SYNTHESIS OF ARYLACETIC ACIDS AND THEIR EFFECT ON ACTIVATION OF FIBRINOLYSIS. QUANTITATIVE RELATIONS BETWEEN STRUCTURE AND BIOLOGICAL ACTIVITY

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A series of arylacetic acids, *III*, has been prepared and evaluated for activation of fibrinolysis and inhibition of heat-denaturation of serum albumin. Regression analysis revealed that either activity was influenced mainly by lipophilicity of the aromatic substituents. The graph showing activation of fibrinolysis by acids *III* takes a linear course up to a maximum, followed by a steep decrease. Also included in the series are *p*-benzyloxy derivatives of the arylacetic acids. Their lipophilicity has been evaluated by thin-layer chromatography, the method of reversed phase being employed. However, the lipophilicity parameters thus obtained fail to describe the hydrophobic bonding of these derivatives to the active site; tabulated values of the parameter π were better in this respect.

Series of β-aryl-n-butyric acids, *I* (refs[1,2]), and the isomeric α-methyl-β-arylpropionic acids, *II* (ref.[3]), were examined for their effect on activation of fibrinolysis. It appeared that in either series it was exclusively lipophilicity of the aromatic substituents that controlled the activation of fibrinolysis. The statistical significance of regression equation (*1*) for the acids *I* and Eq. (*2*) for the acids *II* could not be increased[4] by introducing parameters covering the polar or steric effects of the substituents.

	n	r	s	F	
$\log(1/C^F) = 0{\cdot}601\pi + 0{\cdot}939$	20	0·977	0·099	386	(*1*)
$\log(1/C^F) = 0{\cdot}664\pi + 0{\cdot}834$	10	0·989	0·121	134	(*2*)

In derivatives of the two series of acids the lipophilicity of the substituents did not exceed $\pi = 2{\cdot}4$. The limited solubility or insolubility of the more lipophile derivatives prevented an accurate determination of their fibrinolytic activity in the investigated concentration range. It is probable, however, that a marked decrease in activity would be observed beyond the above-given limit of liphophilicity.

In an attempt to assess the effect of lipophilicity on activation of fibrinolysis in the region of higher lipophilicity of the substituents we have now investigated this rela-

tion in a series of structurally similar arylacetic acids *III*, which were expected more soluble in the conditions of the test employed. Depending on the character of substitution on the aromatic ring, the acids *III* were synthetized by: *a*) the Wilgerodt reaction of substituted acetophenones (method *A*)[5], *b*) reaction of substituted benzyl chlorides with sodium cyanide, followed by hydrolysis of the formed nitriles of arylacetic acids (method *B*)[6], *c*) alkylation of methyl 4-hydroxyphenyl acetate or its 3-chloro derivative, followed by hydrolysis of the alkoxyaryl acetate (method *C*)[7].

Apart from the activation of fibrinolysis we have investigated inhibition of heat-denaturation of serum albumin, as a criterion of binding of the compounds to this protein. With the previous series of acids, *I* and *II*, it was found[2-4] that slopes of the lines relating this activity to lipophilicity (0·503 for acids *I* and 0·564 for *II*) were close to the values calculated for activation of fibrinolysis. In the series of acids *III* we also tested the 4-benzyloxy derivatives *IV*; their lipophilicity was described by the tabulated parameters π, as well as by data obtained from partition chromatography[8,9]. Inclusion of these derivatives in the regression analysis of the activities studied enabled us to get some insight into the nature of their binding to the active centre of the biomacromolecule.

EXPERIMENTAL

Methods

IR spectra of the acids *III* were measured in the region 400—4 000 cm^{-1} in 5% solutions in chloroform, a spectrometer UR-20 (Zeiss, Jena) being used. ^{1}H-NMR spectra were measured with a spectrometer BS 487 C—80 MHz (Tesla, ČSSR) in 6% solutions in deuteriochloroform, with tetramethylsilane as internal standard. Chromatographic behaviour of the acids *I* was examined in a thin layer of silica gel impregnated with 2·5% (w/w) of a silicone oil (in the form of a 5% solution in dioxan), with the use of 50% acetone as the mobile phase. Lipophilicity of the aromatic substituents is expressed by parameters π derived for arylacetic acids[10]. To calculate parameters π of the higher alkyls and alkoxy groups the following increments were used[11]: $\Delta\pi$ (CH_2 aliph.) = 0·5, $\Delta\pi$ (CH_2 ring) = 0·41, $\Delta\pi$ (branching) = —0·2, $\Delta\pi$ (double bond) = = —0·3. According to the original definition[10] the parameters π characterize changes in lipophilicity of monosubstituted derivatives, in which an atom of hydrogen is replaced by a substituent X. As was found by Janssen and Perrin[12], the lipophilicity of hydrogen must be taken into account in calculating $\sum\pi$ of polysubstituted derivatives. Therefore, the $\sum\pi$ of 3,4-disubstituted arylacetic acids were calculated as sums of π parameters of the two substituents from which 1/2 of the logarithm of the partition coefficient of hydrogen in the system n-octanol–water (0·23) was subtracted[13]: With substituents or their combinations where aberrations from the tabulated data could be expected as a result of intramolecular interactions these quantities were calculated from experimental R_M values, substituted into equation (*3*). This equation relates the parameters π and the experimental R_M values of acids *IIIa,b,d—h,j,k,m,q—y*

$$\pi = 3{\cdot}514\, R_M - 0{\cdot}787\, R_M^2 + 2{\cdot}322 \qquad \begin{matrix} n & r & s & F \\ 19 & 0{\cdot}998 & 0{\cdot}076 & 2254 \end{matrix} \qquad (3)$$

The regression coefficients were calculated from experimental data by multiple regression analysis. The statistical significance of the equations was assessed by means of the standard deviation s, correlation coefficient r and the Fischer–Snedecor criterion F. In all equations the level of statistical significance was $\alpha \leq 0.005$.

Biochemical Evaluation

Activation of fibrinolysis was evaluated[14] by the method of "hanging clot", prepared from human plasma and suspended in a solution of the compound to be tested[1]. The activity was expressed by the minimum molarity C^F capable of dissolving the clot after 24-hour incubation at 37°C. Inhibition of heat-denaturation of serum albumin was determined by Mizushima's method[15], described in ref.[1]. The activity was expressed by molarity C^I effecting a 50% inhibition.

Arylacetic Acids

Method A. Acids *IIIf,j,m,q,x* were obtained by the procedure[5] for synthesis of 4-isobutylphenylacetic acid. The crude products were purified by distillation (*IIIj*) or recrystallization. The following data designate respectively: number of the acid, substituent, m.p. (°C), solvent, reported m.p. (°C), yield (%): *IIIf*, 4-C_2H_5, 88—89·5, ethanol–water 1 : 1, rep.[16] m.p. 92, 69·6; *IIIi* 4-iso-C_3H_7, 47 (b.p. 113°C/8·0 Pa), rep.[16] m.p. 53·5, 44·6; *IIIl*, 4-iso-C_4H_9, 85—87, ethanol–water 3 : 2, rep.[5] m.p. 86·5—87·5, 55·7; *IIIp*, 4-cyclo-C_6H_{11}, 76—77, acetic acid–water 1 : 1, rep.[17] m.p. 78, 44·9; *IIIx* — see Table I.

Method B. Acids *IIIb,c,d,h,k* were prepared according to the following modification of a reported procedure[6]: a mixture of 0·05 mol of a substituted benzyl chloride and 0·05 mol of sodium cyanide in 25 ml of dimethyl sulphoxide was heated to 45—50°C for 5 h. It was then cooled down and poured into 200 ml of ice-cold water. The separated oil was taken into 200 ml of ether and the extract was washed with 50 ml of diluted hydrochloric acid (1 : 1) and two 50-ml portions of water. After drying with magnesium sulphate it was concentrated *in vacuo* and the residue was hydrolysed by boiling in a 10% solution of potassium hydroxide in aqueous ethanol. The hydrolysate was concentrated to half the volume, diluted with 50 ml of water, filtered with active carbon and acidified with sulphuric acid (1 : 1) to pH 2. The crude product was purified by crystallization. The following data designate respectively: number of the acid, substituent, m.p. (°C), solvent, reported m.p. (°C), yield (%): *IIIb*, 4-CH_3O, 87, methanol–water 1 : 2, rep.[18] m.p. 86, 75·0; *IIIc*, 3-Cl-4-CH_3O, 94·5—95·5, methanol–water 3 : 1, rep.[19] m.p. 95—96, 61·4; *IIId*, 4-Cl, 106, methanol–water 1 : 1, rep.[20] m.p. 104—106, 78·6; *IIIk*, 4-tert-C_4H_9, 77·5—78·5, methanol–water 1 : 2, rep.[16] m.p. 81·5, 75·5; *IIIh* — see Table I.

Method C adhered to a reported procedure[7]. In addition to the acids given in Table I the following ones were prepared by this method (number, substituent, m.p. (°C), solvent, reported m.p. (°C), yield (%)): *IIIe*, 4-iso-C_3H_7O, 61—63, methanol–water 1 : 2, rep.[21] m.p. 57—59, 88·7; *IIIg*, 3-Cl-4-$CH_2{=}CHCH_2O$, 89—90·5, methanol–water 1 : 1, rep.[6] m.p. 92—93, 78·2; *IIIo*, 4-n-$C_5H_{11}O$, 78—79, benzene–light petroleum 1 : 2, rep.[21] m.p. 71—72, 83·3. To prepare the derivative *IIIs* the method was modified by effecting the alkylation in isopropyl alcohol in the presence of sodium isopropylate; the mixture was kept boiling under a reflux condenser for 16 h.

4-Benzyloxyarylacetic Acids *IVa—e*

These were prepared by a reported procedure[7]. Their melting points agreed with the reported ones[7,22–24].

TABLE I
Characterization of Arylacetic Acids *III*

Number X	Method yield, %[a]	M.p., °C solvent % of methanol	Calculated/Found % C	% H	% Cl
IIIh	*B*	90—91	57·78	5·73	15·51
3-Cl-4-iso-C_3H_7O	63·2	50	57·58	5·65	15·67
IIIi	*C*	61·5—62·5	57·78	5·73	15·51
3-Cl-4-n-C_3H_7O	67·2	50	57·70	5·60	15·49
IIIl	*C*	81—82	59·38	6·23	14·61
3-Cl-4-iso-C_4H_9O	44·5	50	59·38	6·32	14·58
IIIn	*C*	98—100	59·38	6·23	14·61
3-Cl-4-n-C_4H_9O	68·3	50	59·08	6·43	14·55
IIIp	*C*	46—48	60·58	7·04	14·76
3-Cl-4-n-$C_5H_{11}O$	59·0	75	60·83	6·78	13·77
IIIr	*C*	92—94	72·55	8·12	—
4-cyclo-$C_6H_{11}CH_2O$	44·3	65	72·85	8·20	—
IIIs	*C*	103—105	62·57	6·38	13·19
3-Cl-4-cyclo-$C_6H_{11}O$	25·3	60	62·57	6·44	13·43
IIIt	*C*	77—78·5	71·08	8·52	—
4-n-$C_6H_{13}O$	49·8	65	70·84	8·78	—
IIIu	*C*	88—90	63·71	6·77	12·54
3-Cl-4-cyclo-$C_6H_{11}CH_2O$	55·8	50	63·89	6·81	12·70
IIIv	*C*	47—48	62·10	7·07	13·09
3-Cl-4-n-$C_6H_{13}O$	53·5	75	61·89	7·08	13·18
IIIw	*C*	55—56	63·26	7·43	12·45
3-Cl-4-n-$C_7H_{15}O$	50·5	75	63·63	7·38	12·55
IIIx	*A*	—	—	—	—
4-2′-ethylhexyl[b]	36·5				
IIIy	*C*	49—51	64·31	7·76	11·86
3-Cl-4-n-$C_8H_{17}O$	25·4	80	64·62	7·58	11·51
IIIz	*C*	116—118	69·03	7·97	—
3-CH_3O-4-cyclo-$C_6H_{11}CH_2O$	66·4	60	68·93	8·09	—

[a] With method *A* the yield is based on the starting substituted acetophenone, with method *B* on the starting substituted benzyl chloride and with method *C* on the starting methyl 4-hydroxyarylacetate; [b] the oil was identified as cyclohexylammonium salt, m.p. 131—133°C, for $C_{22}H_{37}.NO_2$ (347·5) calculated: 76·03% C, 10·73% H, 4·03% N; found: 76·15% C, 10·81% H, 4·18% N.

RESULTS AND DISCUSSION

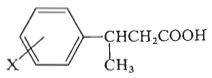
The experimental results showing activation of fibrinolysis and inhibition of denaturation of serum albumin in the individual arylacetic acids are given in Table II. Like with the acids of groups *I* and *II* we examined, by regression analysis, the relations of these activities to the physico-chemical properties of the acids. This involved solutions of equation (*4*) for activation of fibrinolysis and equation (*5*) for inhibition of denaturation of serum albumin. The benzyloxy derivatives *IV* were not included in the regression analysis.

	n	*s*	*r*	*F*	
$\log(1/C^F) = 0{\cdot}636\pi + 0{\cdot}645$	20	0·974	0·108	331	(*4*)
$\log(1/C^I) = 0{\cdot}544\pi + 2{\cdot}710$	22	0·968	0·129	296	(*5*)

In neither case was the statistical significance increased by introduction of the polar constants of substituents on the aromatic ring. Extension of equation (*4*) by π^2 has proved statistically insignificant. The dependence of inhibition of denaturation of serum albumin on lipophilicity was better illustrated by a parabola described by equation (*6*):

	n	*s*	*r*	*F*	
$\log(1/C^I) = 0{\cdot}940\pi - 0{\cdot}114\pi^2 + 2{\cdot}454$	22	0·988	0·082	383	(*6*)

However, interpretation of this parabolic dependence is rather difficult, since only derivative *IIIw*, of maximal lipophilicity, was at variance with the linear equation (*5*). Unfortunately the even more lipophilic derivatives *IIIx*, *y* were not soluble in the conditions of the test, so that further course of this dependence could not be determined. Comparison of equations (*4*) and (*5*) reveals that the slopes of the lines, relating activity to lipophilicity, are very similar. They are also comparable to those calculated for acids of the series *I* and *II* (see equations (*1*) and (*2*)). We believe that the acids *I*, *II* and *III* are similar in character of their hydrophobic bonding to serum albumin and to the biopolymer at the site of activation of fibrinolysis. As the values of these slopes are close to 0·5 it appears that this site is located on the surface of the biomacromolecule[25,26].



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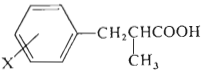

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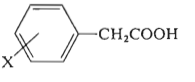


III

TABLE II

Chromatographic Behaviour and Biological Properties of Arylacetic Acids *III*

Number	X	π_{tab}	R_M	C_F [a]	$\log(1/C^F)_{exp}$	$\log(1/C^F)_{calc}$ [b]	C_I [c]	$\log(1/C^I)_{exp}$	$\log(1/C^I)_{calc}$ [d]
IIIa	H	0	−0·59	>100[e]	<1·000[f]	—	—	—	—
IIIb	4-CH_3O	0·01	−0·59	>100[e]	<1·000[f]	—	300	2·523	2·463
IIIc	3-Cl-4-CH_3O	0·46	—	100	1·000	0·937	110	2·960	2·862
IIId	4-Cl	0·70	−0·42	>100[e]	<1·000[f]	—	100	3·000	3·056
IIIe	4-iso-C_3H_7O	0·81	−0·38	100	1·000	1·160	88·0	3·056	3·140
IIIf	4-C_2H_5	0·90	−0·37	60	1·222	1·217	88·0	3·056	3·208
IIIg	3-Cl-4-CH_2=$CHCH_2O$	1·16	−0·32	40	1·398	1·382	42·0	3·377	3·391
IIIh	3-Cl-4-iso-C_3H_7O	1·26	−0·25	40	1·398	1·446	31·5	3·511	3·457
IIIi	3-Cl-4-n-C_3H_7O	1·46	—	30	1·523	1·573	21·0	3·678	3·583
IIIj	4-iso-C_3H_7	1·40	−0·26	32·5	1·488	1·535	32·5	3·488	3·546
IIIk	4-tert-C_4H_9	1·68	−0·16	20	1·699	1·713	21·0	3·678	3·711
IIIl	3-Cl-4-iso-C_4H_9O	1·76	—	10	2·000	1·764	13·2	3·879	3·755
IIIm	4-iso-C_4H_9	1·90	−0·12	20	1·669	1·853	19·8	3·724	3·828
IIIn	3-Cl-4-n-C_4H_9O	1·96	—	10	2·000	1·891	—	—	—
IIIo	4-n-$C_5H_{11}O$	2·01	—	15	1·824	1·923	11·8	3·928	3·882
IIIp	3-Cl-4-n-$C_5H_{11}O$	2·46	—	5	2·301	2·209	7·3	4·137	4·076
IIIq	4-cyclo-C_6H_{11}	2·46	0	5	2·301	2·209	7·5	4·125	4·076
IIIr	4-cyclo-$C_6H_{11}CH_2O$	2·47	0·07	5	2·301	2·216	9·0	4·046	4·080
IIIs	3-Cl-4-cyclo-$C_6H_{11}P$	2·51	0·02	6	2·222	2·235	7·4	4·131	4·091
IIIt	4-n-$C_6H_{13}O$	2·51	0·07	6	2·222	2·235	9·3	4·034	4·094
IIIu	3-Cl-4-cyclo-$C_6H_{11}CH_2O$	2·92	0·18	4	2·398	2·502	4·5	4·347	4·226
IIIv	3-Cl-4-n-$C_6H_{13}O$	2·96	0·18	4	2·398	2·527	6·3	4·201	4·236
IIIw	3-Cl-4-n-$C_7H_{15}O$	3·46	0·36	>100[e]	<1·000[f]	—	5·5	4·260	4·340
IIIx	4-2′-ethylhexyl[h]	3·90	0·47	> 10[e]	<2·000[f]	—	—[g]	—	—
IIIy	3-Cl-4-n-$C_8H_{17}O$	3·96	0·56	>100[e]	<1·000[f]	—	—[g]	—	—
IIIz	3-CH_3O-4-cyclo-$C_6H_{11}CH_2O$	1·91[i]	−0·10	10	2·000	1·860	15·8	3·801	3·833

[a] Concentration in M . 1^{-1} . 10^3; [b] values calculated from equation (*4*); [c] concentration in M . 1^{-1} . 10^5; [d] values calculated from equation (*6*); [e] insoluble at higher concentrations; [f] these compounds are not included in the regression analysis; [g] insoluble in the conditions of the test; [h] the biologic tests of the cyclohexylammonium salt gave the same results; [i] calculated by substitution for R_M in equation (*3*).

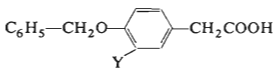
The two activities were also determined with the benzyloxy derivatives *IVa–e*. Their lipophilicities can be characterized either by the tabulated parameters π_{tab}, or by values of π_{calc}, calculated from equation (*3*) by substitution of experimental R_M values (Table III) from partition chromatography. These values are generally lower than the tabulated ones, which is consistent with the determined lipophilicities[27,28] of compounds with sufficiently flexible structures, containing two or more aromatic rings. The lower lipophilicities (than the expected values) can be ascribed[27] to the so-called intramolecular hydrophobic interaction of the two aromatic rings, which reduces the hydrophobic surface of the molecule. Consequently, a prerequisite for this interaction is proximity of the two aromatic rings. By substitution of the π_{tab} values and the values of π_{calc} calculated from equation (*3*) into the regression equations (*4*) and (*6*) we obtained the corresponding activities of the benzyloxy derivatives (Table III). Comparison with the respective experimental values reveals that use of π_{tab} as a measure of lipophilicity of the substituents generally leads to a better accordance of the calculated and the experimentally determined activities. It is probable that in the two studied linkages to the biomacromolecules no hydrophobic interaction of the aromatic rings can occur. This may be due to binding of the benzyloxy derivatives to the surface of the biomacromolecules, in which the planar conformation precludes mutual approach of the two aromatic rings. With the 3-methoxy-4-benzyloxy derivative *IVd* we used $\sum\pi_{tab}$ after subtraction of $\Delta\pi = 0{\cdot}37$, as corresponds to the *ortho*-effect in the dialkoxy derivative *IIIz*. Even with the planar conformation of the aromatic rings the *ortho*-effect of the two alkoxy groups in the acid *IVd* can be operative. Inclusion of the benzyloxy derivatives *IV* into the regression analysis led to equation (*7*) for activation of fibrinolysis and to equations (*8*) and (*9*) for inhibition of denaturation of the albumin:

	n	*r*	*s*	*F*	
$\log(1/C^F) = 0{\cdot}654\pi + 0{\cdot}636$	25	0·969	0·113	359	(*7*)
$\log(1/C^I) = 0{\cdot}547\pi + 2{\cdot}710$	26	0·969	0·119	367	(*8*)
$\log(1/C^I) = 0{\cdot}924\pi - 0{\cdot}109\pi^2 + 2{\cdot}458$	26	0·988	0·077	455	(*9*)

As some of the more lipophilic derivatives were soluble in determining the activation of fibrinolysis we were able to assess the fibrinolytic activity of acids *IIIw* and *IIIy* throughout the concentration range investigated. The two acids, characterized by lipophilicity of aromatic substituents $\sum\pi = 3{\cdot}46$ and 3·96 respectively, had no activating effect whatever and their $\log(1/C^F) < 1{\cdot}000$. Out of the whole series of arylacetic acids the most efficacious ones were *IIIu* and *IIIv*, whose lipophilicity differed from that of *IIIw* by only $\Delta\pi = 0{\cdot}5$. The marked decrease of fibrinolytic activity in this narrow range of lipophilicity practically rules out any parabolic dependence

TABLE III

Chromatographic and Biological Properties of 4-Benzyloxyarylacetic Acids *IV*



Number Y	R_F R_M	π_{tab} π_{calc} [a]	Activation of fibrinolysis			Inhibition of denaturation of serum albumin		
			C^F M . 1^{-1} . 10^3	$\log(1/C^F)_{exp}$	$\log(1/C^F)_{calc}$ [b]	C^I M . 1^{-1} . 10^5	$\log(1/C^I)_{exp}$	$\log(1/C^I)_{calc}$ [c]
IVa	0·65	1·91	9·5	2·022	1·885	17	3·770	3·833
H	—0·29	1·24			1·434			3·444
IVb	0·58	2·36	5	2·301	2·146	9·6	4·018	4·037
Cl	—0·14	1·81			1·796			3·782
IVc	0·67	1·35 [d]	30	1·523	1·504	36·5	3·438	3·460
CH_3O	—0·32	1·10			1·348			3·350
IVd	0·58	2·17	6	2·222	2·025	11·5	3·957	3·957
CH_3	—0·14	1·81			1·796			3·782
IVe	[e]	2·58	4	2·398	2·296	[f]	—	—
Br								

[a] Calculated from R_M values, substituted into equation (*3*); [b] calculated from equation (*4*) by substituting for π_{tab} (upper values) and π_{calc} (lower values); [c] calculated from equation (*6*) by substituting for π_{tab} (upper values) and π_{calc} (lower values); [d] $\Delta\pi = 0{\cdot}37$, corresponding to the decrease in lipophilicity due to the *ortho*-effect in the acid *IIIz*, was subtracted from $\sum\pi$; [e] the acid was not chromatographically tested; [f] the acid was insoluble in the conditions of the test.

of this activity on lipophilicity. It can be inferred that in the given series of arylacetic acids, like in the preceding series *I* and *II*, a maximum of fibrinolytic activity is followed by its steep decrease with further increase in lipophilicity. In all probability, the cationic centre of the active site, to which a carboxylate anion gets bound, is surrounded by a hydrophobic region with a limited binding capacity.

The elemental analyses were carried out at the Microanalytical Department of the Institute (head Dr J. Körbl), the IR *spectra were measured by Mrs P. Vejdělková under the direction of Dr B. Kakáč, the* ^{1}H-NMR *spectra by Dr J. Holubek. Thin-layer chromatography was performed by Mrs M. Jelínková under the direction of Dr V. Rábek.*

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