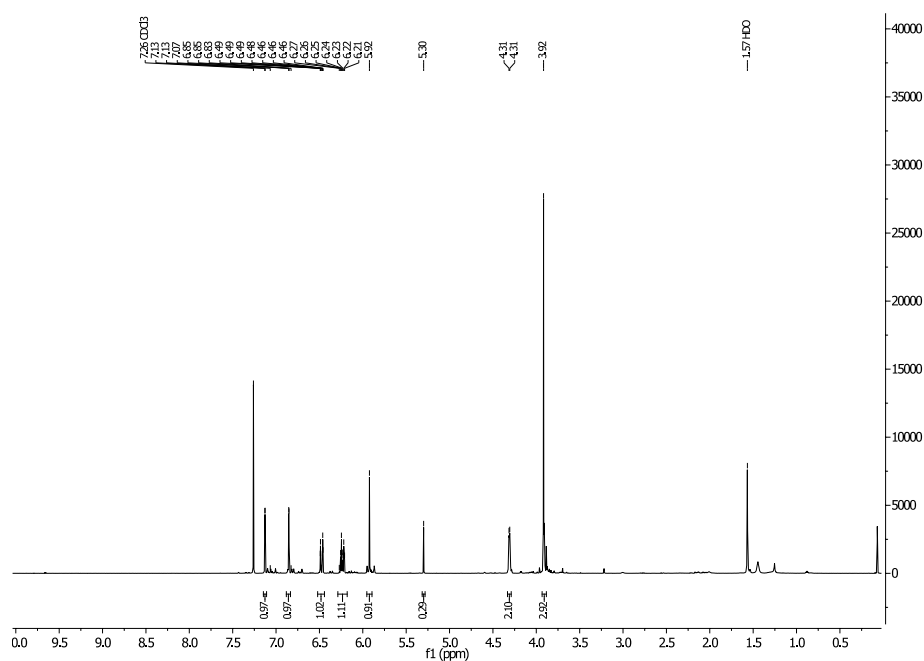
^{13}C-NMR (CDCl$_3$, 151 MHz) δ [ppm]: 56.5 (s, C-10); 63.7 (s, C-9); 107.8 (s, C-6); 108.5 (s, C-4); 123.4 (s, C-2); 127.9 (s, C-8); 123.0 (s, C-7); 130.1 (s, C-5); 142.9 (s, C-1); 147.4 (s, C-3)

Method B

R^1 = H, NO$_2$
R^2 = OMe
R^3 = OAc, OPiv
R^4 = H, OPiv, OMe
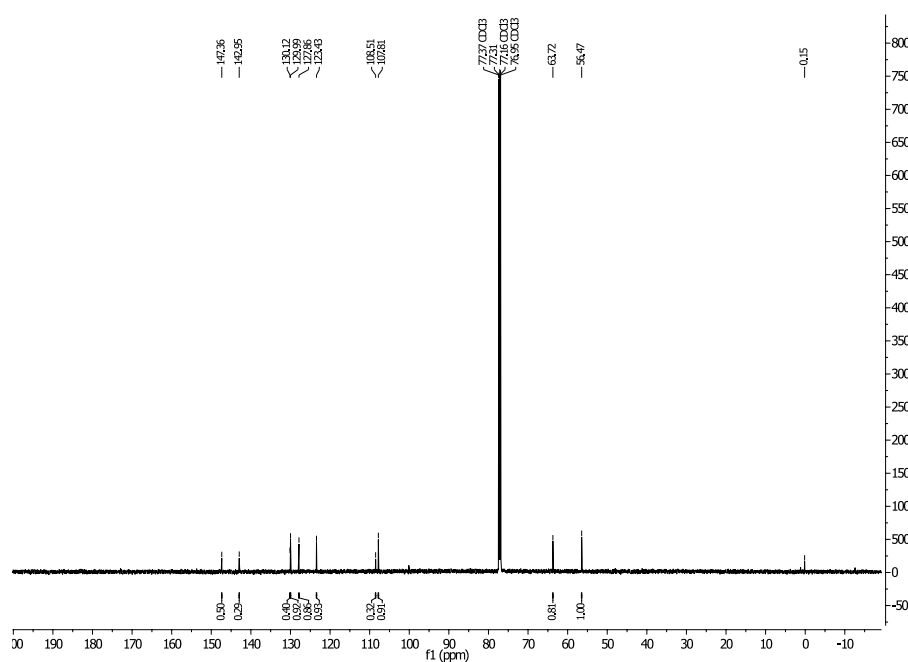
Protection of phenols with acetic anhydride

The aldehyde (6 mmol) together with 0.2 eq. dimethylaminopyridine was solved in 6.4 mL of dichloromethane. Triethylamine (3 eq. per hydroxyl group) and aceti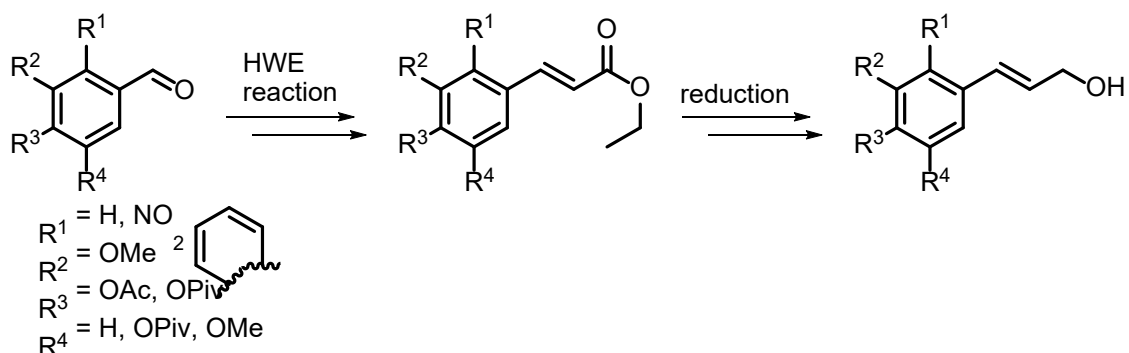c anhydride (1.2 eq. per hydroxyl group) were added to the stirred solution. The reaction was stirred for 1 h at 0 °C, followed by 4 h at 24 °C. After completion the reaction was quenched using water. The phases were then separated and the organic phase was washed with saturated NaHCO$_3$-Solution. Water was removed by washing with brine and through MgSO$_4$ addition. Following filtration, the solvent was removed and pure product could be isolated.[4]

HWE reaction

Triethyl phosphonoacetate (1.7 eq.) was solved in 1.6 mL tetrahydrofuran and the solution cooled to 0 °C. NaH (1.7 eq., 60% suspension in mineral oil) were added in multiple (four-ten) batches. The protected aldehyde (1 mmol) was solved in 1.7 mL tetrahydrofuran and added dropwise to the stirred solution. Conversion was monitored using NMR. The reaction was quenched with 1 mol/L HCl. After phase separation the product was extracted from the aqueous phase three times with ethyl acetate. The combined organic layers were dried with MgSO$_4$ and filtered. The crude product after removal of the solvent was either

directly used for the reduction or purified by column chromatography when necessary.[5]

Reduction using DiBAl-H

DiBAl-H solution (5.5 eq., 1 M in dichloromethane) was diluted with 8.1 mL tetrahydrofuran. The solution was stirred and cooled to -20 °C. The ester (3 mmol) was solved in 2.3 mL tetrahydrofuran and added dropwise over 30 min. The reaction was stirred for 1 h at -20 °C and monitored using TLC. After complete conversion the excess DiBAl-H was converted using ethyl acetate. The resulting yellow solid was treated with 2 mol/L HCl. The product was extracted from the aqueous layer with diethylether (three times). The combined organic layers were washed with saturated NaCl-solution, dried with $MgSO_4$, filtered and the solvent removed under reduced pressure. The crude product was purified using column chromatography.[2]

Reduction using LiAlH₄

LiAlH$_4$ (4 eq.) was added to 10.4 mL diethyl ether and stirred at -30 °C. The (*E*)-5-(3-ethoxy-3-oxoprop-1-en-1-yl)-3-methoxy-1,2-phenylene bis(2,2-dimethyl-propanoate) (1 mmol) in 7.8 mL diethyl ether was added dropwise over 30 min to the solution. The reaction was stirred at -20 °C for 2 h, while being monitored using TLC. After complete consumption of the ester remaining hydride was quenched with ethyl acetate at 0 °C. 2 mol/L HCl was added until the yellow residue was dissolved. The product 14 was extracted from the aqueous phase with diethyl ether (three times). The combined organic layers were washed with brine, dried with MgSO$_4$ and the solvent removed under reduced pressure. The product was immediately purified using column chromatography (*n*-pentane:ethyl acetate 60:40). The pure (*E*)-**3-(3,**4-**dihydr**oxy-**5-methoxy**)phenyl)-prop-2-en-1-ol (14) was stored under argon at -20 °C.

(*E*)-3-(3,4-Dihydroxy-5-methoxy)phenyl)-prop-2-en-1-ol (5-hydroxyconiferyl alcohol) (14)
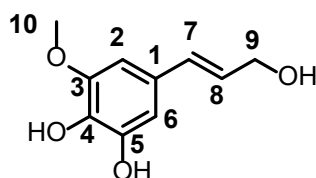
Yield: 33%

¹H-NMR (CDCl$_3$, 600 MHz) δ [ppm]: 3.84 (s, 3 H, 10-H); 4.21 (d, $^3J_{9,8}$ = 4.8 Hz, 2 H, 9-H); 6.21 (dt, $^3J_{8,7}$ = 15.7 Hz, $^3J_{8,9}$ = 5.2 Hz, 1 H, 8-H); 6.45 (d, $^3J_{7,8}$ = 15.9 Hz, 1 H, 7-H); 6.60 (d, $^4J_{2,6}$ = 2.3 Hz, 1 H, 2-H); 6.62 (d, $^4J_{6,2}$ = 2.3 Hz, 1 H, 6-H)

¹³C-NMR (CDCl$_3$, 151 MHz) δ [ppm]: 31.8 (s, C-9); 55.5 (s, C-10); 101.6 (s, C-6); 107.1 (s, C-2); 127.4 (s, C-8); 129.8 (s, C-7); 133.6 (s, C-1); 145.4 (s, C-3); 147.9 (s, C-4 or C-5); 148.1 (s, C-5 or C-4)

(*E*)-3-(4-Hydroxy-3,5-dimethoxy)phenyl)-prop-2-en-1-ol (sinapyl alcohol) (15)
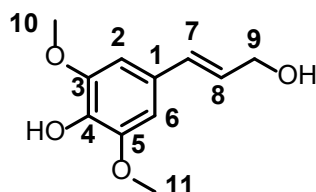
Yield: 71%

¹H-NMR (CDCl$_3$, 600 MHz) δ [ppm]: 3.90 (s, 6 H, 10-H and 11-H); 4.29-4.32 (m, 2 H, 9-H); 5.56 (s, 1 H, 4-O*H*); 6.24 (dt, $^3J_{8,7}$ = 15.8 Hz, $^3J_{8,9}$ = 5.9 Hz, 1 H, 8-H); 6.52 (dt, $^3J_{7,8}$ = 15.9 Hz, $^4J_{8,2/6}$ = 1.5 Hz, 1 H, 7-H); 6.63 (s, 2 H, 2-H and 6-H)

¹³C-NMR (CDCl$_3$, 151 MHz) δ [ppm]: 56.4 (s, C-10 and C-11); 63.9 (s, C-9); 103.5 (s, C-2 and C-6); 126.7 (s, C-1); 128.4 (s, C-8); 131.6 (s, C-7); 134.9 (s, C-4); 147.3 (s, C-3 and C-5)

(*E*)-3-(4-Hydroxy-3-methoxy-2-nitro)phenyl)-prop-2-en-1-ol (2-nitroconiferyl alcohol) (23)
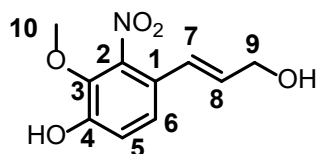
Yield: 39%

¹H-NMR (DMSO-*d$_6$*, 600 MHz) δ [ppm]: 3.81 (s, 3 H, 10-H); 4.06-4.09 (m, 2 H, 9-H); 4.94 (t, $^3J_{9\text{-OH},8}$ = 5.4 Hz, 1 H, 9-O*H*); 6.20 (dt, $^3J_{7,8}$ = 15.7 Hz, $^4J_{7,9}$ = 2.0 Hz, 1 H, 7-H); 6.35 (dt, $^3J_{8,7}$ = 15.7 Hz, $^3J_{8,9}$ = 4.5 Hz, 1 H, 8-H); 7.03 (d, $^3J_{6,5}$ = 8.7 Hz, 1 H, 6-H); 7.33 (d, $^3J_{5,6}$ = 8.7 Hz, 1 H, 5-H)

¹³C-NMR (DMSO-*d₆*, 151 MHz) δ [ppm]: 61.0 (s, C-10); 61.2 (s, C-7); 118.9 (s, C-6); 119.2 (s, C-9); 119.6 (s, C-1); 121.4 (s, C-5); 134.0 (s, C-8); 138.2 (s, C-3); 144.6 (s, C-2); 149.9 (s, C-4)

(*E*)-4-(3-Hydroxyprop-1-en-1-yl)naphthalen-1-ol (23)
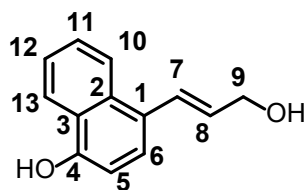
Yield: 52%

¹H-NMR (DMSO-d_6, 600 MHz) δ [ppm]: 4.38 (d, $^3J_{9,8}$ = 6.9 Hz, 2 H, 9-H); 6.32 (dt, $^3J_{8,7}$ = 16.1 Hz, $^3J_{8,9}$ = 6.6 Hz, 1 H, 8-H); 6.95 (d, $^3J_{13,12}$ = 7.8 Hz, 1 H, 13-H); 7.36 (d, $^3J_{7,8}$ = 16.1 Hz, 1 H, 7-H); 7.47-7.56 (m, 3 H, 10-H, 11-H and 12-H); 8.15 (d, $^3J_{5,6}$ = 8.4 Hz, 1 H, 5-H); 8.22-8.34 (m, 1 H, 6-H)
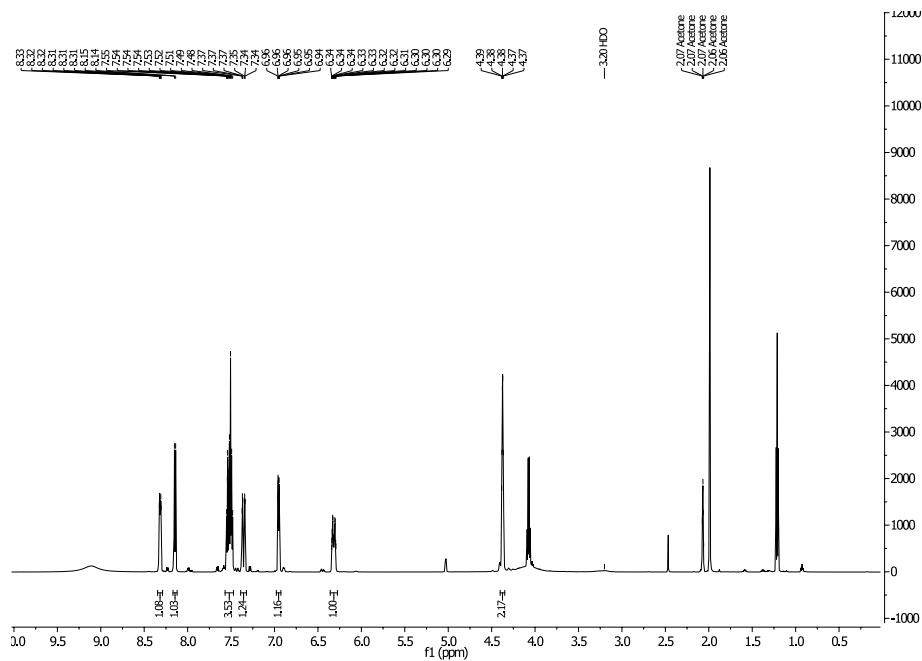
¹³C-NMR (DMSO-d_6, 151 MHz) δ [ppm]: 63.6 (s, C-9); 108.9 (s, C-13); 123.3 (s, C-6); 124.2 (s, C-5); 124.8 (s, C-10 or C-11 or C-12); 125.2 (s, C-10 or C-11 or C-12); 125.6 (s, C-2); 127.0 (s, C-10 or C-11 or C-12); 127.0 (s, C-3); 127.2 (s, C-7); 131.4 (s, C-8); 133.1 (s, C-1); 153.7 (s, C-4)

Synthesis of (*E*)-3-(4-hydroxy-3-methoxy-2-nitrophenyl)acrylic acid

2-Nitrosylation

4-formyl-2-methoxyphenyl acetate (15 mmol) was added slowly under stirring to 0.4 mL of concentrated nitric acid (>90%) at -20 °C. The resulting solution was added to 8 g of ice. After filtration the resulting white solid was washed with water and then solved in 5% (w/v) NaOH-solution. The solution was acidified with 4 M HCl, resulting in precipitation of a white solid. After filtration this solid was washed with water and purified by column chromatography.[6]

HWE reaction

The triethyl phosphonoacetate (1.7 eq.) was solved in 1.6 mL tetrahydrofuran and the solution cooled to 0 °C. The NaH (1.7 eq., 60% suspension in mineral oil) was added in multiple (four-ten) batches. The 4-hydroxy-3-methoxy-2-nitro benzaldehyde (1 mmol) was solved in 1.7 mL tetrahydrofuran and added dropwise to the stirred solution. Conversion was monitored using NMR. The reaction was quenched with 1 mol/L HCl. After phase separation the product was extracted from the aqueous phase three times with ethyl acetate. The

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combined organic layers were dried with MgSO₄ and filtered. The crude product after removal of the solvent was either directly used for the reduction or purified by column chromatography when necessary.[5]

Ester cleavage

The 3 ethyl (*E*)-3-(4-hydroxy-3-methoxy-2-nitrophenyl)acrylate (13 mmol) was solved in 1.5 mL ethanol. The solution was added dropwise to 20 mL 2.5 mol/L NaOH-solution. The reaction was stirred for 20 min at 24 °C and monitored with TLC. After complete conversion the reaction mixture was diluted with water, cooled to 0 °C and acidified with cold HCl. 2-Nitroferulic acid **((*E*)-3-(4-hydroxy-3-methoxy-2-nitrophenyl)acrylic acid)** (20) was collected as a white precipitate after filtration.

(*E*)-3-(4-Hydroxy-3-methoxy-2-nitrophenyl)acrylic acid (2-nitroferulic acid) (20)
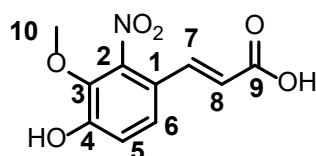
Yield: 38%

¹H-NMR (DMSO-d₆, 600 MHz) δ [ppm]: 3.84 (s, 3 H, 10-H); 6.47 (dd, $^3J_{7,8}$ = 15.7 Hz, $^4J_{7,6}$ = 1.8 Hz, 1 H, 7-H); 7.09 (dd, $^3J_{5,6}$ = 8.8 Hz, $^4J_{5,4\text{-}OH}$ = 1.7 Hz, 1 H, 5-H); 7.13 (d, $^3J_{8,7}$ = 15.7 Hz, 1 H, 8-H); 7.63 (dd, $^3J_{6,5}$ = 8.8 Hz, $^4J_{6,7}$ = 1.8 Hz,, 1 H, 6-H)

¹³C-NMR (DMSO-d₆, 151 MHz) δ [ppm]: 61.4 (s, C-10); 116.5 (s, C-1); 119.1 (s, C-6); 121.1 (s, C-7); 123.2 (s, C-5); 134.9 (s, C-8); 138.6 (s, C-3); 145.7 (s, C-2); 153.0 (s, C-4); 166.9 (s, C-9)

Synthesis of Naphthalene Derivative

Demethylation

The (*E*)-3-(4-methoxynaphthalen-1-yl)acrylic acid (4.4 mmol) were solved in 11.5 mL dichloromethane. BBr_3-solution (3. 5 eq., 1 mol/L in dichloromethane) was added dropwise. The reaction was stirred for 20 h, while being monitored with TLC. After quenching with water the solution was diluted with ethyl acetate and water. The product was extracted from the aqueous layer with ethyl acetate. The combined organic layers were dried with $MgSO_4$, filtrated and the solvent removed under reduced pressure. The crude product 22 was purified with column chromatography.[7]

3-(4-Hydroxynaphthalen-1-yl)prop-2-enoic acid (22)
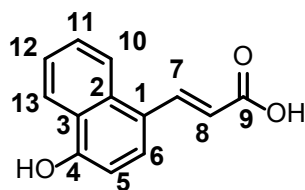
Yield: 35%

¹H-NMR (Acetone-d_6, 600 MHz) δ [ppm]: 6.35 (d, $^3J_{7,8}$ = 15.7 Hz, 1 H, 7-H); 6.88 (d, $^3J_{6,5}$ = 7.6 Hz, 1 H, 6-H); 7.39-7.43 (m, 1 H, 11-H); 7.49-7.53 (m, 1 H, 12-H); 7.72 (d, $^3J_{5,6}$ = 7.6 Hz, 1 H, 5-H); 8.09 (d, $^3J_{10,11}$ = 8.6 Hz, 1 H, 10-H); 8.20 (d, $^3J_{13,12}$ = 8.4 Hz, 1 H, 13-H); 8.34 (d, $^3J_{8,7}$ = 15.5 Hz, 1 H, 8-H)

¹³C-NMR (Acetone-*d₆*, 151 MHz) δ [ppm]: 109.2 (s, C-5); 118.3 (s, C-7); 123.6 (s, C-1); 123.7 (s, C-10); 123.8 (s, C-13); 125.8 (s, C-2); 126.0 (s, C-12); 127.4 (s, C-6); 128.3 (s, C-11); 133.8 (s, C-3); 142.1 (s, C-8); 156.6 (s, C-4); 168.18 (s, C-9)

Absorption maxima and observed retention times of natural and non-natural cinnamic acid derivatives and their corresponding monolignols used for HPLC analyses. Benzoic acid always used as internal standard.

	Retention time [min]	Wavelength [nm]
Natural phenylpropanoids		
Cinnamic acid (**9**)	25.56	275
Cinnamyl alcohol (**6**)	19.38	250
p-Coumaric acid (**2**)	4.60	320
p-Coumaryl alcohol (**5**)	3.83	260
Caffeic acid (**10**)	2.75	325
Caffeoyl alcohol (**7**)	2.39	260
Ferulic acid (**11**)	5.90	325
Coniferyl alcohol (**8**)	4.70	260
Hydroxyferulic acid (**12**)	2.86	320
Hydroxyconiferyl alcohol (**14**)	3.88	250
Sinapic acid (**13**)	5.89	320
Sinapyl alcohol (**15**)	4.67	275
Non-natural phenylpropanoids		
3,4,5-Trimethoxycinnamic acid (**16**)	24.26	320
3,4,5-Trimethoxycinnamyl alcohol (**17**)	15.00	260
5-Bromoferulic acid (**18**)	23.66	325
5-Bromoconiferyl alcohol (**19**)	18.26	260
3-Nitroferulic acid (**20**)	17.30	275
3-Nitroconiferyl alcohol (**21**)	13.34	250
'Bicyclic' *p*-coumaric acid **22**	26.91	260
'Bicyclic' *p*-coumaryl alcohol **23**	28.86	320
Benzoic acid (internal standard)	9.14	230

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