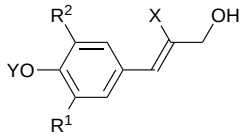
Rational design, synthesis and biological evaluation of the first inhibitor of lignin polymerization

Nicolas Daubresse,[a] Yves Chupeau,[b] Charlette Francesch,[a] Catherine Lapierre,[c] Brigitte Pollet[c] and Christian Rolando*[a]

[a] *Ecole Normale Supérieure, Département de Chimie, URA 1679 du CNRS, Processus d'Activation Moléculaire, 24 rue Lhomond, 75231 Paris Cedex 05, France*
[b] *Laboratoire de Biologie Cellulaire, INRA, Centre de Versailles, 78026 Versailles, France*
[c] *Laboratoire de Chimie Biologique, INRA-CBAI, Institut National Agronomique Paris-Grignon, 78850 Thiverval-Grignon, France*

A fluorinated analogue of coniferin (the glucoside of coniferyl alcohol) has been synthesized in three steps from vanillin tetraacetyl glucoside; bearing a fluorine atom on the β position of the propenyl side chain which is involved in the main site of coupling, it is designed as a potential inhibitor of oxidases involved in the biosynthesis of lignin, and *in vivo* experiments show a pronounced inhibition of lignin biosynthesis in poplar plantlets, with a five-fold decrease in lignin formed.

Lignins, the second major natural biopolymer on earth after cellulose, are biosynthesized by polycondensation of cinnamyl alcohols **1a**–**c**.[1–3] The crucial step consists of an oxidative



1a X = Y = R^1 = H, R^2 = OMe
b X = Y = H, R^1 = R^2 = OMe
c X = Y = R^1 = R^2 = H
2 X = F, Y = R^1 = H, R^2 = OMe
3 X = H, Y = glucose, R^1 = H, R^2 = OMe
4 X = F, Y = glucose, R^1 = H, R^2 = OMe

coupling of these phenols, *via* the formation of radicals[4] by a radical–radical or a radical–anion mechanism.[5,6] The enzymatic route remains unclear, since peroxidases and laccases can both, in artificial medium, catalyse the oxidation step and lead to lignin-like dehydropolymers (DHP).[7–9] To the best of our knowledge, there has been no report on specific inhibition of the polymerization step *in vivo*. Coloration tests on microcuttings, however, have shown that fluoroferulic acid is able to inhibit the activity of peroxidases.[10–12]

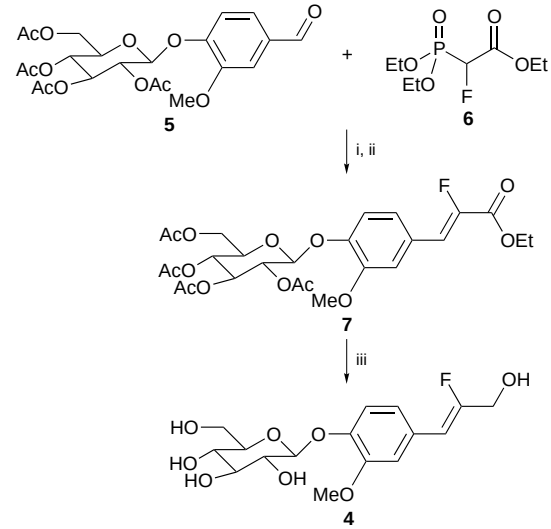
We herein describe the first *in vivo* inhibition of lignin biosynthesis by use of the fluorinated analogue **2** of coniferyl alcohol **1a**, the main natural precursor of lignin. A fluorine atom has been substituted for one hydrogen in the β-vinyl position. The structures of **1a** and **2** are very close, and thus we can expect similar affinities for the enzymes.[13,14] Compounds **1a** and **2** have similar redox potentials [0.11 and 0.16 V respectively](SHE),[15] as might be supposed based on the reported slightly destabilizing effect of fluorine on radical centres.[16,17] Thus no difference should be observed in the ability of the enzyme to oxidize **1a** or **2**. On the other hand, the reactivity of the radical obtained from **2** is expected to be dramatically changed, owing to the interaction between the stabilizing effect of the methoxy group on the aromatic ring[15] and the strong electron-withdrawing effect of the fluorine atom.[18]

Glucosides **3** are the natural protected forms of cinnamyl alcohols **1** and have been extensively used for radiolabelling experiments,[19,20] the glucosyl residue being cleaved *in situ* by a β-glucosidase before the polymerization step.[21–23] β-Fluoroconiferin **4** was thus designed as the stable protected form of fluoroconiferyl alcohol, the actual inhibitor.

Synthesis of **4** was easily achieved, with high yields, from the tetra-*O*-acetylglucoside of vanillin **5** (Scheme 1).[24,25] A Wadsworth–Edmons reaction with triethylfluorophosphonate **6**[26, 27] under phase transfer conditions catalysed by tris[2-(2-methoxyethoxy)ethyl]amine (TDA-1)[28] led, after isomerization of the *E/Z* mixture with bromine,[29] to pure *Z* β-fluoro α,β-unsaturated ester **7**.† The five ester groups were reduced with *in situ* generated lithium borohydride leading to pure **4**.‡

The biochemical assay was performed according to a standard test we have developed on poplar plantlets grown *in vitro* under controlled conditions.§[30] This test is based on the assimilation of the glucosides of cinnamyl alcohols which are administered to the plantlets through the growing artificial medium. Artificial substrates **3** (sample 1) and **4** (sample 2) were delivered to lignifiying zones after diffusion in the medium and crossing of the root barrier. After allowing a three week growing phase, incorporation was followed by a standard lignin analysis of the new stems: lignin was chemically degraded by thioacidolysis and residues were analysed by GC–MS and quantified by GC after silylation.¶[31,32]

After three weeks of growing, no difference was observed on the aspect of plantlets from blanks (no feeding), samples 1 (fed **3**) and 2 (fed **4**). The extent of lignification was, however,



Scheme 1 *Reagents and conditions*: i, TDA-1, aq. K_2CO_3, CH_2Cl_2, reflux, 24 h, 91%; ii, Br_2 (0.05 equiv.), CCl_4, 4 h, 88%; iii, $NaBH_4$ (7.4 equiv.), LiCl (10 equiv.), glyme, 80 °C, 18 h, 69%

Table 1 Thioacidolysis yields in μmol g^{-1} of dried stems in main G (guaiacyl) and S (syringyl) lignin-derived monomers recovered from poplar plantlets cultured *in vitro*

Sample	Precursor administered	G	S	S+ G	S/G
Blank	None	21	10	31	0.50
1	Coniferin	26	15	41	0.58
2	Fluoroconiferin	4	3	7	0.78

significantly lowered in sample 2: the thioacidolysis yield (a reflection of the lignin concentration) was only 7 μmol g^{-1}, compared to a 31 μmol g^{-1} yield for the blank and sample 1 (Table 1).

These results suggest that the designed compound fluoroconiferyl alcohol **2** acts as an inhibitor of the lignification process. As the catalytic activity of the β-glucosidase is most probably unchanged, the lignin biosynthesis must be affected at the oxidative polymerization step. Lignin precursors such as coniferyl alcohol **1a** are oxidized by any kind of oxidizing enzymes, and no specificity has yet been established. The use of labelled derivatives of **4** should allow the identification of the enzymes specifically involved in the oxidative biosynthesis of lignin.

Footnotes and References

* E-mail: chr@roxane.ens.fr

† *Selected data* for **7**: ^{1}H NMR (250 MHz, $CDCl_3$): 7.42–7.28 (m, 3 H, H_{ar}), 7.01 (d, 1 H, ArCH, J_{HF} 34.8), 5.44–5.26 (m, 3 H, H2,3,4), 5.16 (m, 1 H, H1), 4.50 (q, 2H, CH_2CH$_3$, *J* 7.1), 4.43 (dd, 1 H, H6b, *J* 12.3, 5.0), 4.32 (dd, 1 H, H6a, *J* 12.3, 2.6), 3.99 (s, 3 H, MeO), 3.95 (ddd, 1 H, H5, *J* 10.0, 5.0, 2.6), 2.21 (s, 6 H, Ac), 2.19 (s, 6 H, Ac), 1.53 (t, 3 H, CH$_2$CH_3 *J* 7.1); MS (CI, NH_3) *m/z* 588 (100%, M + 18), 331 (23), 240 (14), 110 (43).

‡ *Selected data* for **4**: mp 142–144 °C (Found: C, 48.51; H, 6.32. $C_{16}H_{21}FO_8.2H_2O$ requires C, 48.48, H, 6.36%); ^{1}H NMR (250 MHz, CD_3OD): 7.18 (br s, 1 H, H_{ar}2), 7.09 (d, 1 H, H_{ar}5, *J* 8.5), 7.02 (br d, 1 H, H_{ar}6, *J* 8.5), 5.78 (d, 1 H, ArC*H*CF, J_{HF} 39.1), 4.86 (d, 1 H, H1, *J* 7.0), 4.13 (d, 2 H, ArCHCFCH_2, J_{HF} 15.0), 3.72 (s, 3 H, MeO), 3.67 (m, 1 H, H6b), 3.63 (m, 1 H, H6a), 3.55–3.35 (m, 4 H, H2,3,4,5); ^{13}C NMR (62.5MHz, CD_3OD): 158.1 (d, ArCH*C*F, J_{CF} 265), 148.8 (C_{ar}4), 145.6 (C_{ar}3), 127.8 (C_{ar}1), 121.4 (d, Ar*C*H, J_{CF} 7.0), 116.0 (C_{ar}5), 112.3 (d, C_{ar}6, J_{CF} 7.9), 106.0 (d, C_{ar}2, J_{CF} 6.1), 100.9 (C1), 76.6 (C3), 76.1 (C5), 73.2 (C2), 69.7 (C4), 60.9 (C6), 60.8 (d, CF*C*H$_2$OH, J_{CF} 32.3), 55.1 (MeO). MS (CI, NH_3) *m/z* 378 (83%), 360 (10), 198 (74), 182 (14), 181 (100), 180 (48), 163 (12), 102 (22).

§ *Plant material and administration* of **3** and **4**. Microcuttings from hybrid poplar (*Populus alba* × *Populus tremula*, clone 717-B4) were cultured aseptically on solid medium as previously described (ref. 30). One day after the sterile deposition of nodes into culture tubes, 2.5 mg of coniferin **3** (sample 1) or fluoroconiferin **4** (sample 2) in DMSO (30 μl) were added carefully on the surface of the solid medium without any contact with the nodes. After three weeks of controlled growing, samples were collected, new shoots were detached, freeze-dried and directly subjected to thioacidolysis. Each administration was repeated for five culture tubes, and the amount of collected sample varied from 3 to 5 mg.

¶ *Thioacidolysis and GC–MS analysis*. Thioacidolysis was performed as previously described (refs. 31, 32). The amount of C22 docosane internal standard was 0.02 mg per analysis of *ca.* 3 mg of dried sample.

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