Pergamon

Tetrahedron 57 (2001) 371–378

TETRAHEDRON

Asymmetric biomimetic oxidations of phenols: the mechanism of the diastereo- and enantioselective synthesis of dehydrodiconiferyl ferulate (DDF) and dehydrodiconiferyl alcohol (DDA)

Marco Orlandi,[a,*] Bruno Rindone,[a] Giorgio Molteni,[b] Petteri Rummakko[c] and Gösta Brunow[c]

[a]*Department of Environmental Sciences, University of Milan-Bicocca, Piazza della Scienza 1, I-20126 Milan, Italy*
[b]*Department of Organic Chemistry, University of Milan, Via Golgi 19, I-20133 Milan, Italy*
[c]*Department of Chemistry, P.O. Box 55 FIN-00014, University of Helsinki, Helsinki, Finland*

Received 30 June 2000; revised 1 September 2000; accepted 19 September 2000

Abstract—Stereoselective bimolecular radical coupling of enantiopure phenylpropenoidic phenols are described, starting from enantiopure amidic derivatives of ferulic acid. The latter were prepared from ferulic acid by reaction with (*S*)-alanine or Oppolzer camphor sultam. The oxidation step was performed both enzymatically (HRP/H_2O_2) and chemically (Ag_2O). The observed enantioselectivity in the oxidation step encompasses the range 65–84% and is consistent with the conformational analysis of the quinone methide intermediates at the PM3 level.

1. Introduction

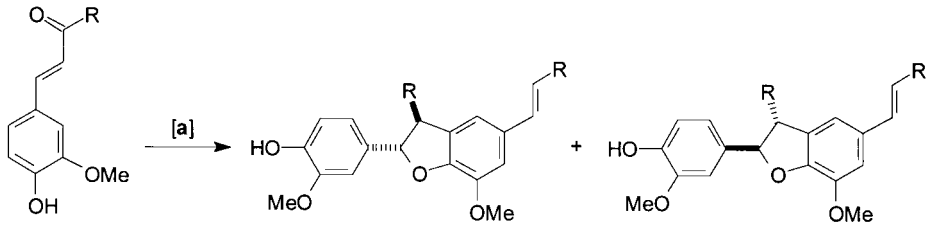
Organic compounds obtained from radical coupling of phenylpropenoidic phenols account for nearly 30% of the organic carbon circulating in the biosphere, and display important biological roles. In fact, they constitute organic polymers such as lignin,[1] suberin[2] and algal cell wall[3] and an important class of bioactive substances, the lignans.[4] Since the 1950's, oxidative coupling of monolignols have been postulated to be a stereorandom process,[5] but naturally occurring lignans and neolignans are known to be homochiral.

Recently, it has been found that neolignans characterised by the presence of the phenylcoumaran skeleton appear to be ubiquitous in the plant kingdom, being found in plants as diverse as loblolly pine,[6] tobacco,[7] ferns,[8] and hornworts,[9] thus spanning all major groups of vascular plants and suggesting their involvement in an universal chemical defense system.[10] Moreover, the dilignol 3′-4-di-*O*-methylcedrusin[11] is a wound healing agent and an inhibitor of thymidine incorporation in endothelial cells[12] and dehydrodiconiferyl alcohol plays a role in promoting cell division in tobacco tissue cultures.[13]

The total synthesis of phenylcoumarans are known to involve multistep procedures with low overall yields;[14] nevertheless, the oxidative phenol coupling is often used as the key step of the synthetic sequence. Although a lot of different methodologies involving radical phenol coupling have been described to prepare molecules with 2,3-dihydrobenzo[*b*]furan skeleton (Scheme 1, Table 1), the bimolecular phenoxy radical coupling does not appear to be regio- and stereo- controlled.[25] This is probably due to the fact that phenoxy radicals are very persistent species,[26] and the dimerization reaction is rather slow. Hence, the new stereogenic centres which arises from the in vitro oxidative phenol coupling are racemic[27,28] but naturally occurring lignans are homochiral.[4] The biosynthetic pathway to enantiopure lignans have been proposed quite recently. A protein isolated from *Forsythia* species has been suggested to be responsible for the formation of enantiopure pinoresinol from achiral coniferyl alcohol,[29] and another protein which enantiospecifically converts (+)-pinoresinol into (−)-secolarciresinol[30] has also been isolated. We have recently reported that regio- and diastereoselectivity are operative in the oxidative phenol coupling reaction with horseradish peroxidase (HRP) as catalyst and hydrogen peroxide as the oxidant.[19] This reaction takes advantage from mild conditions and fast reaction rates. It is possible to enhance the selectivity of this reaction by tuning the pH and using the appropriate organic cosolvent, but stereoselection is missing under these conditions. The same negative results have been obtained with cyclodextrine as external chiral auxiliary.[31] It is important to note the fact that the enzyme does not effect any stereocontrol.

Keywords: enantioselection; radicals; lignans; mechanism.
* Corresponding author. Tel.: +39-02-64474301; fax: +39-02-64474300; e-mail: maorl@disat.unimib.it

0040–4020/01/$ - see front matter
PII: S0040-4020(00)00944-3



Scheme 1.

Table 1. Literature radical phenol coupling methods for the synthesis of dihydrobenzo[*b*]furan

Authors	Reactants [a]	Yields
Cousin 1909[15]	$FeCl_3$	–[a]
Neish 1968[16]	Laccase/O_2	–[a]
Fukuzumi 1978[17]	HRP/H_2O_2water/acet	45%
Drago 1986[18]	Co(II)smpt/O_2	30%
Brunow 1993[19]	HRP/H_2O_2 water/methanol.	56%
Quideau1994[20]	Ag_2O /CH_2Cl_2	50%
Lemiere 1995[21]	Ag_2O acetone/benzene	40%
Backa 1996[22]	hν $^{60}Co/O_2$	40%
Ralph 1988[23]	HRP/H_2O_2acetate buffer 40°C	50%
Bolzacchini 2000[24]	HRP/H_2O_2 buffer Ph3/dioxane	70%

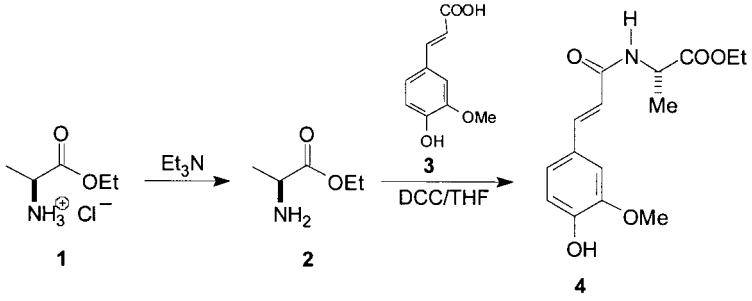
[a] Yields not reported.

Nevertheless, we recently observed stereocontrol in the enzymatic oxidative coupling of phenylpropenoidic phenols;[32,33] furthermore, Charlton et al. have reported that chiral sinapate esters can be coupled with $FeCl_3$ to aryltetralin lignans with moderate stereoselectivity.[34] We report here the synthesis of enantiopure phenylcoumarans by means of stereoselective phenol coupling oxidation of enantiopure ferulic acid derivatives catalyzed by HRP.

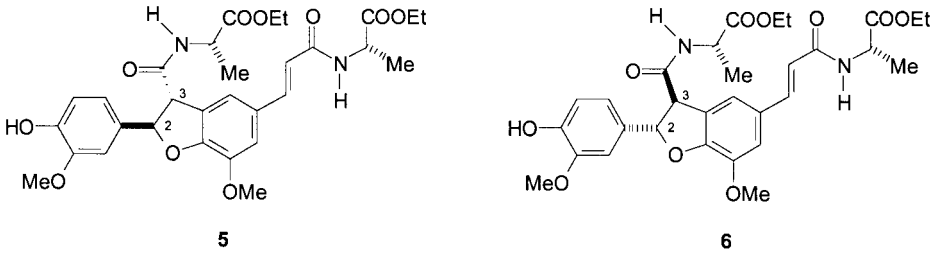
2. Results

The first synthetic approach adopted by us involved the oxidation of ferulic acid amidic derivative **4** containing the ethyl (*S*)-alaninate pendant as the chiral auxiliary. The starting amide **4** was prepared through condensation with an equimolecular amount of ferulic acid **3** and ethyl (*S*)-alaninate **2** and in the presence of dicyclohexylcarbodiimide (Scheme 2). The HPR-catalyzed oxidative phenol coupling was performed in a dioxane-aqueous buffer at pH 3, obtaining a mixture of the two diastereoisomers **5** and **6** with 70% yield (Scheme 3). Separation by silica gel flash chromatography followed by crystallization and final purification by preparative RP-HPLC, allowed to obtain enantiopure **5** and **6**. The diastereomeric excess (d.e.=65%) was evaluated by RP-HPLC analysis of the reaction crude.

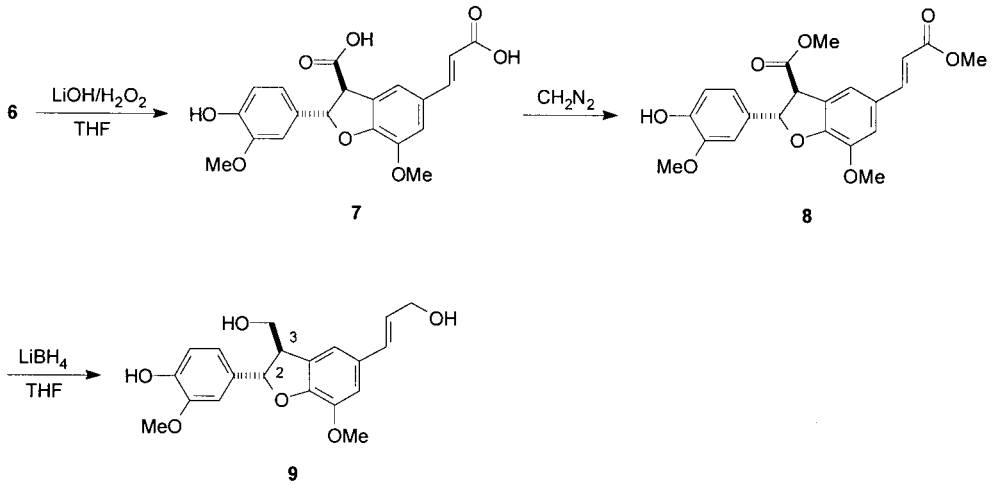
The absolute configuration of the newly formed stereocentres of the major diastereoisomer **6** was attributed by chemical methods. Hydrolysis of **6** with $LiOH/H_2O_2$ in THF[35] gave the diacid **7**; subsequent treatment with diazomethane gave the dimethylester **8**, which was then reduced with $LiBH_4$ to optically pure dehydrodiconiferyl alcohol (DDA) **9** (Scheme 4). Comparison of **9** (chiral HPLC) with authentical specimens of both enantiomers of



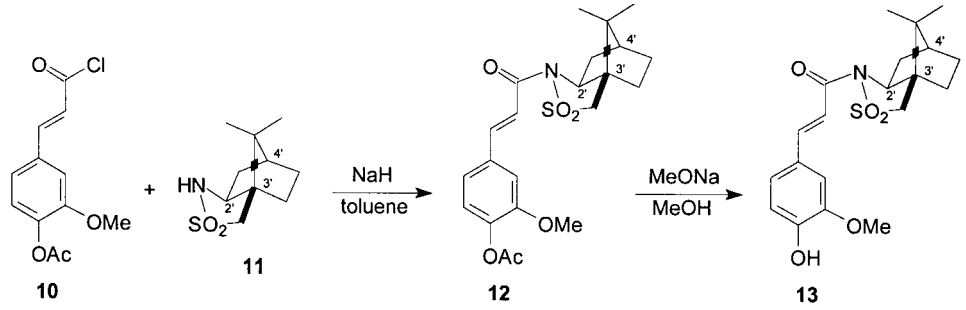
Scheme 2.



Scheme 3.



Scheme 4.

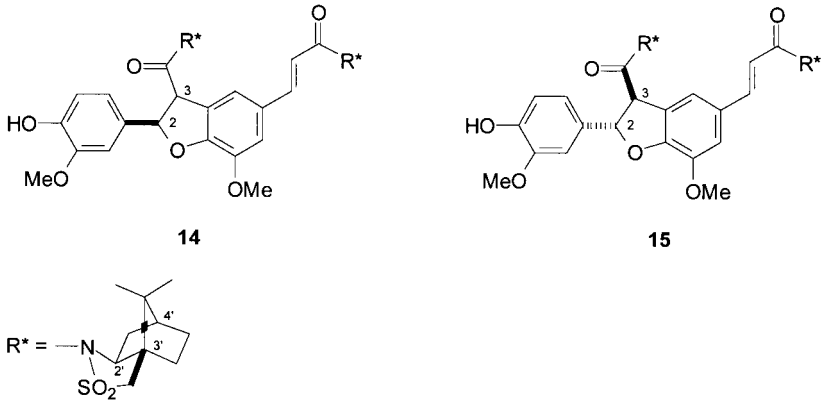


Scheme 5.

dehydrodiconiferyl alcohol[36] allowed to assign the absolute configuration (2*S*,3*R*).

The second approach was performed by using Oppolzer's sultam[37] **11** as the chiral auxiliary. The derivative **13** was prepared from *O*-acetylferulic acid chloride **10**[38] with Oppolzer's sultam followed by deacetylation of intermediate **12**[38,39] (Scheme 5). Compound **13** was coupled oxidatively in two different ways to obtain the diastereoisomers **14**,**15**: (i) enzymatically, by using HRP/H_2O_2 with 40% yield (ii) chemically, by using silver oxide[20,21] also with 40% yield (Scheme 6). After the dimerization, and separation of the pure diastereoisomers **14** and **15** by preparative RP-HPLC the absolute configuration of the newly formed stereocentres of the major diastereoisomer **15** was attributed by chemical method. The camphor sultam auxiliary of phenylcoumaran **15** was removed by reduction with $LiAlH_4$/THF[37] to DDA **9**. Comparison of **9** (chiral HPLC) with authentical specimens of both enantiomers of dehydrodiconiferyl alcohol[36] allowed to assign the absolute configuration (2*S*,3*R*).

The yields of the diastereoisomeric phenylcoumarans **14**, **15** and the d.e. are given in Table 2. Diastereomeric ratios were determined by ^{1}H NMR from the reaction mixtures.



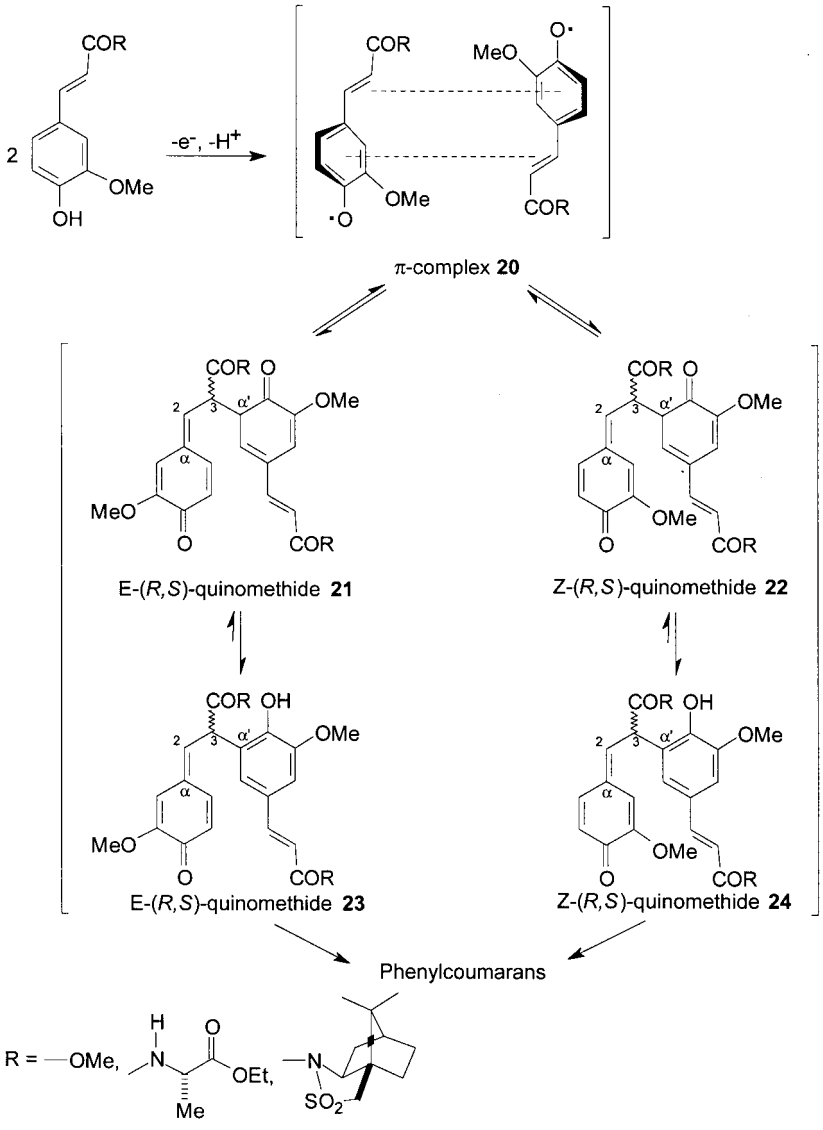
Scheme 6.

Table 2. Oxidations of chiral phenols

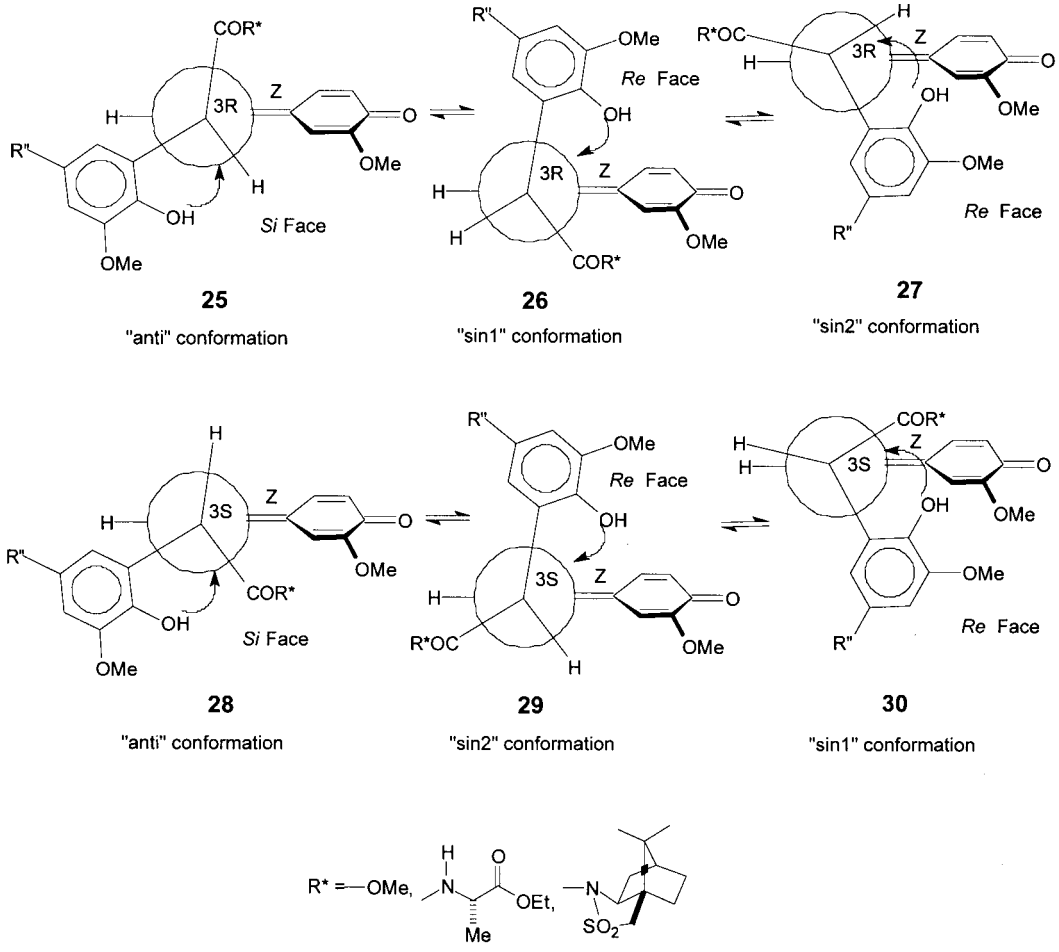
Substrate	Oxidant	Solvent	pH	T/°C	Yield in phenylcoumaran mixture %	d.e. %	Absolute configuration of the major diastereoisomer after reduction to DDA
4	HRP/H_2O_2	Dioxane/Buffer	3.5	25	70	65	*trans* 2*S*,3*R*-(+)
13	HRP/H_2O_2	Acetone/Buffer	3.5	0	40	81	*trans* 2*S*,3*R*-(+)
13	Ag_2O	CH_2Cl_2		−20	40	80	*trans* 2*S*,3*R*-(+)
13	Ag_2O	CH_2Cl_2		25	35	84	*trans* 2*S*,3*R*-(+)

Scheme 7 shows the postulated mechanism[40] for the oxidative dimerization of phenylpropenoic phenols, which is based upon the formation of a very persistent phenoxy radical.[14,36] An initial single electron transfer gives the intermediate π-complex **20**, which undergoes carbon–carbon bond formation in a reversible way[41,19,24] to give isomeric quinomethides **21**,**22** (R=OCH_3) possessing, respectively, the (*E*) and (*Z*) configuration to the hexocyclic quinonoid double bond and (*R*,*S*) configuration to the stereogenic C-3 center. Hence, in principle, two pairs of enantiomers, i.e. *E*-(*R*,*S*)-**23** and *Z*-(*R*,*S*)-**24** can be produced. Then, a suitable conformation of *E*-(*R*,*S*)-**23** and *Z*-(*R*,*S*)-**24** undergoes nucleophilic attack from the phenolic oxygen to the quinomethide double bond giving the phenylcoumaran products.

The analysis of conformations **25**–**30**, which arises from the rotation about the C2–C3 bond of *E*-(*R*,*S*)-**23** and *Z*-(*R*,*S*)-**24**, is of central importance to elucidate the stereochemistry of the nucleophilic attack from the phenolic oxygen to the quinomethide double bond (Scheme 8). Two diastereofaces



Scheme 7.



Scheme 8.

can be involved in the final cyclization to the dihydrofuran. In fact, the *anti* conformation **25** leads to the nucleophilic attack of the phenolic hydroxyl group to the quinomethide double bond from the *Si* face, whereas the two *syn* conformations **26**,**27** (*syn*1, **26** and *syn*2, **27**), give nucleophilic attack from the *Re* face.

In order to account for the finding that racemic *trans*-phenylcoumaran was obtained from the cyclization of methyl ferulate, we undertook the conformational analysis on rotating about the C2–C3 bond by PM3 calculations of the MM2 optimized geometries. The observed *trans*-stereoselectivity suggests that the formation of diastereoisomeric intermediates **23**–**24** occurs under thermodynamic control. This implies the equilibration to the most stable quinomethide, thus leading to diastereoselection in the final cyclization step. Our calculations revealed (Table 3) that all the conformations leading to a *trans*-phenylcoumaran were nearly 5 kcal/mol more stable than those leading to a *cis*-phenylcoumaran. This accounts very well for the observed ring closure to a *trans*-phenylcoumaran, if the assumption is made that the energy of activation for

Table 3. Conformational analysis on rotating the C2–C3 bond of quinomethides **21** and **22** (R=COOMe) using semiempirical calculations with PM3 on the geometries optimized with MM2

Compound (configuration)	C2–C3 conformation	PM3 Heat of formation (kcal/mol)	Angle Cα∇C2–C3–Cα′ (°)	Distance C–O (Å)	Predicted phenylcoumaran (DDF)
Z,3*S*	*anti*	−231	−141	3.84	*trans* 2*S*,3*S*
Z,3*S*	*syn*1	−226	−82	3.84	*cis* 2*R*,3*S*
Z,3*S*	*syn*2	−225	21	3.67	*cis* 2*R*,3*S*
Z,3*R*	*anti*	−229	−140	3.75	*trans* 2*R*,3*R*
Z,3*R*	*syn*1	−223	94	3.81	*cis* 2*S*,3*R*
Z,3*R*	*syn*2	−230	−65	4.00	*trans* 2*R*,3*R*
E,3*R*	*anti*	−231	−131	3.81	*trans* 2*R*,3*R*
E,3*R*	*syn*1	−225	97	3.88	*cis* 2*S*,3*R*
E,3*R*	*syn*2	−228	−61	4.03	*trans* 2*R*,3*R*
E,3*S*	*anti*	−229	131	3.45	*trans* 2*S*,3*S*
E,3*S*	*syn*2	−223	−57	4.32	*trans* 2*S*,3*S*

Table 4. Conformational analysis on rotating the C2–C3 bond of quinomethides **23** and **24** (R=*S*-phenylalaninate) using semiempirical calculations with PM3 on the geometries optimized with MM2. The conformation at C2–C3 bond is *anti*

Compound (configuration)	PM3 Heat of formation (kcal/mol)	Angle Cα–C2–C3–Cα′ (°)	Distance C–O (Å)	Predicted phenylcoumaran (DDF)	Predicted phenylcoumaran (DDA)
(*SS*),*E*,3*S*	−268	137	3.83	*trans* 2*S*,3*S*	*trans* 2*S*,3*R*
(*SS*),*Z*,3*S*	−270	130	3.81	*trans* 2*S*,3*S*	*trans* 2*S*,3*R*
(*SS*),*E*,3*R*	−267	−123	3.15	*trans* 2*R*,3*S*	*trans* 2*R*,3*S*
(*SS*),Z,3R	−268	−116	3.14	*trans* 2*R*,3*R*	*trans* 2*R*,3*S*

the cyclization reaction is similar for all the conformations of all the intermediate quinomethides. These findings suggested that a similar computational approach could explain the observed enantioselection when using starting phenols derivatized with a chiral auxiliary. In fact, the presence of the chiral auxiliary should generate an equilibrium mixture of diastereoisomeric quinomethides of different stability. It can be noted however that in conformations **25**–**30** which predict the stereochemical course of the cyclization reactions the topological indexes such as bond angles and C–O distances can hardly be related with the observed selectivity.

Again, the conformational analysis on rotating the C2–C3 bond was performed by using semiempirical PM3 calculations on the MM2 optimized geometries of the alaninate amide-derivatized quinomethides **23**,**24** (R=(*S*)-phenylalaninate). Three conformations of different stability was found: one *anti* and two *sin* conformations. Calculations performed on the *anti* conformations (Table 4) showed that, because of the presence of the chiral auxiliary, there is a difference of 2.0 kcal/mol among the conformations generating the (2*S*,3*S*)-*trans*-phenylcoumaran and those generating the (2*R*,3*R*)-*trans*-diastereoisomer. This accounts for the 65% d.e. observed in the oxidative cyclization.

In the case of camphor sultam-derivatized quinomethides **23**,**24** (R=camphor sultam) more than 5 kcal/mol energy difference was calculated between the quinomethide *E*-(3*S*)-*anti* conformation generating the (2*S*,3*S*)-*trans*-phenylcoumaran and the quinomethide *E*-(3*R*)-*anti* conformation (Table 5) generating the *trans*-(2*R*,3*R*)-phenylcoumaran. This fitted well with the observed diastereomeric excess of 81–84% in favour of the *trans*-(2*S*,2*R*)-DDA phenylcoumaran.

3. Conclusions

These results show that chiral auxiliaries provide significant levels of diastereoselection in bimolecular coupling reactions of phenoxyl radicals, and this results in enantioselection in the final product. It is expected that this methodology could be extended to various lignan structures thus providing a new approach to the synthesis of valuable lignans. Experiments to find the optimal chiral auxiliaries and reaction conditions are underway.

4. Experimental

Melting points were determined with a Büchi apparatus and are uncorrected. IR spectra were recorded with a FT-IR Jasco spectrophotometer. Mass spectra were determined by the direct injection system mode with positive electron impact a VG 7070 EQ instrument. ^{1}H NMR spectra were taken with a Bruker AC 300 or a Bruker AMX 300 instrument (in $CDCl_3$ solutions). Chemical shifts are given as ppm from tetramethylsilane and *J* values are given in Hz. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter at the sodium D line at 25°C. HPLC analysis were performed on a WATERS 600 E instrument by using an HP 1040 Diode Array Detector.

4.1. Oxidative phenol coupling of compound 4 catalyzed by Horseradish Peroxidase (HRP)

Ethyl *N*-ferulyl (*S*)-alaninate **4** (1.00 g, 2.7 mmol) was dissolved in dioxane (30 mL) and phosphate/citric acid buffer pH 3.5 (0.002 M, 70 mL) was added. 1 M Aqueous hydrogen peroxide (1.35 mL, 1.35 mmol) and HRP (1230 U) were added over 20 min. The mixture was stirred for 2.5 h at room temperature and then extracted with ethyl acetate (4×40 mL). The combined organic extracts were washed with 5% aqueous $NaHCO_3$ (20 mL), water (25 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was chromatographed on a silica gel flash column with hexane–ethyl acetate (gradient mode, from 4:1 to 1:1) yielding a mixture of the phenylcoumarans **5** and **6** (0.55 g, 70%) with 1:4 ratio. The minor diastereomer **5** and the major one **6** were then separated by fractional crystallisation from ethanol.

Table 5. Conformational analysis on rotating the C2–C3 bond of quinomethides **23** and **24** (R=camphorsultame) using semiempirical calculations with PM3 on the geometries optimized with MM2. The conformation at C2–C3 bond is *anti*

Compound (configuration)	PM3 Heat of formation (kcal/mol)	Angle Cα–C2–C3–Cα′ (°)	Distance C–O (Å)	Predicted phenylcoumaran (**14**–**15**)	Predicted phenylcoumaran (DDA)
(*SRS*), E,3S	−270	159	3.72	*trans* 2*S*,3*S*	*trans* 2*S*,3*R*
(*SRS*), Z,3S	−275	138	3.59	*trans* 2*S*,3*S*	*trans* 2*S*,3*R*
(*SRS*), E,3R	−269	−96	2.70	*trans* 2*R*,3*R*	*trans* 2*R*,3*S*
(*SRS*), Z,3R	−268	−90	2.66	*trans* 2*R*,3*R*	*trans* 2*R*,3*S*

Further purification by preparative HPLC (isocratic gradient $CH_3CN–H_2O$ 1:1) yielded analytically pure phenylcoumaran **6**: (0.44 g, 56% yield), mp 205°C; ^1H NMR: 7.58 (d, J=15.0 Hz, 1H), 6.35 (d, J=8.0 Hz, 1H), 6.31 (d, J=15.0 Hz, 1H), 6.12 (d, J=8.0 Hz, 1H), 5.95 (d, J=7 Hz, 1H), 5.60 (s, 1H), 4.71 (dq, J=7.0, 8.0 Hz, 1H), 4.60 (dq, J=7.0, 8.0 Hz, 1H), 4.30 (q, J=8.0 Hz, 2H), 4.30 (q, J=8.0 Hz, 2H), 3.95 (s, 3H), 3.90 (s, 3H) 1.45 (d, J=7.0 Hz, 3H), 1.40, (d, J=7.0 Hz, 3H), 1.25 (t, J=8.0 Hz, 3H),1.20 (t, J=8.0 Hz, 3H); MS (EI) m/z 584 (M^+) (100), 495 (20), 481 (20); IR (nujol): 3200, 1462 (cm^{-1}); Anal. Calcd for $C_{30}H_{36}O_{10}N_2$: C, 61.64; H, 6.16; N, 4.79. Found: C, 61.68; H, 6.19; N, 4.75. $[\alpha]_D^{25}$=+48.3 (AcOEt, c 0.1).

4.2. Hydrolysis of major phenylcoumaran 6 and esterification of the diacid 7

Phenylcoumaran **6** (0.10 g, 0.24 mmol) was dissolved in THF (20 mL), and aqueous 10 M hydrogen peroxide (1.0 mL, 10.0 mmol) was added. LiOH (70 mg, 2.4 mmol) in water (3 mL) was added over 10 min under stirring. After 18 h at room temperature the reaction mixture was ice-cooled, then saturated aqueous sodium bisulfite was added dropwise until a negative response was observed with starch-iodide paper. THF was removed by rotary evaporation, and aqueous 0.1 M HCl was added dropwise to pH 5. The aqueous mixture was extracted with ethyl acetate (3×30 mL), and the combined organic extract were washed with water (25 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure affording crude **7**, which was dissolved in 40 mL of saturated ethereal diazomethane. The solvent was removed and the residue was purified by preparative HPLC (isocratic $CH_3CN–H_2O$ 1:1) giving analytically pure (−)-*trans*-(2*S*,3*S*)-dehydrodiferulate **8** (10 mg, 10% yield), $[\alpha]_D^{25}$=−74.0 ($CHCl_3$, c 0.2).

4.3. Preparation of camphor sultam derivative 13

NaH (37 mg 1.54 mmol) was suspended in toluene (50 mL) and **11** (0.30 g, 1.4 mmol) was added under magnetic stirring. After 30 min, 4-acetylferulic acid chloride **10**[42] (0.39 g, 1.7 mmol) was added, and the mixture was stirred for 2 h at room temperature. The organic layer was washed with water (20 mL), dried over Na_2SO_4 and evaporated. The residue was dissolved in CH_3OH (8 mL), and 1 M CH_3ONa in CH_3OH (0.75 mL, 0.75 mmol) was added. The mixture was stirred for 30 min; water (5 mL) was added, the organic layer was dried over Na_2SO_4 and evaporated affording crude **13**. Silica gel flash chromatography of the residue (isocratic; hexane–AcOEt 1:1) gave pure **13** (0.52 g, 85% yield). ^1H NMR 7.73 (d, J=15.0 Hz, 1H), 6.88–7.19 (m, 4H), 5.89 (s, 1H), 3.99 (dd, J=5.5, 7.0 Hz), 3.92 (s, 3.0), 3.51 (d, J=4.0 Hz, 2H), 2.15–2.20 (m, 2H), 1.85–1.96 (m, 3H), 1.25–1.45 (m, 3.0), 1.21 (s, 3.0), 0.99 (s, 3.0). MS of **12** (EI) m/z 433 (M^+) (10), 391, (65), 177 (100). $[\alpha]_D^{25}$=+63.0 ($CHCl_3$, c 0.043); HREIMS of **12** calculated for $C_{22}H_{27}O_6NS$: 433.1559; found 433.1573.

4.4. Horseradish Peroxidase (HRP) promoted oxidative phenol coupling of compound 13

A solution of **13** (0.40 g, 1.0 mmol) in acetone (14 mL) and 0.02 M phosphate/citric acid buffer (4.0 mL, pH 3.5) was cooled to 0°C. 0.86 M Aqueous hydrogen peroxide (0.60 mL, 0.5 mmol) and aqueous HRP (0.93 mL, 837 U) were added to the reaction vessel in small portions over 15 min. The mixture was stirred at 0°C for 4 h, and saturated aqueous NaCl (20 mL) was added. Acetone was removed by rotary evaporation, and the resulting solution was extracted with AcOEt (4×20 mL). The combined organic extracts were washed with 5% aqueous $NaHCO_3$ (25 mL), water (25 mL), and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel flash cromatography with toluene–AcOEt (gradient mode, from 4:1 to 1:1) yielding a mixture of phenylcoumarans **14** and **15** (156 mg, 40%) with 1:9 ratio. The minor diastereomer **14** and the major one **15** were then separated by preparative HPLC (isocratic mode, $CH_3CN–H_2O$ 1:1) yielding pure **15** (140 mg, 36% yield). ^1H NMR: 7.73 (d, J=15.0 Hz,1H), 6.88–7.19 (m, 5H), 6.10 (d, J=8.0 Hz, 1H), 5.90 (s, 1H), 5.80 (d, J=8.0 Hz, 1H), 4.20 (d, J=8.0 Hz, 1H), 4.00 (dd, J=5.5, 7.0 Hz), 3.99 (dd, J=5.5, 7.0 Hz), 3.92 (s, 3.0), 3.85 (s, 3.0), 3.60 (d, J=4.0 Hz, 2H), 3.51 (d, J=4.0 Hz, 2H), 2.15–2.20 (m, 4H), 1.85–1.96 (m, 6H), 1.25–1.45 (m, 6H), 1.30 (s, 3H), 1.21 (s, 3H), 0.99 (s, 3H), 0.80 (s, 3H); MS (FAB^+) m/z 781 $(M+1)^+$ (10), 564 (20), 536 (20), 351 (100). Anal. Calcd for $C_{40}H_{48}O_{10}N_2S_2$: C, 61.54; H, 6.15; N, 3.59. Found: C, 61.60; H, 6.20; N, 3.50. The minor diastereoisomer **14** was not characterized.

4.5. Ag_2O Promoted oxidative phenol coupling of compound 13

A solution of **13** (0.20 g, 0.5 mmol) in dry CH_2Cl_2 (5.0 mL) was added with silver(I)oxide (0.18 g, 0.8 mmol) under argon atmosphere at room temperature. After stirring for 24 h, the mixture was filtered through a Celite pad and evaporated under reduced pressure. The residue was purified by silica gel flash chromatography with toluene–AcOEt (gradient mode, from 4:1 to 1:1) yielding a mixture of phenylcoumarans **14** and **15** (62 mg, 35%) with 1:12 ratio. The minor diastereomer **14** and the major one **15** were separated by preparative HPLC (isocratic mode, $CH_3CN–H_2O$ 1:1) yielding pure phenylcoumaran **15** (57 mg, 32% yield). The minor diastereoisomer **14** was not characterized.

4.6. Reduction of major phenylcoumaran 8

trans-(2*S*,3*S*)-Dehydrodiferulate **8** (10 mg, 0.024 mmol) was dissolved in dry THF (5 mL) under argon at −78°C. $LiBH_4$ (1 mg, 0.054 mmol) was suspended in dry THF (1.0 mL) and added to the reaction mixture, which was further stirred for 2 h at −78°C. 80% Aqueous THF (10 mL) was added slowly, and aqueous 0.1 M ammonium chloride (5 ml) was then added. The mixture was extracted with AcOEt (2×10 mL), and the combined organic extracts were washed with water (10 mL) and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was analysed by HPLC with a chiral column (Chiralcell OF; isocratic mode, hexane–isopropanol 1:1) in comparison with authentical specimens of both enantiomers of dehydrodiconiferyl alcohol.[36] The major diastereoisomer is *trans*-(2*S*,3*R*)-(+)-dehydrodiconiferyl alcohol.[43,44]

4.7. Reduction of phenylcoumaran 15

$LiAlH_4$ (1 mg, 0.026 mmol) was suspended in THF (10 mL) and cooled to −20°C. Compound **15** (20 mg, 0.025 mmol) in THF (5.0 mL) was added slowly, and the mixture was stirred for 2 h at −20°C. 80% Aqueous THF (1.0 mL) was added, followed by treatment with 0.1 M aqueous ammonium chloride (5.0 mL). The mixture was extracted with AcOEt (2×10 mL), and the combined organic extracts were washed with water (20 mL), dried over Na_2SO_4 and evaporated. The residue was analysed by HPLC with chiral column (Chiralcell OF, isocratic; hexane–2-propanol 1:1) in comparison with authentical specimens of both enantiomers of dehydrodiconiferyl alcohol[36] The major diastereoisomer is *trans*-(2*S*,3*R*)-(+)-dehydrodiconiferyl alcohol.[43,44]

Acknowledgements

Financial support for this work was provided by the Technology Development Centre of Finland (TEKES) and The Academy of Finland. We thank also the National Research Councils and the Ministry for Scientific Research of Italy for financial support. We thank Professor D. G. Lynn and Mrs J. Zhang of University of Chicago for the helpful discussion.

References

1. Higuchi, T. In *Biosynthesis and Biodegradation of Wood Components*; Higuchi, T., Ed.; Academic: New York, 1985; pp 141–148.
2. Bernards, M. A.; Lopez, M. L.; Zajicek, J.; Lewis, N. G. *J. Biol. Chem.* **1995**, *270*, 7382.
3. Ragan, M. A. *Phytochemistry* **1984**, *23*, 2029.
4. Ayres, D. C.; Loike, J. D. *Lignans. Chemical, biological and clinical properties*; Cambridge University Press: Cambridge, 1990, pp 278–373.
5. Freudenberg, K. *Nature* **1959**, *183*, 1152.
6. Nose, M.; Bernards, M. A.; Furlan, M.; Zajicek, J.; Eberhardt, T. L.; Lewis, N. G. *Phytochemistry* **1995**, *39*, 71.
7. Binns, A. N.; Chen, R. H.; Wood, H. N.; Lynn, D. G. *Proc. Nat. Acad. Sci. USA* **1987**, *84*, 980.
8. Satake, T.; Murakani, T.; Saiki, Y.; Chen, C. F. *Chem. Pharm. Bull.* **1978**, *26*, 1619.
9. Asakawa, Y. *Heterocycles* **1997**, *47*, 795.
10. Gang, D. R.; Kasahara, H.; Xia, Z.; Mijnsbrugge, K. V.; Bauw, G.; Boerjan, W.; Van Montagu, M.; Davin, L. B.; Lewis, N. G. *J. Biol. Chem.* **1999**, *274*, 7516.
11. Antus, S.; Baitz-Gacs, E.; Bauer, A.; Gottsegen, A.; Seligmann, O.; Wagner, H. *Liebigs Ann. Chem.* **1990**, 495.
12. Pieters, L.; De Bruyne, T.; Claeys, M.; Vlietnick, A.; Calomme, M.; Van den Berghe, D. *J. Nat. Prod.* **1993**, *56*, 899.
13. Teutonico, R. A.; Dudley, M. W.; Orr, J. D.; Lynn, D. G.; Binns, A. N. *Plant Physiol.* **1991**, *97*, 288.
14. Ward, R. S. *Nat. Prod. Rep.* **1997**, *14*, 43.
15. Fengel, D.; Wenger, G. *Wood chemistry. Ultrastructure reactions*; Walter de Gruyster: Berlin, 1994.
16. Neish, A. C. *Monomeric intermediates in the biosynthesis of lignin and related compounds: the formation of wood in forest trees*; Academic: New York, 1995; pp 219–239.
17. Katayama, Y.; Fukuzumi *Mokuzay Gakkaishi* **1978**, *24*, 664.
18. Drago, R. S.; Corden, B. B.; Barnes, C. W. *J. Am. Chem. Soc.* **1986**, *108*, 2453.
19. Chioccara, F.; Poli, S.; Rindone, B.; Pilati, T.; Brunow, G.; Pietikainen, P.; Setala, H. *Acta Chem. Scand.* **1993**, *47*, 610.
20. Quideau, S.; Ralph, J. *Holzforshung* **1994**, *48*, 12.
21. Lemiere, G.; Gao, M.; De Groot, A.; Domisse, R.; Lepoivre, J.; Pieters, B. *J. Chem. Soc., Perkin Trans. 1* **1995**, 1775.
22. Backa, S. *Coupling of Coniferyl Alcohol in the presence of Ca^{2+} at different pH*; Fourth European Lignocellulosic and Pulp Stresa, 1996, 323.
23. Ralph, J.; Conesa, M. T.; Wiliamson, G. *J. Agr. Food Chem.* **1988**, *46*, 2531.
24. Bolzacchini, E.; Meinardi, S.; Orlandi, M.; Rindone, B.; Brunow, G.; Pietikainen, P.; Rummakko, P. *Green Chemistry*; Tundo, P., Anastas, P., Eds.; Oxford University Press: London, 2000, pp 23–35.
25. Iqbal, I.; Bhatia, B.; Nayyar, N. K. *Chem. Rev.* **1994**, *94*, 519.
26. Sheldon, R. A.; Kochi, J. K. *Metal-Catalyzed Oxidation of Organic Compounds*; Academic: New York, 1981.
27. Aulin-Erdtman, G.; Tomita, Y. *Acta Chem. Scand.* **1963**, *17*, 535.
28. Nakatsubo, F.; Higuchi, T. *Holzforschung* **1975**, *29*, 95.
29. Davin, L. B.; Wang, H.; Crowell, A. L.; Bedger, D. L.; Martin, D. M.; Sarkanen, S.; Lewis, N. G. *Science* **1997**, *275*, 362.
30. Fujita, M.; Gang, D. R.; Davin, L. B.; Lewis, N. G. *J. Biol. Chem.* **1999**, *274*, 618.
31. Ichihara, A., Kawagishi, H., Sakamura, S. *24th Symposium on the Chemistry of Natural Products*; Osaka, 1981; p 490
32. Bolzacchini, E.; Brunow, G.; Meinardi, S.; Orlandi, M.; Rindone, B.; Rummakko, P.; Setälä, H. *Tetrahedron Lett.* **1998**, *39*, 3291.
33. Rummakko, P.; Brunow, G.; Orlandi, M.; Rindone, B. *Synlett* **1999**, 333.
34. Bogucki, D. E.; Charlton, J. L. *Can. J. Chem.* **1997**, *75*, 1783.
35. Hung, R. R.; Straub, J. A.; Whitesides, M. *J. Org. Chem.* **1991**, *56*, 3849.
36. Hiray, N.; Okamoto, M.; Udagawa, H.; Yamamuro, M.; Kato, M.; Koshimizu, K. *Biosci. Biotech. Biochem.* **1994**, *58*, 1678.
37. Oppolzer, W.; Chapuis, C.; Bernardinelli, G. *Helv. Chim. Acta* **1984**, *67*, 1397.
38. Boiadjiev, S. E.; Lightner, D. A. *Tetrahedron: Asymmetry* **1996**, *7*, 2825.
39. Doerge, D. R.; Taurog, A.; Dorris, M. L. *Arch. Biochem. Biophys.* **1994**, *315*, 90.
40. Chance, M.; Powers, L.; Poulos, T.; Chance, M. *Biochemistry* **1986**, *25*, 1266.
41. Amstrong, D. A.; Cameron, C.; Nonhebel, D. C.; Perkins, P. G. *J. Chem. Soc., Perkin Trans. 2* **1983**, 563.
42. Ogawa, H. *Chem. Zentralbl.* **1927**, *98*, 2068.
43. Vermes, B.; Seligmann, O.; Wagner, H. *Phytochemistry* **1991**, *30*, 3087.
44. Kikuchi, T.; Matsuda, S.; Kadota, S.; Tai, T. *Chem. Pharm. Bull.* **1985**, *33*, 1444.