Proc. Estonian Acad. Sci. Chem., 1992, 41, 1, 1—5
https://doi.org/10.3176/chem.1992.1.01
UDC 577.1

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INHIBITORY EFFECTS OF ALKYLRESORCINOLS ON PROSTAGLANDIN-H_2-SYNTHETASE

Several inhibitors of prostaglandin-H_2-synthetase (E. C. 1. 14. 99. 1.) are well-known anti-inflammatory drugs, as, for example, aspirin or indomethacin [1]. At the same time, some resorcinols with alkylic substituents are used for the same purpose externally [2], although mainly because of their antiseptic properties. Dewhirst [3] showed the existence and structural dependence of the inhibitory effect of substituted monophenols on the same enzyme. In our work similar effects of 1,3-dihydroxybenzene with various alkylic substituents are described.

Materials and Methods

Substituted resorcinols were synthesized in the Oil-Shale Research Institute, Kohtla-Järve, Estonia. Arachidonic acid was a product of the Pilot Plant of the Institute of Chemistry, Estonian Academy of Sciences. The enzyme was prepared from sheep vesicular glands obtained from a local slaughter-house. Membrane proteins were solubilised by 1% TWEEN 20 (Ferak). The residue was removed by ultracentrifugation. According to the chromatographic data the supernatant contained about 5% of useful protein. The separation method is described in [4]. Arachidonic acid was used as a 15 mM ethanol solution. Phenol (Reakhim, analytical grade) was used as an electron donor. Other reagents were purchased from Serva, analytical grade. The heme solution was made and its concentration measured as described in [5].

Experimental

The reaction mixture is given in Table 1. The enzyme was saturated with all substrates except arachidonic acid. Phenol concentration was sufficient for saturation without showing an inhibitory effect which occurs at higher levels. Under these conditions the enzyme catalyzed reaction follows the Michaelis equation like a monosubstrate reaction [6].

The kinetic measurements were carried out in a 1.17 ml vessel closed with a Clark electrode having a side channel. The reactives were added by means of 10 and 50 μL Hamilton syringes. The EDTA buffer was previously saturated with air at normal pressure (oxygen content 21 vol%) in a thermostated laboratory glass by stirring for 1 hour. The substrates were added 4—5 min before starting the reaction with enzyme to avoid their non-enzymatic oxidation. During this period the baseline drift was registered. Data on the oxygen consumption rate were collected with the help of a PA-2 polarographic analyser (Laboratorni Pristroje,

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Czechoslovakia) on a recorder or, directly, via an A/D converter (Adalab, IMI), to Apple IIe computer. The initial velocities were determined using the "Direct Linear Plot" method by Eisenthal and Cornish-Bowden

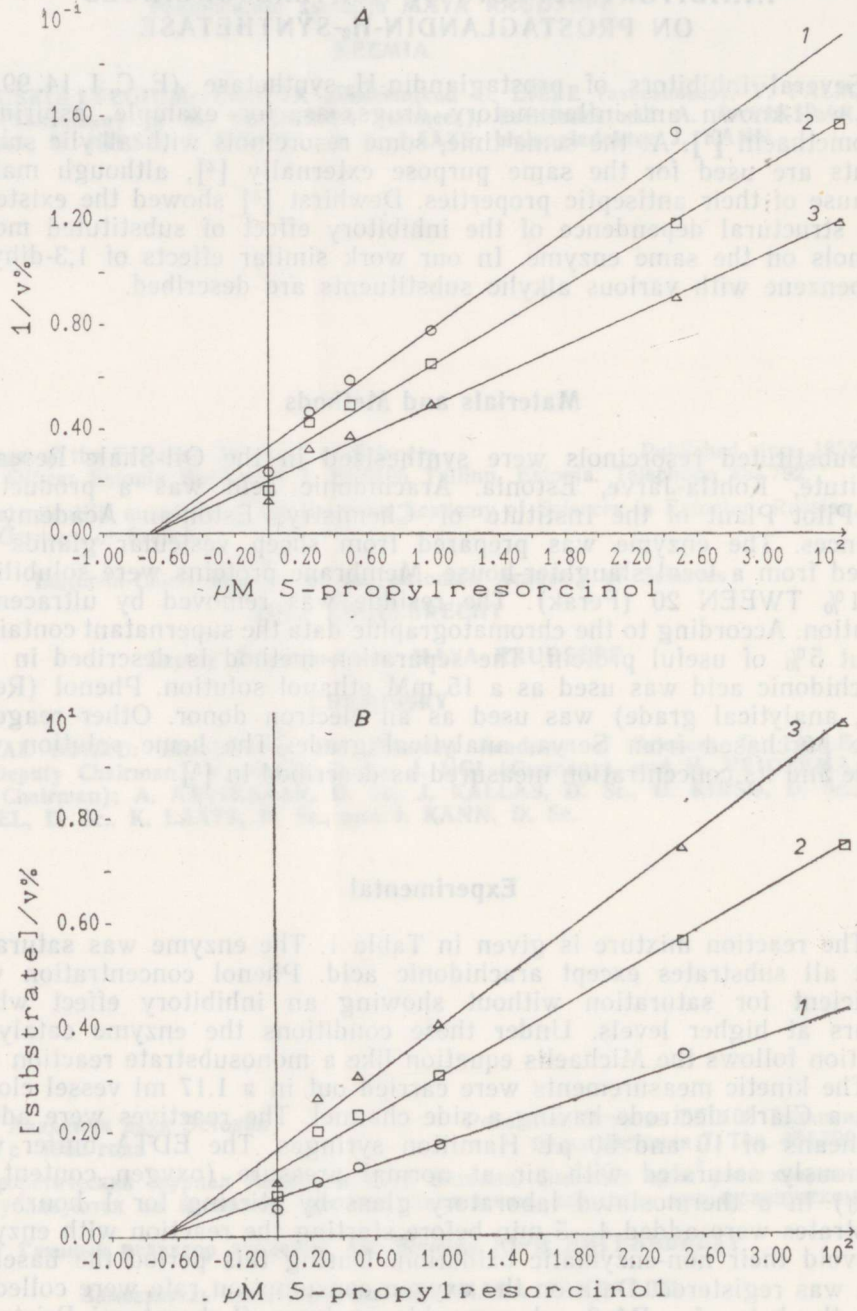

Fig. 1. An example of determining kinetic parameters. *A* — in Dixon coordinates, *B* — in the coordinates of Cornish-Bowden. Inhibitor 5-propylresorcinol; *v* is given in relative units.
Arachidonic acid: *1* — 22.3 μM, *2* — 47.0 μM, *3* — 61.8 μM.

[7, 8], which is less influenced by self-inactivation of the enzyme during reaction than the classical ones. Other kinetic investigations were performed on an Olivetti 290 with "Enzfitter" software. Two methods of estimating the type of inhibition were used: the Dixon method ($1/v$ vs. i) and that of Cornish-Bowden (s/v vs. i) [9]. For each inhibitor 5—10 experiments were carried out to get I_{50} values, for K_i it was also done at three different arachidonic acid concentrations. An example is given in Fig. 1.

Table 1

Reactives and conditions	Values
Arachidonic acid	$2.23—9.4\times10^{-5}$ M
Hemin	7×10^{-7} M
Phenol	7×10^{-4} M
Oxygen	2.514×10^{-4} M
EDTA	5×10^{-2} M
TWEEN 20	0.1%
Protein	10—20 mg/ml
Resorcinols	$10^{-6}—10^{-3}$ M
Cyclooxygenase activity of protein	3—5 μmol/min×mg
pH	8.0
Temperature	298±0.1 K

Results

The main results are given in Table 2. It was experimentally proved that the alkylresorcinols tested are not substrates for prostaglandin-H_2-synthetase. Only 2,5-dimethyl resorcinol caused oxygen consumption in the reaction mixture without arachidonic acid. This effect was not in correlation with the enzyme concentration but with the pH value. Resorcinols do not act as electron donors for this enzyme, not replacing phenol even at high levels (mM). Kinetic analysis led to a conclusion that they display an inhibitory activity of a noncompetitive or mixed type. The fact that in the Dixon coordinates the experimental points lack linearity at higher inhibitor concentrations suggests a nonspecific binding of resorcinols to the enzyme. As the latter is multisubstrate, it

Table 2

No.	Substitution position	Substituent	pI_{50}	π	I_{50}, μM	K_i, μM
1.	4	$—CH_3$	3.959	0.56	110	23.3
2.	4	$—C_2H_5$	3.967	1.02	108	
3.	4	$—C_3H_7$	4.762	1.55	17.3	
4.	4	$—C_4H_9$	4.857	2.13	13.9	
5.	4	$—C_5H_{11}$	5.06	2.67	8.7	
6.	4	$—C_6H_{13}$	5.143	3.22	7.2	7.0
7.	4	$—C_9H_{19}$	4.047	4.87	89.7	30.1
8.	4	$—C_{10}H_{21}$	4.250	5.42	56.2	
9.	2	$—C_2H_5$	4.807	1.02	15.6	
10.	5	$—C_2H_5$	4.796	1.02	16	
11.	2,5	$—CH_3$	4.053	1.12	88.6	
12.	4,6	$—CH_3$	4.082	1.12	82.8	47.8
13.	5	$—CH_3$	3.367	0.56	430	
14.	2,4	$—CH_3$	3.305	1.12	495	
15.	5	$—C_3H_7$	4.101	1.55	79.2	75.0
16.	—	—	3.377	0	420	

Remarks: The values of I_{50} are given at arachidonic acid concentration 47 μM. The values of K_i are given in case the statistical error was under 20%.

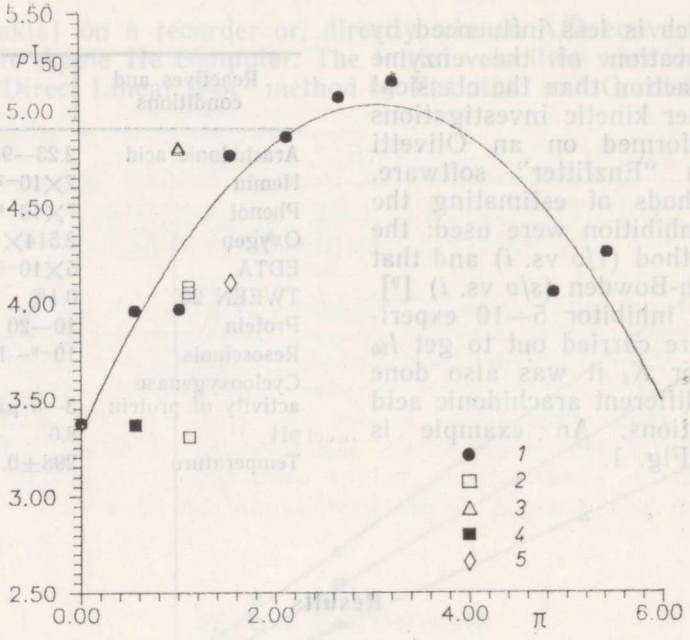

Fig. 2. Correlation between hydrophobic constant π and pI_{50} of resorcinols. π for non-substituted resorcinol was conventionally taken zero.

may also bind more than one molecule of the inhibitor to its active site. Dewhirst in his above-mentioned work proved that in the case of phenols a sterically free —OH group is responsible for their inhibitory properties. Having one more —OH in the third position in resorcinols has no dramatic effect. The I_{50} values are for phenol 1600 μM and for resorcinol 420 μM. Substituents which increase hydrophobicity of phenolic compounds lower their I_{50} value correspondingly. This dependence has a minimum in case of resorcinols but for monophenols only substituents with π values lower than three were used. According to the information in [10] the use of hydrophobicity as the basis of correlation analysis is justified. The electronic and steric constants have low and slightly different values for our substituent groups. According to [11] we suggested a parabolic shape of dependence between pI_{50} and π. The nonlinear regression of the data for 4-substituted alkylresorcinols yielded:

$$pI_{50} = -0.181\,(\pm 0.028)\,\pi^2 + 1.105\,(\pm 0.16)\,\pi + 3.33\ (\pm 0.18),$$
$$R = 0.94.$$

Other variants do not correlate with π (Fig. 2).

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Received
Oct. 10, 1991

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ALKÜÜLRESORTSIINIDE INHIBEERIV TOIME PROSTAGLANDIIN-H_2-SÜNTETAASILE

On määratud mitmete alküülresortsiinide inhibeeriv toime prostaglandiin-H_2-süntetaasile (E.C.1.14.99.1) ning täheldatud sõltuvust hüdrofoobsuse ja inhibeerimisvõime vahel 4-asendatud ühenditel.

Пеэтер КЯЭМБРЕ, Нигулас САМЕЛ, Юло ЛИЛЛЕ

ИНГИБИРОВАНИЕ ПРОСТАГЛАНДИН-H_2-СИНТЕТАЗЫ АЛКИЛРЕЗОРЦИНАМИ

Изучено ингибирующее действие ряда алкилрезорцинов на PCH_2-синтетазу (К.Ф.1.14.99.1). Обнаружена взаимозависимость гидрофобности и ингибирующих свойств 4-алкилрезорцинов.