# HORSERADISH PEROXIDASE-MEDIATED PREPARATION OF DIMERS FROM EUGENOL AND ISOEUGENOL*

Andrzej R. KRAWCZYK, Ewa LIPKOWSKA, Jerzy T. WRÓBEL

*Department of Chemistry, Warsaw University, 1 L. Pasteur' St. 02093 Warsaw, Poland*

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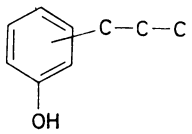
*Dedicated to the memory of Professor František Šorm.*

Coupling of eugenol (*I*) and isoeugenol (*II*) by means of horseradish peroxidase and hydrogen peroxide resulted in ~20% yield of dimeric compounds: 5,5'-diallyl-2,2'-dihydroxy-3,3'-dimethoxy-biphenyl (*III*) and 2-(4'-hydroxy-3'-methoxy)phenyl-7-methoxy-3-methyl-5-propenyl--2,3-dihydrobenzofurane (*IV*), respectively.

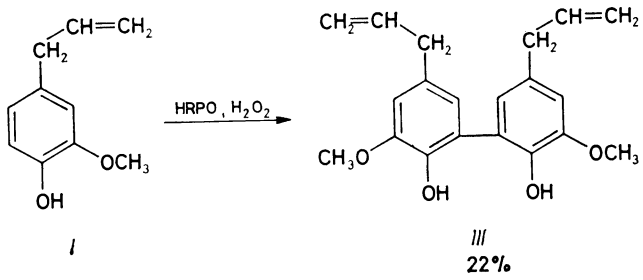
An enzymatic coupling of phenol compounds has been recognized as a possible route to the synthesis of certain classes of alkaloids[1]. It is believed that macrocyclic lactone or ester part in *Lythraceae* alkaloids[2] can arise from oxidative coupling of phenol precursors. It should be mentioned that chemical methods of such couplings in many cases gave very poor yields or failed completely[1-6]. On the other hand, phenolpropanoid derivatives' oxidative dimerization/polymerization has been studied previously with the aim of understanding of the biogenesis of lignins[7,8]. For the above two reasons appropriate substrates for enzymatic oxidative coupling[9-11] belong to a class of compounds of a general formula of phenol with differently functionalized carbon side chain.




We have examined a couple of phenolesters in the reaction catalyzed by HRPO (horseradish peroxidase, EC 1.11.1.7) directing to enzymatic synthesis of biphenyl alkaloids of *Lythraceae* family (Part I). In this communication we would like to present two more substrates for the oxidative coupling with HRPO/$H_2O_2$ system, namely 4-allyl-2-methoxy-phenol (eugenol, *I*) and 2-methoxy-4-propenyl-phenol (isoeugenol, *II*).

* Part II in the series Enzymatic Coupling of Phenol Compounds; Part I: Bull. Pol. Acad. Sci. *34*, 115 (1986).

In order to establish the best conditions for preparative-scale reactions a few series of analytical experiments were made with eugenol as a substrate. Solutions of the latter in pH 6 buffer containing some amounts of methanol were treated with different amounts of enzyme and hydrogen peroxide in reactions carried for different time. E/S ratio varied from $1/10^6$ to $1/10^3$ while $H_2O_2/S$ ratio varied in the range $3 . 10^{-2}$ to 1 in these analytical experiments. On the basis of these results the preparative-scale oxidation of eugenol was performed using 1 g (6 mmol) of the substrate in the mixture of pH 6 buffer (400 ml) and methanol (140 ml) solution with 0·3 mg ($7{\cdot}5 . 10^{-6}$ mmol) of HRPO and $H_2O_2$ (0·5 mmol) for 25 h at room temperature. A crude precipitate (300 mg) was filtered off the post-reaction mixture, dried and crystallized from the mixture of benzene and hexane to give 220 mg of the product (*III*). In summary, the reaction which is taking place gives rise to the product of *ortho–ortho* coupling (Scheme 1) – the most common product of oxidative coupling of phenol substrates[3,9].




*I* *III* 22%

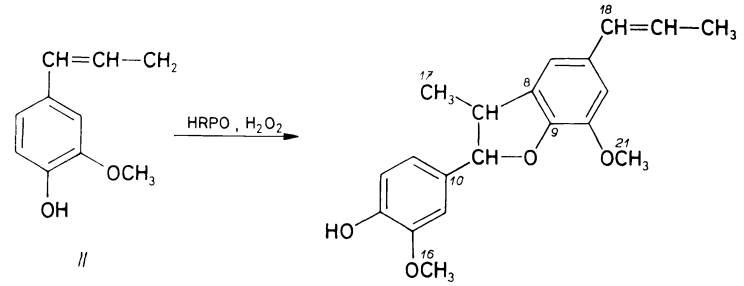
SCHEME 1

Oxidation of isoeugenol (1 g, 6 mmol) was done in the same conditions as above to give the product (*IV*) after chromatographic purification. Its structure was based on full spectroscopic and elemental analyses and it was evaluated as a dimeric compound resulting from the coupling of phenol group and *ortho*-carbon of one molecule with propenyl side chain of the second molecule of the substrate[7], as it is seen from Scheme 2.

A discussion of the product structure in this case has to take into account the following: $^1$H NMR spectrum showed: (i) two different alkyl-type methyl groups; (ii) two closely similar methoxy groups and, moreover, one proton α to oxygen; (iii) one proton doublet at ~5 ppm; (iv) $D_2O$ exchangeable proton; (v) CH=CH system; (vi) aromatic protons. The similarly significant differences were observed in $^{13}$C NMR spectra. The mass ion determination gave value $m/z$ 326 while IR absorption at 3 450 cm$^{-1}$ indicates OH group.

Up to now we have dealt with compounds giving opportunities of inter-molecular coupling. In order to make an enzymatic synthesis of alkaloids with biphenyl linkage

it is crucial to couple appropriate substrates intra-molecularly. In the light of relatively satisfying yields of reactions presented here the above seems promising in the area of eugenol-type substrates.




*II* *IV*
19%

SCHEME 2

## EXPERIMENTAL

Melting points were determined on "Boetius" apparatus and were not corrected. $^1$H NMR spectra ($\delta$, $J$ in Hz) were recorded on a JOEL JNM-4H-100 and Tesla 100 MHz, and $^{13}$C NMR spectra on a JOEL FX 90 Q spectrometers. IR spectra were obtained using UR 20 spectrometer. Mass spectra were determined on LKB 9000 spectrometer. Type VI of 250 U/mg activity horseradish peroxidase, EC 1.11.1.7, HRPO, "Sigma" was used in the reactions.

### Purification of Substrates

Eugenol (*I*) was isolated from cloves by a steam distillation and purified through its benzoate derivative. $^1$H NMR ($CDCl_3$): 3·29 (d, 2 H, $J$ = 6·5); 3·84 (s, 3 H); 4·9—5·2 (dd, 2 H); 5·51 (s, 1 H, $D_2O$ exchangeable); 5·7—6·2 (m, 1 H); 6·6—6·9 (m, 3 H). $^{13}$C NMR ($CDCl_3$): 39·9 (C-1′), 55·8 ($C_{OMe}$), 111·2 (C-3), 114·3 (C-6), 115·4 (C-3′), 121·2 (C-5), 131·9 (C-4), 137·7 (C-2′), 144·0 (C-1), 146·5 (C-2).

Isoeugenol (*II*, Merck) was purified on silicagel (Merck, 230—400 mesh) with benzene as eluent, prior to the reaction. $^1$H NMR ($CDCl_3$): 1·85 (d, 3 H, $J$ = 5); 3·88 (s, 3 H); 5·57 (s, 1 H, $D_2O$ exchangeable); 6·1—6·4 (m, 2 H); 6·8 (aromatic, 3 H). $^{13}$C NMR ($CDCl_3$): 18·2 (C-3′), 55·9 ($C_{OMe}$), 102·2 (C-3), 114·5 (C-6), 119·4 (C-5), 123·3 (C-2′), 130·8 (C-4), 130·9 (C-1′), 144·9 (C-1), 146·7 (C-2).

### Enzymatic Oxidation

*General procedure*: A substrate (1 g, 6 mmol) in methanol (40 ml) was added to the mixture of pH 6 phosphoric buffer (400 ml) and methanol (100 ml). A reaction was started with HRPO (0·2—0·3 mg, 4·5—7·5 . $10^{-6}$ mmol) and 0·03% $H_2O_2$ (60 ml, 0·5 mmol) and the mixture was stirred for 24—25 h at room temperature. A precipitate was filtered off and crystallized or purified chromatographically.

5,5′-Diallyl-2,2′-dihydroxy-3,3′-dimethoxy-biphenyl (*III*)

From 1 g of *I* 220 mg (22%) was obtained; m.p. 103—104°C (benzene–hexane). IR (KBr): 3 270 to 3 360 $cm^{-1}$. $^1H$ NMR ($CDCl_3$): 3·38 (d, 4 H, $J = 6·5$); 3·92 (s, 6 H); 5·0—5·20 (dd, 4 H); 5·8—6·1 (m and s, s $D_2O$ exchangeable); 6·7—6·8 (4 H). $^{13}C$ NMR ($CDCl_3$): 39·9 ($CH_2$); 56·1 ($OCH_3$); 110·8 (C-4, 4′); 115·7 (=$CH_2$); 123·2 (C-6, 6′); 124·5 (C-1, 1′); 131·9 (C-5, 5′); 137·7 (CH=); 141·1 (C-2, 2′); 147·7 (C-3, 3′). MS (70 eV, *m/z*, %): 326 ($M^+$, 100), 327 (20), 328 (1·9), 297 (18·7), 253 (19·8), 244 (10·9). For $C_{20}H_{22}O_4$ (326·4) calculated: 73·62% C, 6·75% H; found: 73·12% C, 7·11% H.

2-(4′-Hydroxy-3′-methoxy)phenyl-7-methoxy-3-methyl-5-propenyl-[2,3]-dihydrobenzofurane (*IV*)

From 1 g of *II* 185 mg (19%) was obtained after chromatography on silicagel (Merck, 230—400 mesh) with benzene–ethyl acetate (95 : 5). M.p. 133—134°C (ref.[7]). IR (KBr): 3 450 $cm^{-1}$, $^1H$ NMR ($CDCl_3$): 1·37 (d, 3 H, $J = 6·5$); 1·86 (d, 3 H, $J = 5·5$); 3·45 (m, 1 H); 3·85 (s, 3 H); 3·88 (s, 3 H); 5·09 (d, 1 H, $J = 9·4$); 5·66 (s, 1 H, $D_2O$ exchangeable); 6·1—6·5 (m, 2 H); 6·8—7·0 (m, 5 H). $^{13}C$ NMR ($C_6D_6$): 17·7 and 18·5 (C-17, 20); 46·2 (C-3); 55·3 and 56·1 (C-16, 21); 93·6 (C-2); 109·0 (C-6); 111·3 (C-11); 114·1 (C-14); 114·6 (C-4); 120·0 (C-15); 122·9 (C-19); 131·9 (C-18); 132·6, 132·9 and 134·0 (C-5, 8, 10); 145·0 (C-13); 146·5, 147·1 and 147·9 (C-7, 9, 12). MS (70 eV, *m/z*, %): 326 ($M^+$, 100), 327 (28·2), 328 (4·0), 311 (9·7), 202 (9·4), 151 (11·3), 149 (12·0), 137 (15·4), For $C_{20}H_{22}O_4$ (326·4) calculated: 73·62% C, 6·75% H; found: 73·18% C, 6·63% H.

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