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# ELECTROCHEMICAL OXIDATION AND ANTIBACTERIAL PROPERTIES OF PHENOLIC COMPONENTS OF ESSENTIAL OILS

A systematically related group of phenolic substances the majority of which is found in essential oils was investigated for a correlation between their bactericide potential and electrochemical oxidizability. For this purpose we have determined the effective concentrations of the 50% growth inhibition of E. coli and the half peak potentials of the oxidation by cyclovoltammetry in diluted phosphate buffer at glassy carbon for phenol, guajacol, catechol as well as for their p-allyl- and p-propenyl derivatives. We found a relation between the effectivity of the substances, presented as logarithm of the reciprocal effective concentration log  $(EC_{50})^{-1}$ , the logarithm of the octanol/water distribution coefficient log K<sub>ow</sub>, and the half peak potential  $E_{p/2}$  of the electrochemical oxidation.

#### INTRODUCTION

Essential oils are volatile plant components which may occur in different parts of plants. Some essential oils are not only bactericide, but also fungicide. Experiments have shown, that phenols are among the most active antimicrobial components of the essential oils [1]. Little is known about the mechanisms of effect on microorganisms of essential oils so far. Teuscher *et al.* [2] assume that the type of mechanism depends on their concentrations. In the present paper we therefore investigate the question of whether the antimicrobial activity of phenols correlates with their electrochemical oxidizability and their octanol/water distribution coefficients. The phenols to be studied were chosen considering the following three aspects: occurrence in essential oils, strong antimicrobial effect and arrangement of systematically related group. This led to phenol, chavicol and anol, to guajacol, eugenol and isoeugenol and to catechol and 4-allylcatechol [3a–3h]. The basis of a variety of correlations known from literature is a structure-effect relationship first published by Hansch [4] (Equation (1) ).

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 $\log (EC_{50})^{-1} = -a (\log K_{ow})^{2} + b \log K_{ow} + \log K_{A} + c \sigma + d$ (1)

where  $EC_{50}$  is the effective concentration causing a 50% inhibition of a biological process,  $K_{ow}$  is the octanol/water distribution coefficient,  $K_A$  is the affinity constant between substance and receptor,  $\sigma$  is the substituent constant according to Hammet, and a-d are general constants. Equation (1) is based on the following assumptions:

- the substance reaches the place of action via diffusion through lipophilic and hydrophilic phases (-a (log  $K_{ow}$ )<sup>2</sup> + b log  $K_{ow}$ ),

- an interaction between the substance and a receptor takes place (log KA),

– a reaction between the substance and the receptor leads to the biological effect of the substance (c  $\sigma$ ).

In case of phenols, equation (1) can be simplyfied. It is known from the literature [5], that the binding force of phenols to bovine serum albumine can be described by equation (2).

$$\log (EC)^{-1} = a \log K_{ow} + b \tag{2}$$

Here, EC is the concentration required to form a complex in a predetermined ratio. Therefore it can be assumed, that the formation of the complex (log  $K_A$ ) as well as the diffusion of the substance can be described by the logarithm of the distribution coefficient. Additionally, there is a linear relationship between the substituent constant  $\sigma$  and the half step potential  $E_{1/2}$  of the electrochemical oxidation for m- and p-substituted phenols [6]. The substituent constant may thus be replaced by a parameter of the electrochemical oxidation, thus the equation (1) can be transformed to equation (3).

$$\log (EC_{50})^{-1} = -a (\log K_{ow})^{2} + b \log K_{ow} + c E_{1/2} + d$$
(3)

Finally, in case all log  $K_{ow}$  values are either below or above 4.3, according to [7] it may be assumed, that there is a linear relationship between  $(EC_{50})^{-1}$  and log  $K_{ow}$  instead of a parabolic one. Hence we determined  $EC_{50}$  values, electrochemical oxidizability and log  $K_{ow}$  values in order to establish a correlation.

### METHODS AND MATERIALS

#### 1. Determination of EC<sub>50</sub> values

Determination of the effective concentrations leading to a 50% inhibition of a biological process was done by means of a growth inhibition test with the strain Escherichia coli K12 "wild type" ATCC 23716. The medium used was the DSM 1

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medium (5 g peptone (Merck, from casein) and 3 g meat extract (Merck) are added to distilled water to yield 1 dm<sup>3</sup>) proposed for E. coli by the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [8]. The pH value was adjusted to 7.4. The medium was autoclaved at 393.15 K and 1.2-1.4 bar for 20 minutes.

The sample of 0.5 cm<sup>3</sup> of an overnight culture (17 hours, 310.15 K) of the bacterial strain E. coli K 12 was added to 25 cm<sup>3</sup> of the medium and incubated at 310.15 K for 3 hours. Subsequently this preculture was diluted to an optical density (percentage of intensity loss due to turbidity) of 5% . 0.25 cm<sup>3</sup> of this solution was added to 2.25 cm<sup>3</sup> of the medium (as reference) and to 2.25 cm<sup>3</sup> of the medium containing a certain concentration of one of the eight substances under study (optical density 0.5%). In addition to four parallel samples in both cases a blank (without bacteria) was prepared.

The samples were incubated at 310.15 K for four hours on a rotating shaker in the dark, followed by chilling in ice water. The optical density was determined using a spectrophotometer Shimadzu UV-120-01 (wavelength 550 nm, path length 1 cm). The inhibitory effect on cell growth was calculated in dependence of the concentration of the phenolic compounds from the optical data of the reference, the samples containing the compounds and the blank, following the procedure described in [9]. In case of some compounds (catechol and 4-allylcatechol) the samples showed different discolouring, depending on presence or absence of bacteria. The optical density corresponding to the blank was therefore determined following centrifugation of the samples. In the case of isoeugenol this was not possible, since it forms an emulsion due to its low water solubility. In this case the blank had to be used. The EC<sub>50</sub> values needed were obtained with the help of a computer program [10].

## 2. Determination of data concerning the electrochemical oxidizability

The electrochemical oxidizability was studied by cyclovoltammetry in a buffer solution of pH 7.4. All measurements were performed in an usual laboratory H-cell of 25 cm<sup>3</sup> volume. The electronic equipment consisted of a standard Potentiostat (Wenking STP 84) in combination with a CP/M MAC 80 (Spectradata) (program CV 2.0 [11]).

To obtain reproducible cyclovoltammograms of the phenolic compounds (concentration range from 0.025 to 5.0 mmol dm<sup>-3</sup>) it was necessary to use a highly polished glassy carbon electrode. The electrode was polished (0.05 mm Alumina) and a reference cyclovoltammogram of 1.0 mmol dm<sup>-3</sup> hexacyanoferrate (II) was taken before each measurement to ensure identical surface conditions. According to our observations this system is very sensitive to surface changes. The graphically determined [12] half peak potential  $E_{p/2}$  of the positive sweep (rate 0.007 V s<sup>-1</sup>) was taken as significant.

# 3. Determination of log Kow values

Data for phenol, guajacol and catechol are available from literature [13, 14]. The log  $K_{ow}$  values of the remaining compounds were calculated according to [14] employing fragment constants and factors taking the type of bonding into account.

#### 4. Chemicals

The following compounds were purchased from commercial sources: phenol (Fluka p. a. > 99.5%), guajacol (Merck reinst > 99%), eugenol (Fluka > 99%), isoeugenol (Merck 98%, 75% trans, 25% cis), catechol (Merck > 99%).

A sample of chavicol was made available through Givaudan [15]. A further amount of chavicol (98.9%) was synthesized according to Zemplen and Gerecs [16] via the demethylation of 1-methoxy-4-allylphenol with the use of methyl magnesium iodide. Anol (99.7%) was obtained via the demethylation of anethol using potassium hydroxide according to Stoermer and Kahlert [17].

The 4-Allylcatechol (98.5%) was prepared via the catechol monoallyl ether [18] and a Claisen rearrangement [19]. Trans isoeugenol (99.1%) was isolated from the commercial isoeugenol via the sodium salt [20]. The progress of the reactions and the purity of the compounds were monitored via thin layer and gas chromatography. For the buffer solutions (adjustment of pH value) potassium dihydrogen phosphate (Janssen Chimica p. a.), disodium hydrogen phosphate (Merck p. a.) and 18 M $\Omega$  water (Seral pro 90 c) were used.

#### RESULTS AND DISCUSSION

Table 1 shows the substances in a decreasing order of effective concentrations of growth inhibition, logarithm of the octanol/water distribution coefficient and half peak potential.

In the growth inhibition test guajacol exhibits an activity similar to that of phenol, whereas catechol is twice as active as phenol. The p-alkenyl-substituted compounds are more active than the respective parent compounds. The higher activity of isoeugenol compared to eugenol corresponds with results of agar diffusion tests for antifungicide effects [21]. The fact that chavicol is more active than eugenol against E. coli agrees with results of another agar diffusion test [22].

The octanol/water distribution coefficients are lowest for the parent compounds. On introduction of the p-alkenyl substituents the log  $K_{ow}$  value changes by an average of 1.25.

Table 1

List of substances in a decreasing order of effective concentrations of growth inhibition, logarithm of the octanol/water distribution coefficient and half peak potential (mean values).

The phenol coefficients are stated in parentheses in the first column following the substance names. **Parent compounds are printed boldface** 

EC <sub>50</sub> /mmol dm <sup>-3</sup>	log K <sub>ow</sub>	Ep/2/ Vvs. NHE
phenol (1.0)	anol	phenol
12.8	2.78	0.787
guajacol (1.1)	isoeugenol	chavicol
11.2	2.65	0.685
catechol (2.1)	chavicol	guajacol
6.1	2.65	0.592
eugenol (7.1)	eugenol	anol
1.8	2.53	0.555
chavicol (8.6)	4-allylcatechol	eugenol
1.5	2.04	0.552
isoeugenol (10.5)	phenol	isoeugenol
1.2	1.46	0.415
4-allylcatechol (12.1)	guajacol	catechol
1.0	1.33	0.380
anol (14.4)	catechol	4-allylcatechol
0.9	0.85	0.335

The half peak potentials of the parent compounds conform with values known from literature [23–26]. The effect of the p-propenyl group on the oxidizability is stronger than the effect of the p-allyl group. The effect of p-substituents on the half peak potential decreases from phenol via guajacol to catechol.

In order to determine whether there is a correlation between the antibacterial effect of phenolic substances and their physicochemical behaviour – represented by the half peak potential of the electrochemical oxidation and by the octanol/water distribution coefficient – the values obtained can be inserted in equation (3). Since all values of log  $K_{ow}$  are below 4.3, the equation reads as follows (considering  $E_{p/2}$  instead of  $E_{1/2}$ ):

$$\log (EC_{50})^{-1} = a \log K_{ow} + b E_{p/2} + c$$
(4)

The constants were adjusted to the data of Table 1 by multiple regression [10]. The result obtained is shown in equation (5). Please note that in equation (5) the concentration has to be inserted in mol  $dm^{-3}$  and the potential in Volts versus NHE.

$$\log (EC_{50})^{-1} = 0.559 \log K_{ow} - 1.34 E_{p/2} + 2.14$$
(5)

The correlation coefficient is 0.97. In addition a probability > 99% for the existence of a correlation is indicated by the so called F-Test. This test is based on the number of parameters (2) and the number of substances under study (8).

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The determined linear correlation between the effectivity of the substances in the growth inhibition test and the logarithm of the octanol/water distribution coefficient agrees with data found in literature [7]. The determined slope is within the range to be expected and points to an unspecific action by formation of complexes with bacterial proteins. However, the correlation presented with  $E_{p/2}$  is a new finding.

A correlation performed, as shown above, allows the determination of the effectivity of related not investigated substances. For instance, the effectivity of 4-propenylcatechol [27] can be estimated. Equation (5) leads to a value of 0.87 mmol dm<sup>-3</sup> for EC<sub>50</sub> using log K<sub>ow</sub> = 2.17 (determined according to [14]), and  $E_{p/2} = 0.220$  V versus NHE (extrapolated from the  $E_{p/2}$  data of Table 1). Hence 4-propenylcatechol would have been most effective.

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#### ELEKTROCHEMISCHE OXIDIERBARKEIT UND ANTIBAKTERIELLE EIGENSCHAFTEN PHENOLISCHER BESTANDTEILE ÄTHERISCHER ÖLE

Eine systematisch zusammenhängende Gruppe phenolischer Substanzen, von denen die Mehrzahl in ätherischen Ölen vorkommt, wurde in bezug auf eine Korrelation zwischen antibak-terieller Wirkung und elektrochemischer Oxidierbarkeit untersucht. Zu diesem Zweck wurden die effektiven Konzentrationen, welche eine 50%ige Hemmung des Wachstums von E. coli hervorrufen, sowie die Halbpeakpotentiale für die Oxidation bestimmt (letzteres mittels Zyklovoltammetrie in wässrigem Phosphatpuffer an Glaskohlenstoff). Eingesetzt wurden Phenol, Guajacol und Brenz-katechin sowie deren p-allyl- und p-propenyl- Abkömmlinge. Wir fanden eine lineare Beziehung zwischen der antibakteriellen Wirkung (dargestellt als Logarithmus der reziproken effektiven Konzentration log  $(EC_{50})^{-1}$ ), dem Logarithmus des Oktanol/Wasser-Verteilungskoeffizienten K<sub>ow</sub> und dem Halbpeakpotential E<sub>p2</sub> der elektrochemischen Oxidation.

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### ELEKTROCHEMICZNE UTLENIANIE I WŁASNOŚCI ANTYBAKTERYJNE FENOLOWYCH POCHODNYCH OLEJKÓW ETERYCZNYCH

Opisano wyniki badań zależności między bakteriobójczymi własnościami wybranych fenoli, a ich podatnością na eletrochemiczne utlenianie. Badania elektrochemiczne dziewięciu związków z grupy fenoli prowadzono w układzie woda-oktanol. Wyniki badań wskazują na liniową zależność między własnościami antybakeryjnymi (jako odwrotność logarytmu stężenia efektywnego (EC<sub>50</sub>)<sup>-1</sup>, logarytmu współczynnika podziału (K<sub>ow</sub>) i potencjalu półfali (E<sub>p/2</sub>) eletrochemicznego utleniania.