Nonlinearity in Biology, Toxicology, and Medicine, 2: 129–141, 2004

ISSN: 1540-1421
DOI: 10.1080/15401420490464466

Taylor & Francis
healthsciences

EFFECT OF LOW AND VERY LOW DOSES OF SIMPLE PHENOLICS ON PLANT PEROXIDASE ACTIVITY

Elżbieta Malarczyk, Janina Kochmańska-Rdest, and Marzanna Paździoch-Czochra
□ Department of Biochemistry, M. Curie-Skłodowska University, Lublin, Poland

□ *Changes in the activity of horseradish peroxidase resulting from an addition of ethanol water dilutions of 19 phenolic compounds were observed. For each compound, the enzyme activity was plotted against the degree of dilution expressed as* $n = -\log_{100}$ *(mol/L) in the range* $0 \leq n \geq 20$. *All the curves showed sinusoidal activity, more or less regular, with two to four peaks on average. Each analyzed compound had a characteristic sinusoidal shape, which was constant for samples of peroxidase from various commercial firms. This was clearly visible after function fitting to experimental results based on the Marquadt–Levenberg algorithm using the least-squares method. Among the 19 phenolics, the highest amplitudes were observed for phenol and iso- and vanillate acids and aldehydes. The specific character of each of the analyzed curves offers a possibility of choosing proper dilutions of phenolic compound for activating or inhibiting of peroxidase activity.*

Keywords. peroxidase, activity, phenolics, low doses, oscillations

INTRODUCTION

Horseradish peroxidase (HRP) is a very common enzyme in nature (Urrutigoity *et al.*, 1991; Silaghi-Dumitrescu, 1999). This important hemoprotein oxidoreductase catalyzes the process of aromatic substrate oxidation in the presence of H_2O_2. It catalyzes the cleavage of hydrogen peroxide and the oxidation of the catalytic center to a cationic state known as compound I. This compound accepts one electron from the organic substrate and in this process the enzyme gets converted to compound II, which, in turn, can accept a second electron from a second organic substrate molecule to produce compound III. This is created in the form of oxyperoxidase. It is completed with an excess of H_2O_2 as the resonance hybrid balancing between Fe(II)-$)_2$ and Fe(III)-O_2^- forms (Silaghi-Dumitrescu, 1999). Compound III gradually degrades to compound I because of its lower reactivity. The presence of these three compounds can be observed by spectral analysis. Two histidine residues located in positions 42 and 170, as well as arginine-38 and asparagin-43, are very active in the conformational stabilization of the heme-containing center

The authors thank Dominik Szałkowski for his excellent mathematical assistance. This work was supported by the Polish Committee for Scientific Investigations BS/BiNoZ/2003.

Address correspondence to Elzbieta Malarczyk, Department of Biochemistry, M. Curie-Sklodowska University, pl. M. Curie-Sklodowskiej 3, 20-031 Lublin, Poland. Tel: +48-081-5375770. Fax: +48-081-5375200. E-mail: malar@hermes.umcs.lublin.pl

of the peroxidase. All the aforementioned reactions are connected with the formation of a substrate radical existing together with the reducing form of the organic substrate complexes with His-170, present in the active center (Urrutigoity *et al.*, 1991). According to Silagi-Dumitrescu (1999), "the high reactivity and low selectivity... make the chemistry of peroxidase products really complicated." She analyzed 171 substrates, among them those of phenolic origin like phenol, benzoic acid, and protocatechuic acid, catechol and pirogallol, and guaiacol (Saunders, 1957), which were used just during the last 50 years of the preceding millenium.

In our previous paper (Malarczyk *et al.*, 2003), the dose-dependent activation and inhibition of the peroxidase in fungal cultures were described when the medium contained low and very low doses of ethanol or guaiacol. The pure enzyme changed its activity in the presence of very low doses of guaiacol and enzymatic activity oscillated between three maxima and three minima when the concentration of guaiacol ranged from 100^{-1} to 100^{-20} mol/L. The present paper analyzes the effect of low doses of 19 phenolic compounds on the activity of the pure enzyme. The observations show how the same enzyme behaved in the presence of low doses of simple phenolic substances, which vary in the type of functional groups at the aromatic ring. These were $-OH$, $-OCH_3$, $-COOH$, and $-CHO$ in various configurations common in natural phenols, methoxyphenols, phenolic acids, and aldehydes.

MATERIALS AND METHODS

Enzyme Assay

HRPs were purchased from Sigma (peroxidase I), Boehringer Mannheim GmbH (peroxidase II), and from Merck (peroxidase III). The activities of peroxidases were determined with 4 μM of *o*-dianisidine in methanol and 10 used b mmoles of H_2O_2 in 0.1 M acetate Na buffer, pH 5.5, as the substrate according to the method used by Clairborne and Fridovich (1979). The sample volumes were 0.2 mL. The activity was expressed in nkatals/L as described previously (Malarczyk *et al.*, 2003).

Preparation of Phenolic Substance Dilutions

Nineteen phenolics, well known and very popular in nature (Figure 1), were purchased mainly from Fluka Company. The 1 M solutions that were prepared were used for making serial dilutions (1 part phenolic solution to 99 parts 75% ethanol) according to Malarczyk *et al.* (2003). The serial dilutions ranged from 100^{0} (1 mol/L) to 100^{-20}. They were prepared according to the

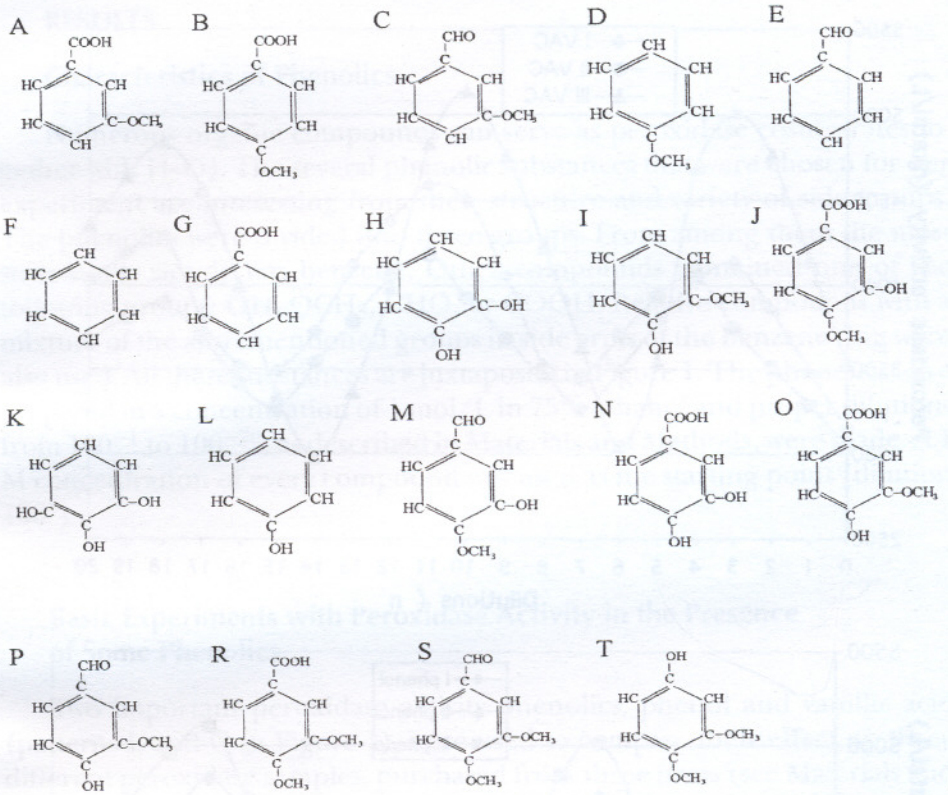


FIGURE 1 Patterns of phenolics used in dilutions for a 1-hr incubation of the peroxidase before the determination of its activity. A-m-anisic acis, B-p-anisic acid, C-m-anisic aldehyde, D-anisol, E-benzaldehyde, F-benzen, G-benzoic acid, H-catechol, I-guaiacol, J-isovanillic acid, K-isovanllic aldehyde, L-phenol, M-pyrogallol, N-protocatechuic acid, O-vanillic acid, P-vanillic aldehyde, R-veratric acid, S-veratric aldehyde, T-veratrol.

classical homeopathic method with 10-fold succussion between dilutions and they corresponded to the notation $C_{11/2}C_{20}$ (or 1CH–20CH) (see also Linde *et al.*, 1994; Dittmann and Harisch, 1996; Elia and Niccoli, 1999). One hour before measurement, 20 μl of serial dilutions were added to each reaction vessel containing 200 μl of peroxidase in 700 μl of 0.1 M acetate Na buffer, pH 5.5, and enzyme activities were measured colorimetrically at 460 nm on a Shimadzu 160 spectrophotometer.

Statistical Analysis

All experiments were replicated three times and (±SD) value was calculated but omitted on Figures 2 and 4–6 because the range between repetitions was not more than 30 nkatals/L and they are not visible on figures where the scale is in thousands of nkatals/L. For time-dependent curves, five repetitions were used. To fit the experimental curves to their sinusoidal

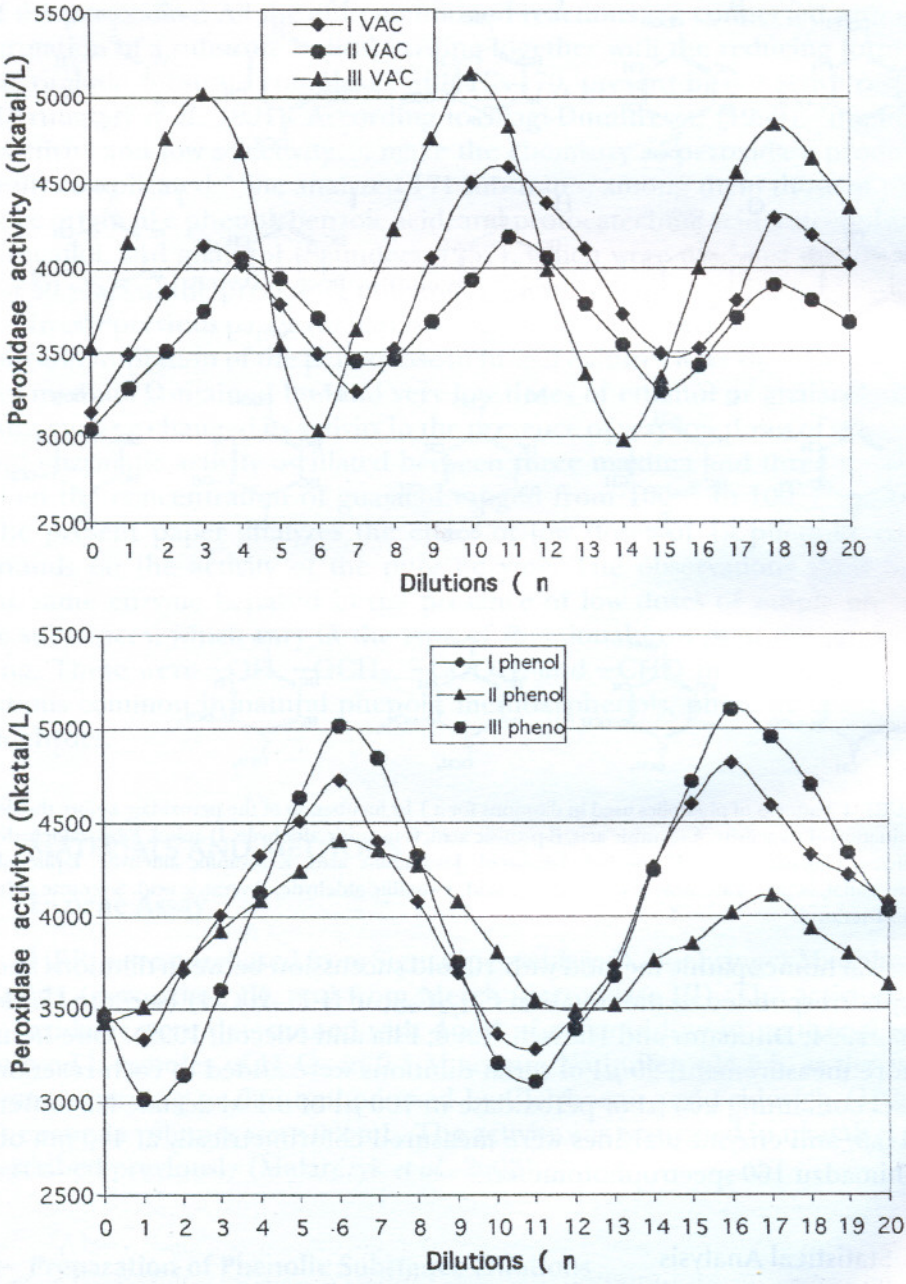


FIGURE 2 Effects of dilutions of VAC and phenol on peroxidase activity for three various samples of enzyme, I, II, and III (for details see Materials and Methods); $n = -\log_{100}$ (mol/L).

shapes, all the curves were recalculated using a four-parameter equation: $y = y_0 + A\cos(\beta n - \gamma)$ with Marquadt–Levenberg algorithm by the method of least squares (Gill and Murray, 1981). These recalculated curves are presented in the figures as dotted lines.

RESULTS

Characteristics of Phenolics

Numerous organic compounds can serve as peroxidase cosubstrates together with H_2O_2. The several phenolic substances that were chosen for our experiment are interesting from their structure and variety of side groups. The phenolics were divided into three groups. From among them the most structurally simple was benzene. Other compounds contained one of the following groups: OH, OCH_3, CHO, or COOH. Relative compounds with a mixture of the aforementioned groups in side arms of the benzene ring were also used. All these substances are juxtaposed in Figure 1. The phenolics were prepared in a concentration of 1 mol/L in 75% ethanol and proper dilutions from 100^{-1} to 100^{-20}, as described in Materials and Methods, were made. A 1 M concentration of every compound was used as the starting point (dilution 100°).

Basic Experiments with Peroxidase Activity in the Presence of Some Phenolics

Two important peroxidase activity phenolics, phenol and vanillic acid (patterns L and O in Figure 1), were used to compare their effect on three different peroxidase samples, purchased from three firms (see Materials and Methods). Their activities were comparable at the beginning of the experiments. For comparison of the effect of diluted phenolics on these three kinds of enzymes, three distinct serial dilutions of phenol and vanillic acid were prepared. Figure 2 shows the resulting curves, about three for vanillic acid (VAC) and three for phenol. All curves from the part with vanillic acid had nearly the same shape as the curves from the part with phenol, but they differ between parts, giving the special shape for the individual compound.

The other controversial point concerned the preparation of the mentioned curves in time. For this reason the same experiments were repeated when the successive dilutions were used in order from 100° to 100^{-20} or when they were administrated in a random manner. In Figure 3 these two types of curves for vanillic acid and for phenol are visible and the data in white point curves were measured in a chaotic manner. In this way, the five data points from five various random measurements were placed for one dilution. Also in these types of experiments the shape of curves for individual compounds was preserved.

All these experiments illustrated in Figures 2 and 3 were important to the conclusion that, for comparative analysis with various phenolics, the same conditions must be retained. The peroxidase (III) sample from Merck was chosen for all of these measurements.

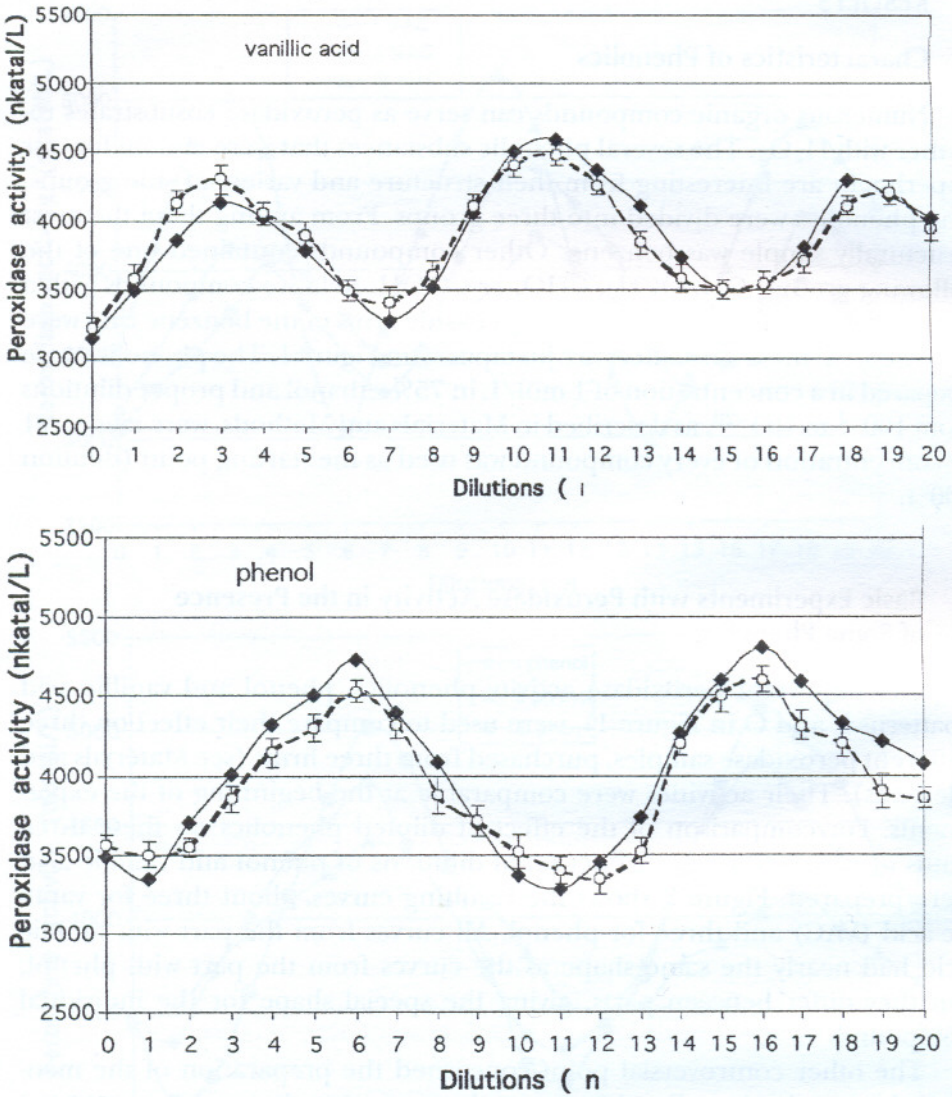
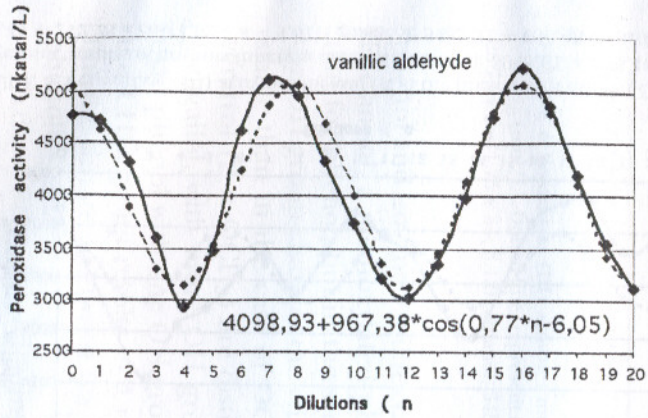
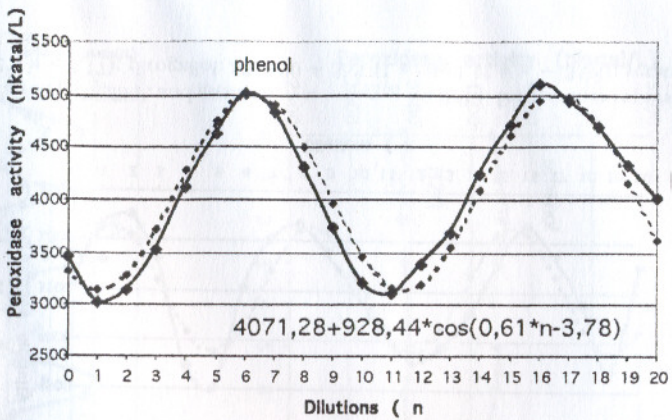


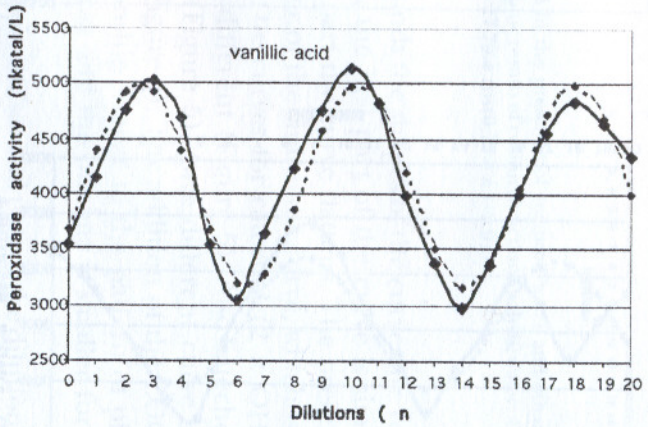
FIGURE 3 The comparison between the same results of the effect of phenol and VAC on peroxidase (I) activity in agreement with order from 0 to 20 (solid line) or with random times (see also text); $n = -\log_{100}$ (mol/L).

Relations Between Peroxidase Activity and Presence of Phenolics

One can compare the effect of the chosen compounds by looking at Figures 4–6, where experimental relationships for all the tested dilutions are given. All the studied substances exert a dynamic influence on the changes in the enzymatic activity, and maxima and minima are observed from two to four times. In Figures 4 and 5, the shape of the curves reflects the effect of the successive dilutions of phenolic compounds on the peroxidase activity. For







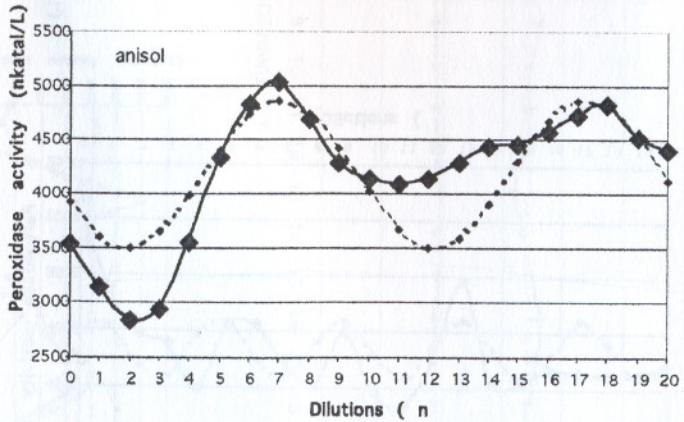


FIGURE 4 Peroxidase (III) activities measured in the presence of diluted vanillic aldehyde, VAC, phenol, and anisol; $n = -\log_{100}$ (mol/L). Dotted lines represent the graphic results of cosinus function analysis; vanillic aldehyde: $4098.93 + 967.38 * \cos(0.77 * n - 6.05)$; VAC $4076.38 + 920.14 * \cos(0.81 * n - 2.03)$; phenol: $4071.28 + 928.44 * \cos(0.61 * n - 3.78)$; anisol: $4181.71 + 686.20 * \cos(0.61 * n - 4.31)$.

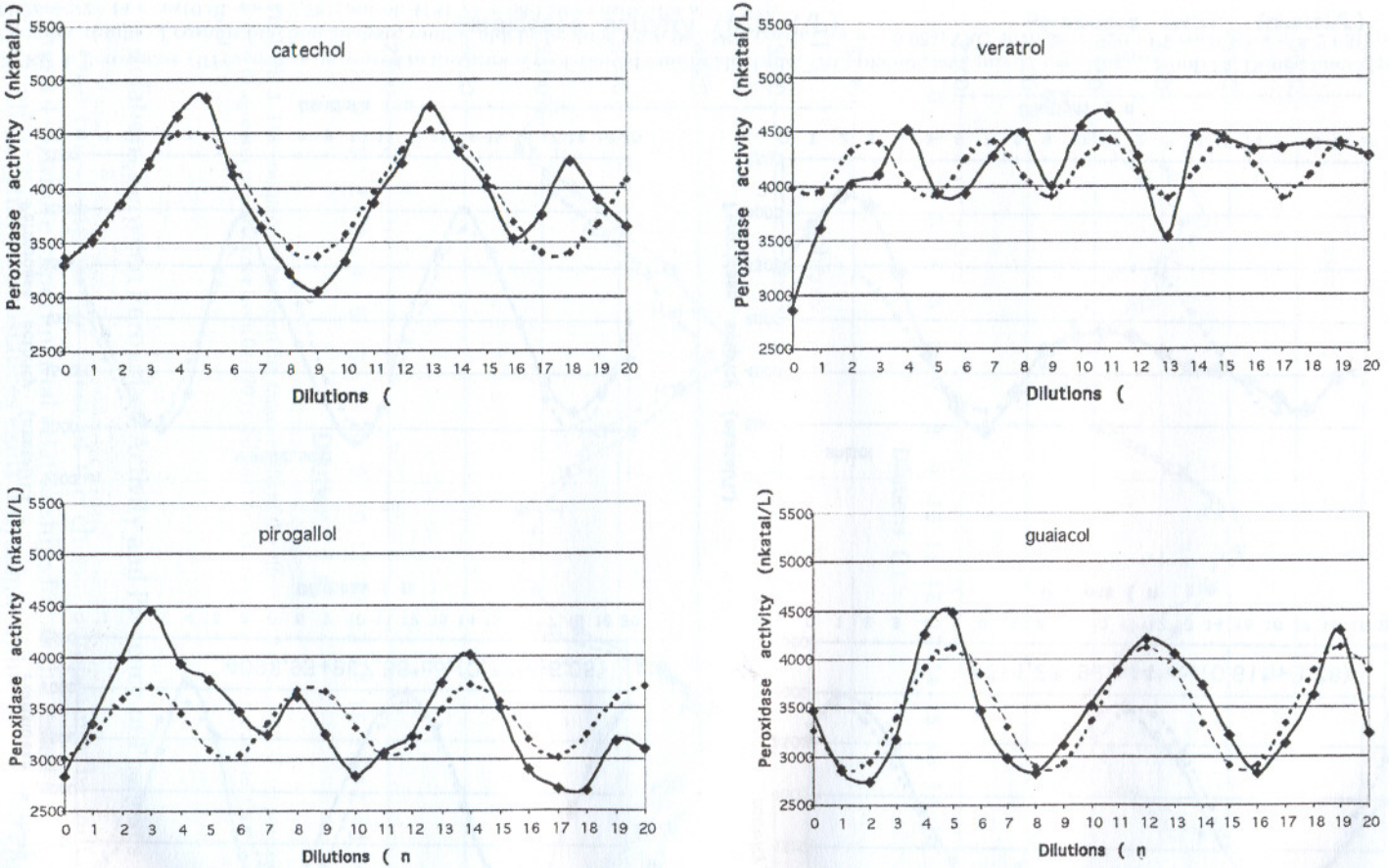


FIGURE 5 Peroxidase (III) activities measured in the presence of diluted catechol, veratrol, pirogallol, and guaiacol; $n = -\log_{100}$ (mol/L). Dotted lines represent the graphic results of cosinus function analysis; catechol: $3948.61 + 583.46 * \cos(0.72 * n - 3.17)$; pirogallol: $3369.30 + 353.41 * \cos(1.11 * n - 3.08)$; veratrol: $4158.44 + 272.28 * \cos(1.52 * n - 4.01)$; guaiacol: $3482.31 + 636.48 * \cos(0.89 * n - 4.38)$.

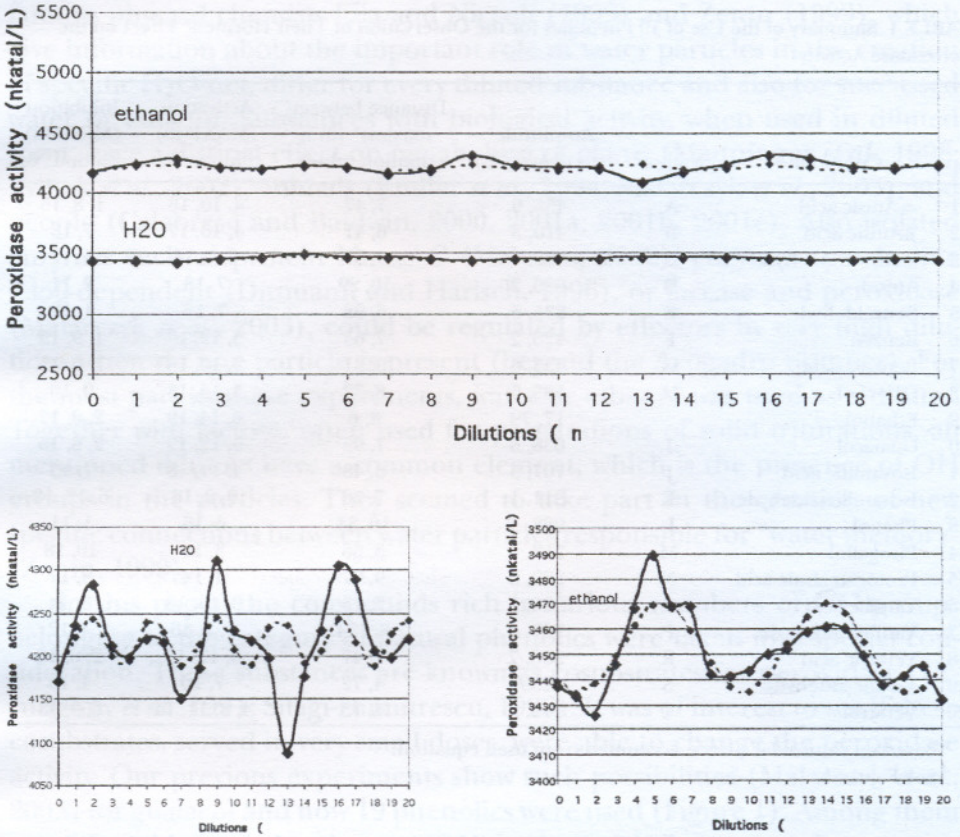


FIGURE 6 Peroxidase (III) activities measured in the presence of sucussed water and ethanol; $n = -\log_{100}$ (mol/L). Dotted lines represent the graphic results of cosinus function analysis; water: $4215.89 + 28.75 * \cos(1.75 * n - 3.12)$; ethanol: $3452.27 + 17.79 * \cos(0.73 * n - 3.91)$.

phenol and iso- and vanillic compounds the shapes of the curves are regular. For anisol, a methoxylated phenol, the first part of the curve is similar to that for phenol, but in the second part the curve flattens. The presence of the OH group changes the character of the sinusoidal curve, which is more stable than in the case of OCH_3. The curves for catechol ($2 \times$ OH) and veratrol ($2 \times OCH_3$) are distinctly different (Figure 5). Also catechol and pirogallol, with more OH groups (Figure 5), gave different curve shapes than phenol with one OH group (Figure 4). The addition of the COOH or the CHO group results in a relationship similar to the pattern of phenol, but the distance between the maxima and the minima is smaller for these phenolics.

A decrease in the oscillating effect in the second part of the dilution curve was observed for anisol (Figure 4) so that mainly the activating functions of dilutions are visible. The highest activation/inhibition effect was observed for phenol and the lowest for veratrol (Figures 4 and 5 and Table 1). In Table 1

TABLE 1 Summary of the Use of 19 Phenolics for the Observation of Their Hormetic Effect on the Peroxidase Activity

Lp	Compound	Symbol†	Amplitude* (in nkatals/L)	Distance between maxims* (in n dilution value)	Activation (MAXIMS) dilution No	Inhibition (MINIMS) dilution No
1	*m*-Anisic acid	A	456, 9	7, 47	4, 10, 18	1, 8, 15
2	*p*-Anisic acid	B	410, 3	6, 41	4, 10, 17	7, 13
3	*m*-Anisic aldehyde	C	582, 7	9, 37	2, 7, 16	4, 12
4	Anisol	D	686, 2	10, 29	7, 18	2, 11
5	Benzaldehyde	E	335, 5	6, 98	7, 16	1, 12
6	Benzen	F	426, 2	7, 65	5, 12, 19	1, 9, 15
7	Benzoic acid	G	435, 6	9, 81	3, 10, 18	1, 6, 15
8	Catechol	H	583, 5	8, 72	5, 13, 18	9, 16
9	Ethanol		17, 79	8, 6	5, 14, 19	2, 9, 17
10	Guaiacol	I	636, 5	7, 05	5, 12, 19	2, 8, 16
11	Isovanillic acid	J	707, 5	8, 48	4, 11, 18	7, 15
12	Isovanillic aldehyde	K	811, 1	7, 56	2, 8, 16	6, 12, 18
13	Phenol	L	928, 4	10, 34	6, 16	1, 11
14	Pirogallol	M	353, 4	5, 66	3, 14	10, 18
15	Protocatechuic acid	N	289, 6	9, 97	4, 14	8, 17
16	Vanillic acid	O	920, 1	7, 75	3, 10, 18	6, 14
17	Vanillic aldehyde	P	967, 4	8, 134	1, 7, 16	4, 12
18	Veratric acid	R	386, 7	7, 47	6, 12, 18	2, 8, 16
19	Veratic aldehyde	S	239	4, 72	7, 16	4, 13
20	Veratrol	T	272, 3	4, 13	4, 8, 11, 14	6, 9, 13

*Based on sinusoidal curves, accounted from used equation.
†See Figure 1.

there are data accounted from theoretical sinusoidal curves resulted from fitting of cosinus functions to the experimental data. Using the equation, the curves for individual phenolics can be compared in the amplitude value, as well as distances between maximal points and also in the dilutions, characteristic for maxima and minima of individual phenolics. The most characteristic points are written in bold characters in Table 1. In Table 1 the comparison between the effect of 19 phenolics, ethanol, and water on the peroxidase activity can be observed very quickly. Figure 6 consists of three parts; one situates the patterns for water and ethanol on the picture in the same scale as previous Figures (4 and 5), but the other two figures give the same curves on a smaller scale. It could be stressed that the sinusoidal curves were characteristic also for these two dilutors.

DISCUSSION

The mood of action of substances in their highly-diluted solutions as regulators in many biological reactions is more often connected with the uncial properties of dilutors as water or ethanol, used in the process of succussion, which is well known in homeopathic pharmacology. Papers by

famous physical chemists Elia and Niccoli (1999) and Zenin (1999), which give information about the important role of water particles in the creation of specific H_2O-net, differ for every diluted substance and also for succussed water or ethanol. Substances with biological activity, when used in diluted form, have a distinct effect on metabolism of plants (Manninger *et al.*, 1998; Tyihak *et al.*, 2002), animals (Endler *et al.*, 1994; Malarczyk *et al.*, 2003), and people (Calabrese and Baldwin, 2000, 2001a, 2001b, 2001c). Also isolated enzymes such as protein kinase C (Maltseva, 2002), enzymes cytochrome P450-dependent (Dittmann and Harisch, 1996), or laccase and peroxidase (Malarczyk *et al.*, 2003), could be regulated by effectors in very high dilutions when no one particle is present (beyond the Avogadro number). For the most part in these experiments, water or ethanol was used as a diluter. Together with lactose, often used for preparations of solid triturations, all mentioned dilutors have a common element, which is the presence of OH groups in the particles. They seemed to take part in the creation of new specific connections between water particles responsible for "water memory" (Zenin, 1999).

In this paper the compounds rich in various numbers of OH groups belonging to the category of natural phenolics were taken into special consideration. These substances are known as cosubstrates for peroxidase (Urrutigoity *et al.*, 1991; Silagi-Dumitrescu, 1999). It was of interest to see if these cosubstrates, served in very small doses, were able to change the peroxidase activity. Our previous experiments show such possibilities (Malarczyk *et al.*, 2003) for guaiacol and now 19 phenolics were used (Figure 1). Among them aromates with methylated group OH, such as anisol or veratrol, were presented, which corresponded to their hydroxyl equivalents such as phenol or catechol. According to our results all tested phenolics change the peroxidase activity in a sinusoidal manner and the shape of the curve was characteristic for the kind of phenolics. This shape was the same for various samples of enzymes, which were purchased from different places.

Basically the phenolic dilutions were used in order from 100° to 100^{-20} but in some experiments they were used in random sequence. In these experiments the shape of the sinusoid stayed the same. As was visible for phenol and VAC, the shape of the curves was characteristic for them. For comparison, ethanol and water caused only very small oscillations in the same experimental conditions when the amplitude was not more than 30 nkatals/L.

The experimental curves fitted very well to the earlier mentioned cosinusoid transformation of Levenberg–Marquardt (−). The amplitude values for these new curves are collected in Table 1. The highest values were noticed for phenol and for vanillic and isovanillic acids and aldehydes. All tested acids, with the exception of protocatechuic, showed three spikes on sinusoidal curves, but aldehydes created only two. It seems that the OH groups can play a role in the stabilization of the enzymatic center, but OCH_3

makes the curve flat. The compounds without the COOH group, pirogallol (3 × OH), catechol (2 × OH), and phenol (1 × OH), resulted in the creation of, respectively, four, three, and two spikes. The experimental curve for veratrol and anisol were asymmetric and rather flat. Benzene (Table 1) also created three maximum curves but their value was half of the maximum value. Characteristics of curves for additional phenolics are shown in Table 1.

Presented experiments lead to the conclusion that phenolics in very high dilutions can act as a cosubstrate for peroxidase. The shape of the peroxidase activity curve was strongly dependent on the type of phenolic compound present in the reaction medium according to its chemical structure. Completed results seemed to show evidence that the oscillatory behaviors in the peroxidase–phenolics connections are reproducible, nonartefacted, and repeated for various samples of peroxidase. The knowledge about dynamics for the opposite inversion of a phenolic compound from activator to inhibitor by succussed dilution only could also have a practical aspect because of the very economical status of omitting the addition of other activators or inhibitors for the regulation of peroxidase activity.

REFERENCES

Calabrese E, Baldwin L. Chemical hormesis: Its historical foundations as a biological hypothesis. *Hum Exp Toxicol.* 2000;19:2–32.

Calabrese E, Baldwin L. Hormesis: A subordination generalizable and unifying hypothesis, *Crit Rev Toxicol.* 2001a;31:353–424.

Calabrese E, Baldwin L. The frequency of U-shaped dose responses in the toxicological literature. *Toxicol Sci.* 2001b;62:330–338.

Calabrese E, Baldwin L. U-shaped dose-responses in biology, toxicology, and public health. *Annu Rev Pub Health.* 2001c;22:15–23.

Clairborne AC, Fridovich I. Chemical and enzymatic intermediates in the peroxidation of o-dianisidine by horseradish peroxidase. *Biochemistry.* 1979;18:2324–2330.

Dittmann J, Harisch G. Characterization of differing effects caused by homeopathically prepared and conventional dilutions using cytochrome P450 2E1 and other enzymes as detection systems. *J Altern Complem Med.* 1996;2:279–290.

Elia V, Niccoli M. Thermodynamics of extremely diluted aqueous solutions. *Ann NY Acad Sci.* 1999;879: 241–248.

Endler PC, Pongratz W, Kastberg G. The effect of highly diluted agitated tyroxine on the climbing activity of frogs. *Vet Hum Toxicol.* 1994;36:56–57.

Gill PR, Murray W, Wright MH. The Levenberg-Marquardt method. In: *Practical Optimization.* Soc 4.7.3. London: Academic Press; 1981;136–137.

Linde K, Jonas WB, Melchart D. Critical review and meta-analysis of serial agitated dilutions in experimental toxicology. *Hum Exp Toxicol.* 1994;13:481–492.

Malarczyk E, Kochmanska-Rdest J, Jarosz-Wilkolazka A. Effect of low doses of guaiacol and ethanol on enzymatic activity of fungal cultures. *Nonlinearity.* 2003;1:167–178.

Maltseva L. Bimodal type of regulation of protein kinase C by antioxidants down to ultra-low doses. Materials of Intern. Conference: Non-linear dose-response relationships in biology, toxicology and medicine. Amherst MA. 11–13 June 2002.

Manninger K, Csösz M, Tyihak E. Induction of resistance of wheat plants to pathogens by pretreatment with N-methylated substances. *Acta Biol Hung.* 1998;49:275–281.

Saunders BC. *Peroxidase-Action and Use in Organic Synthesis* Lect. Monograph Report, London; 1957.

Silaghi-Dumitrescu RL. HRP: A summary of its structure, mechanism and substrate diversity. LABPV—Peroxidase Biotechnology and Application 1–17 (http://www.unige.ch/LABPV/publications/silaghi/Dumitrescu.html).

Tyihák E, Steiner U, Schönbeck F. Time- and dose-dependent double immune response of plants to pathogens. *Modern Fungicide and Antifungal Compounds III, 13th International Reinhardsbrunn Symposium*, 14–18 May 2001:187–196. Friedrichroda, Germany (AgroConcept GmbH Bonn).

Urrutigoity M, Baboulene M, Lattes A, Souppe J, Seris J.-L. Effect of linking allyl and aromatic chains to histidine 170 in horseradish peroxidase. *Biochim Biophys Acta*. 1991;1079:209–213.

Zenin SV. The complex formation of acetonitril and methyl alcohol with water. *Russ J Phys Chem*. 1999;73:835–838.

ERRATA

PLACE	IS:	OUGHT TO BE:
5	……of ethanol water	……of ethanol – water
58	10 **used b** mmoles…	10 mmoles…….
Figure 1 (second line)	…..A-m-anisic acis,	…….A-m-anisic acid….
Figure 1 (third line)	….K-isovanllic aldehyde,	…….K-isovanillic aldehy
69	……notation $C_{11/2}$ C_{20} (or…..	…….notation C_1- C_{20} (or…..
101	Two important peroxidase……	Two important **for** peroxidase
116	….the data **in** white point	…the data **on** white point
138	..(Figure 5), g**a**ve….	..(Figure 5), g**i**ve….
163	. Papers **by**	. Papers **of**

Table 1

Lp	Compound	Symbol**	Amplitude* (in nkatals/L)	Distance between maxims* (in **n** dilution value)	Activation (MAXIMS) dilution No	Inhibition (MINIMS) dilution No
1	*m*-anisic acid	A	456,9	7,47	4, **10**, 18	1, **8**,15
2	*p*-anisic acid	B	410,3	6,41	4, **10**, 17	**7**,13
3	*m*-anisic aldehyde	C	**582,7**	**9,37**	2, **7**, 16	**4**, 12
4	anisol	D	**686,2**	**10,29**	**7**, 18	**2**, 11
5	benzaldehyde	E	335,5	6,98	7, **16**	**1**, 12
6	benzen	F	426,2	7,65	5, **12**, 19	**1**, 9, 15
7	benzoic acid	G	435,6	**9,81**	3, **10**, 18	1, **6**, 15
8	catechol	H	**583,5**	**8,72**	**5**, 13, 18	**9**, 16
9	ethanol		17,79	**8,6**	**5**, 14, 19	**2**, 9, 17
10	guaiacol	I	**636,5**	7,05	**5**, 12, 19	**2**, 8, 16
11	isovanillic acid	J	**707,5**	**8,48**	4, **11**, 18	7, **15**
12	isovanillic aldehyde	K	**811,1**	7,56	2, **8**, 16	6, **12**, 18
13	phenol	L	**928,4**	**10,34**	6, **16**	**1**, 11
14	pirogallol	M	353,4	5,66	**3**, 14	10, **18**
15	protocatechuic acid	N	289,6	**9,97**	**4**, 14	**8**, 17
16	vanillic acid	O	**920,1**	7,75	3, **10**, 18	**6**, 14
17	vanillic aldehyde	P	**967,4**	**8,134**	1, 7, **16**	**4**, 12
18	veratric acid	R	386,7	7,47	**6**, 12, 18	2, 8, **16**
19	veratric aldehyde	S	239	4,72	**7**, 16	**4**, 13
20	veratrol	T	272,3	4,13	4, 8, **11**, 14	6, 9, **13**

* - based on sinusoidal curves, accounted from used equation

** - see Fig.1