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Complexation of Nitrostyrenes with Soluble β-Cyclodextrin Polymer Studied by Reversed-Phase Thin-Layer Chromatography

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Abstract. Cyclodextrin complexation decreases the apparent lipophilicity of hydrophobic guest molecules. A higher complex stability results in a larger decrease of lipophilicity as determined by reversed-phase thin-layer chromatography. The method was applied to study the complex formation of 33 nitrostyrene derivatives with a water soluble cross linked β-cyclodextrin polymer (weight average molecular weight: 4300). The substituents in the *para* position of the benzene ring had a higher impact on the complex stability than those in the *meta* and *ortho* positions. The substituents on the alkyl side chain influenced the complex stability to the same extent as those on the benzene ring.

Key words: Nitrostyrenes, soluble cyclodextrin polymer, complex stability, thin-layer chromatography.

1. Introduction

The biological activity of a compound is controlled by many factors, one of the most important being its lipophilicity because its penetration through membranes of the target organism is governed chiefly by the molecular lipophilicity [1]. Since cyclodextrins are hydrophilic and the bioactive molecules to be complexed are generally lipophilic, complex formation will decrease the lipophilicity of molecules included in the cavity of cyclodextrins. It is assumed that the higher the decrease of lipophilicity the stronger is the complex stability [2].

The drawback of this method is that it does not give information about the stoichiometry of the complex because the complex stoichiometry does not change within a homologous series. Therefore the sequence of the stability values determined by this method coincides with that determined by other methods. The lipophilicity of a substance in solution can be estimated by reversed-phase thin-layer chromatography (RPTLC) [3,4], the method having been succesfully applied to study the cyclodextrin inclusion complex formation (with soluble β-cyclodextrin polymer) with the antibiotics polymyxine [5], some symmetric triazine [6] and triphenylmethane derivatives [7].

The present paper reports on the interaction between a soluble β-cyclodextrin polymer and some nitrostyrene derivatives with marked antifungal activity [8,9] and on the effect of various substituents on the complex stability.

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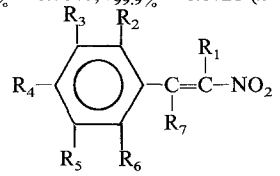
2. Materials and Methods

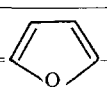
The chemical structures of the nitrostyrene derivatives studied are shown in Table I. Polygram UV_{254} (Macherey–Nagel) plates were impregnated by the method of [5]. Paraffin oil was

Table I. Chemical structure and parameters of the linear correlations between the lipophilicity of nitrostyrene derivatives and the concentration of the soluble β-cyclodextrin polymer in the eluent. $n = 10$, $r_{95\%} = 0.6319$, $r_{99\%} = 0.7646$, $r_{99.9\%} = 0.8721$ (n = number of observations).



R_1, R_2, R_3, R_4, R_5, R_6 and R_7 = H unless stated otherwise

N° of compound	Substituents		a	$-b$	r
1			31.2	13.83	0.8748
2	$R_1 = -CH_3$		61.0	10.98	0.9199
3	$R_2 = -Cl$		52.7	19.78	0.9406
4	$R_1 = -CH_2-O-CO-C_2H_5$		60.6	6.65	0.8769
5	$R_1 = -CH_2-O-CO-C_6H_5$		99.1	17.63	0.9576
6	$R_1 = -CH_2-O-CO-C_{11}H_{23}$		196.8	11.48	0.9534
7	$R_1 = -CH_2-O-CO-CH_3$		40.0	6.48	0.8364
8	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -F$	39.6	4.57	0.7489
9	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = -Cl$	69.8	5.59	0.8398
10	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -Cl$	63.0	4.88	0.8002
11	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -Cl$	62.4	6.85	0.8414
12	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = R_6 = -Cl$	65.3	0.94	0.9009
13	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = R_5 = -Cl$	99.3	5.32	0.7893
14	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -Br$	67.6	8.79	0.9229
15	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = -NO_2$	34.4	5.68	0.8316
16	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -NO_2$	46.7	5.11	0.8404
17	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -NO_2$	34.9	5.93	0.8436
18	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = -CH_3$	54.9	1.60	0.5142
19	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -CH_3$	63.7	3.33	0.7830
20	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -CH_3$	62.6	4.59	0.8653
21	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = -OCH_3$	51.2	1.55	0.4686
22	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -OCH_3$	50.0	3.67	0.8152
23	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -OCH_3$	49.9	5.43	0.8602
24	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = R_4 = -OCH_3$	38.8	1.19	0.2699
25	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -O-CH_2-O-(= R_4)$	39.6	6.64	0.8336
26	$R_1 = -CH_2-O-CO-CH_3$	$R_2 = -OCH_3$, $R_6 = -OCOCH_3$	36.1	0.15	0.0384
27	$R_1 = -CH_2-O-CO-CH_3$	$R_3 = -C_4H_9$	124.4	7.45	0.8911
28	$R_1 = -CH_2-O-CO-CH_3$	$R_4 = -C_4H_9$	128.9	18.12	0.8662
29*	$R_1 = -CH_2-O-CO-CH_3$		10.2	2.66	0.6363
30**	$R_1 = -CH_2-O-CO-CH_3$		48.7	11.68	0.9750
31†	$R_1 = -CH_3$		30.2	10.59	0.9187
32†	$R_1 = -CH_2OH$		8.9	32.49	0.9516
33†	$R_1 = -CH_2OH$	$R_1 = -OH$	−24.7	34.00	0.9909

* =  instead of benzene ring.

** = H instead of $-NO_2$ group.

† = saturated bond in the alkyl chain.

applied as hydrophobic agent for the impregnation. The compounds were dissolved in acetone at a concentration of 2 mg/cm^3; 5 mm^3 of each solution was spotted onto the plates. Methanol : water 1 : 1 (vol.) was applied as eluent containing various quantities of water soluble β-cyclodextrin polymer (SCDP). Because of the fairly low solubility of the β-cyclodextrin monomer in the eluent, its water soluble polymer (weight-average molecular weight: 4300 D; β-CD content: 64%; intrinsic viscosity: 5.7×10^{-3} l g^{-1}) was prepared by cross-linking with epichlorohydrin [10]. This polymer was soluble in the eluent. Methanol was chosen as the organic solvent miscible with water because it forms only a very weak inclusion complex with β-CD [11] and thus does not modify the character of the interaction between β-cyclodextrin polymer and nitrostyrene derivatives.

After development, the plates were dried at 105 °C and the compounds were detected by their UV absorption. The compounds were visible as dark spots on the fluorescent plate background under a CAMAG ultraviolet lamp at 254 nm. For each experiment five independent parallel determinations were carried out, the quantity of SCDP changed from 0.5 to 4.5 g at 0.5 g intervals for 30 cm^3 eluent. Linear correlations were calculated between the lipophilicity values ($100 \times R_M$) and the quantity of SCDP in the eluent for each compound:

$$100 \times R_M = a + b \times g_{\mathrm{SCDP}} \tag{1}$$

The '*b*' value (change of lipophilicity caused by unit change of concentration of SCDP in the eluent) was considered to be related to the complex stability. To evaluate the influence of various substituents Fujita–Ban analysis [12] was applied to the complex stability (*b*) values. Fujita–Ban analysis is a computer-assisted method to calculate the activity contributions of separate substituents considering the less substituted compound as reference. The method works provided that the substituents do not interact with each other and their activity contributions are independent of each other. Due to its different character, compound (**29**), has been omitted from the calculations.

3. Results and Discussion

The parameters of Equation 1 are compiled in Table I. In cases where the significance level of the linear correlation was below 95%, the compound was considered as not complexable with SCDP (i.e. **18**, **21**, **24** and **26**). The compound with the bromo substituents in the *para* position (**14**) formed a more stable complex with SCDP than the corresponding chloro derivative (**11**). In the *meta* position chloro substitution (**10**) increased the inclusion complex stability to a greater extent than did fluoro substitution (**8**); apparently the bulkier the halogen atom, the higher is the stability of the complex. Also the site of substitution considerably influences the complex formation. For chloro (**9**, **10** and **11**) and nitro substituents (**15**, **16** and **17**) the stability order is *para* > *ortho* > *meta*. For methyl (**18**, **19** and **20**) and methoxy groups (**21**, **22** and **23**) the order is changed to *para* > *meta* > *ortho*.

Since the dimensions of these four substituents do not differ greatly from each other [13] the phenomenon cannot be explained by simple steric reasoning; factors other than steric parameters must influence the complex stability.

In different positions the stability influencing the orders of substituents are fairly similar. For the *para* position:

$$n\text{-butyl} > \mathrm{Br} > \mathrm{Cl} > \mathrm{NO_2} > \mathrm{CH_3O} > \mathrm{CH_3}$$

For the *meta* position:

$$n\text{-butyl} > \mathrm{NO_2} > \mathrm{Cl} > \mathrm{F} > \mathrm{CH_3O} > \mathrm{CH_3}$$

For the *ortho* position:

$$NO_2 > Cl > CH_3O = CH_3$$

It is interesting to note that the differences between the effects of the same substituents (e.g. *n*-butyl and methyl in *para* and *meta* positions) in different positions are dissimilar (**19**, **20**, **27** and **28**). The differences are the greatest with the *para* position; that is the complex stability depends more strongly on substituents in the *para* than in *meta* and *ortho* positions.

Moreover not only the ring substituents (which are expected to be inserted into the cyclodextrin cavity) but the side chain substituents also markedly influence the complex stability. The presence of double bonds has a negligible effect on the complex formation (*cf.* **2** and **31**).

Compared to the unsubstituted side chain the small substituents (NO_2, CH_3, CH_2—O—CO—CH_3 etc.) decrease the complex stability. The bulkier CH_2—O—CO—$C_{11}H_{23}$ substituent gives a higher complex stability than the CH_2—O—CO—CH_3 group. Introduction of a second ring into the structure (**5**) greatly increases the complex stability. It is assumed that in such cases the side chain interacts with a second cyclodextrin cavity. The presence of hydroxyl groups enhances the complex formation (**32** and **33**). This effect is probably not due to its dimensions, but more probably to the formation of hydrogen bonds with the hydroxyl groups of the cyclodextrin.

The results of the Fujita–Ban analysis are shown in Table II. Only the substituents that exert the greatest effect on the complex stability have been listed. Among all the substituents on the benzene ring studied the *n*-butyl group gives the highest positive effect when in the *para* position. In the *meta* and *ortho* position some substituents (Cl, CH_3O and CH_3) considerably reduce the complex stability (as compared to the unsubstituted compound) these derivatives are probably too big to fit comfortably in the β-cyclodextrin cavity.

Table II. Results of Fujita–Ban analysis. Activity of unsubstituted compound: 23.03. $r = 0.9914$, $s = 1.54$, $F = 50.93$

Substituents			
Position	Type	Activity contribution	Standard deviation
	$-NO_2$	−5.95	1.60
R_1	$-CH_3$	−6.29	1.41
R_1	$-CH_2OH$	16.17	1.41
R_1	$-CH_2-O-CO-CH_3$	−11.35	0.99
R_1	$-CH_2-O-CO-C_2H_5$	−10.43	1.78
R_1	$-CH_2-O-CO-C_{11}H_{23}$	−5.60	1.78
R_2	$-Cl$	−4.79	1.60
R_2	$-CH_3$	−5.73	1.60
R_2	$-OCH_3$	−5.73	1.60
R_3	$-OCH_3$	−3.90	1.17
R_4	$-C_4H_9$	12.39	1.60

The substituents on the side chain exhibit the same (or slightly greater) effect on the complex formation as the substituents on the benzene ring. Most of them decrease the complex stability irrespective of their dimensions. The introduction of a polar hydroxyl group had the highest influence on the complex stability. The assumed hydrogen bond seems to be more important in the complex formation than the hydrophobic interactions within the β-cyclodextrin cavity.

It is generally accepted that besides steric factors hydrophobic interactions play an important role in the inclusion complex formation. Sometimes a good linear correlation was found between the lipophilicity and CD complex stability of compounds [7]. In our case no linear correlation was observed between the lipophilicity (*a* values of Table I) and complex stability (*b* values of Table I) of nitrostyryl derivatives, the regression coefficient was $r = 0.1596$. This finding supports our hypothesis concerning the importance of non hydrophobic interaction in the CD complex formation of nitrostyryl derivatives.

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