THE SYNTHESIS OF ARYLPROPIONIC ACIDS AND THE QUANTITATIVE RELATIONSHIP BETWEEN THE STRUCTURE AND THE ACTIVATION OF FIBRINOLYSIS

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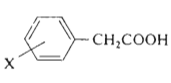
A number of substituted 2-arylpropionic (*IV*) and 3-arylpropionic (*V*) acids were prepared and their activity in the activation of fibrinolysis and the inhibition of heat denaturation of serum albumin was evaluated. The results were worked up using the method of regression analysis. From the regression equations obtained it may be considered that both activities are affected mainly by the lipophilicity of the aromatic substituents. The effect of the branching in the connecting chain between the carboxyl group and the aromatic ring is negligible in both activities. The linear dependence of the fibrinolytic capacity on lipophilicty is in both series of acids, *IV* and *V*, characterized by a distinct decrease in activity, following the attainment of the optimum value of lipophilicity. In the series of cinnamic acids (*VI*) regression equations concerning the inhibition of the denaturation of serum albumin and the activation of fibrinolysis were also calculated, showing a linear dependence of these activities on the lipophilicity of the varying substituents R and X. Summary regression equations were derived for both activities in the whole set of acids *I*—*VI*. Both the inhibition of the denaturation of serum albumin, and the activation of fibrinolysis depends on the lipophilicity of the mentioned acids exclusively. The modification of the connecting chain between the carboxyl group and the aromatic ring affects both activities primarily by the corresponding change in lipophilicity.

Considerable attention is devoted[1,2] to various types of organic acids from the point of view of their ability to increase endogenous fibrinolytic activity in plasma *in vitro*. The studies in this field represent one of the trends of development of thrombolytic active substances. A number of antiinflamatory substances of acidic nature also activates fibrinolysis in systems *in vitro*. For example derivatives of salicylic acid[3,4], anthranilic acid[5], 3-indolylacetic acid[6] and 1,2-diphenyl-3,5-dioxopyrazolidine[6,7] were also investigated and among the substances of the mentioned type very active fibrinolysis activators were found. Therefore, in connection with the synthesis of antiinflamatory arylaliphatic acids, we focussed our attention on the evaluation of their fibrinolytic activity as well. The experimental results were worked up using the method of regression analysis, with the aim of finding quantitative relationships[8] between the activation of fibrinolysis and the physico-chemical properties of the acids studied. These relationships not only permit the determination of optimal

physico-chemical parameters in the series of acids studied, but they also may serve as a valuable tool in the interpretation of the mechanism of activation of fibrinolysis.

During the study[9-11] of the effect of 3-arylbutyric acids (*I*) and 2-methyl-3-arylpropionic acids (*II*) on the activation of fibrinolysis we found on the basis of the regression equations obtained that this activity is in both series affected by the lipophilicity of the aromatic substituents exclusively. The fibrinolytic capacity increases linearly with lipophilicity up to the value $\sum\pi$ about 2·4. Further increase of lipophilicity brought about a decrease in the solubility of these acids under the conditions of the test to such an extent that it was no longer possible to determine their fibrinolytic capacity accurately. From the measurements in the region of lower concentrations it may be judged, however, that beyond the limit of lipophilicity mentioned a clear decrease in the activation of fibrinolysis takes place. In the series of more soluble arylacetic acids *III* even considerably lipophilic derivatives could be evaluated[12], in which the lipophilicity of the aromatic substituents attained the value $\sum\pi = 3{\cdot}96$. It became evident that after the attainment of the optimum a distinct decrease of the fibrinolytic activity takes place with a further increase in lipophilicity. This decrease within the narrow limits of lipophilicity $\Delta\pi = 0{\cdot}5$ practically excludes a parabolic dependence of this activity on lipophilicity. Now we have determined fibrinolytic capacity in the series of 2-arylpropionic (*IV*) and 3-arylpropionic (*V*) acids; we used again the method of the "hanging clot"[13] in the *in vitro* system. Regression analysis showed in both series of acids *IV* and *V* the effect of physico-chemical parameters on the activation of fibrinolysis. We also submitted to regression analysis the results of the inhibition of the denaturation of serum albumin[14]



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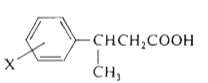

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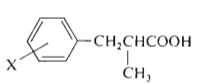

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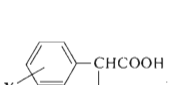

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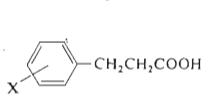

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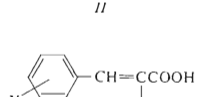
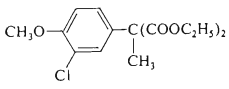


VI

which may be considered as a criterion of the binding of acids *IV* and *V* on this protein. The dependences of both activities on the physico-chemical properties were also calculated in the series of acids *VI*. From the data for acids *VI* described earlier[15,16] we included into our regression analysis those for which both the activation of fibrinolysis and the inhibition of denaturation of serum albumin could be determined. In view of the similarity of the relationships of both activities to the physico-

-chemical properties of the acids studied, summary regression equations were calculated for both activities in all the series of arylaliphatic acids *I*–*VI* prepared so far.

The acids *IV* were obtained from the substituted acetophenones by the rhodanine synthesis[17,18] (method *A1*), or from the corresponding arylacetic acids which were converted with diethyl carbonate to corresponding malonates, which then afford the required acids *IV* by methylation, hydrolysis and decarboxylation[19] (method *A2*). Using the reaction of substituted benzyl chlorides with ethyl malonate, hydrolysis and decarboxylation (method *B1*) we prepared the acids *V* which were also obtained by hydrogenation of corresponding cinnamic acids (method *B2*). Alkoxy derivatives of acids *IV* and *V* were prepared on alkylation of methyl esters of corresponding 4-hydroxy derivatives (methods *A3*, *B3*) by a similar method[20] as in the series of arylacetic acids. Methyl esters of 4-hydroxyphenylpropionic acids *IX* and *XI* were prepared by demethylation of corresponding methoxyphenylpropionic acids *IVb* and *Va* and subsequent esterification with methanol in the presence of hydrogen chloride. Methyl esters of 3-chloro-4-hydroxyphenylpropionic acids *X* and *XII* were obtained on demethylation and simultaneous hydrolysis of corresponding malonates *VII* and *VIII* and further decarboxylation and esterification. Acids *IV* and *V* were identified by elemental analysis, IR and ^{1}H-NMR spectra which agreed with the assumed structure in all instances. For the preparation of cinnamic acids *VI* we applied Wittig's reaction the performance of which and the physico-chemical data of the acids prepared and evaluated are presented elsewhere[15,16].



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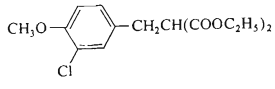

VIII

EXPERIMENTAL

Methods

The IR spectra of acids *IV* and *V* were measured in the 400—4000 cm^{-1} region using 5% solutions in chloroform and a UR-20 (Zeiss, Jena) spectrometer. The ^{1}H-NMR spectra were measured on a BS 487c—80 MHz spectrometer (Tesla, Czechoslovakia), in a 6% solution in deuteriochloroform, with tetramethylsilane as internal reference. The p*K* values of acids *IV* and *V* were determined at 25°C in 80% methylcellosolve with a potentiometer Titrigraph Radiometer SBR-2c (Copenhagen, Denmark). The evaluation of esters *X*—*XII* and ethyl 2-aryl-2-methylmalonates (method *A2*) by gas chromatography was carried out on Fractometer chromatograph (Perkin–Elmer F7), using a stainless-steel column (diameter 3 mm, length 2 m), packed with Gas-Chrom Q (125—150 μm), wetted with 3% polyethylene glycol (mol. mass about 20000).

For the expression of the lipophilicity of aromatic substituents in the series of acids *IV* and *V* parameters π were used[21], derived for arylacetic acids, while in the series of acids *VI* the parameters derived for benzoic acids were employed. For the calculation of the parameters π of higher alkyls and alkoxy groups the following increments were used[22]: $\Delta\pi$ (CH_2 aliphatic) = 0·5; $\Delta\pi$ (CH_2 cyclic) = 0·41; $\Delta\pi$ (branching) = —0·2. For the calculation of $\Sigma\pi$ of 3,4-disubstituted derivatives the difference[23] between the lipophilicity of the aromatic fragments $C_6H_4<$ and $C_6H_3<$ was taken into consideration. This value ($\Delta\pi$ = —0·23), which is in agreement with the lipophilicity of hydrogen[24], was subtracted from the sum of parameters of both substituents. For 3,4-dialkoxy derivatives *Vb* and *Vf* in which deviation from the tabulated parameters π occur in consequence of intramolecular interactions $\Sigma\pi$ values were employed which were obtained[25] from partition chromatography of 2-methyl-3-arylpropionic acids. The logarithms of partition coefficients log *P* of acids *I*—*VI* were calculated according to equation (*1*):

$$\log P = \log P_H + \Sigma\pi \,, \qquad (1)$$

where P_H are the partition coefficients of unsubstituted acids *I*—*VI* the experimental values of which were taken from ref.[22].

For the evaluation of electronic effects of substituents polar constants σ were made use of, mostly taken over from refs[26,27]. For the substituents 4-iso-C_3H_7O, 4-n-$C_6H_{13}O$, 4-cyclo-$C_6H_{11}CH_2O$ and 4-$C_6H_5CH_2O$ these values were calculated[28] from the relationships p*K*–σ in the series of cinnamic and arylacetic acids. The utilizability of the values σ obtained in this manner was checked by putting them into the regression relationship between the p*K* values of acids *IV* and the polar constants σ, expressed by the equation (*2*).

	n	*r*	*s*	*F*	
$pK = -0{\cdot}767\,\Sigma\sigma + 6{\cdot}837$	15	0·997	0·012	1948	(*2*)

In the series of acids *V* the similar relationship is expressed by equation (*3*).

	n	*r*	*s*	*F*	
$pK = -0{\cdot}294\,\Sigma\sigma + 6{\cdot}911$	15	0·939	0·017	97	(*3*)

The regression coefficients were calculated from the experimental data by multiple regression analysis. The statistical significance was evaluated by the standard deviation *s*, correlation coefficient *r* and Fischer–Snedecor's criterion *F*. Individual parameters in multiparameter relationships were evaluated statistically using Student's t-test at maximum level of statistical significance $\alpha \leqq 0{\cdot}001$.

Biochemical Evaluation

The activation of fibrinolysis[13] was estimated using the method of "hanging clot", prepared from human plasma suspended in a solution of the compound tested[9]. The activity was expressed as minimum molar concentration C^F which dissolves the coagulum after 24 h incubation at 37°C. The inhibition of the denaturation of serum albumin is described in ref.[18]. The activity was expressed as molar concentration C^I causing 50% inhibition.

2-Arylpropionic Acids *IV*

Method A1: using this method we already prepared acids *IVa*, *b*, *d*, *e*, *f*, *i*; the reaction procedure and the physico-chemical characteristics of the mentioned acids are described in ref.[18].

Method A2: this is analogous to the procedure described in ref.[19] for the preparation of acids *IV*. As intermediates 2-aryl-2-methylmalonates were isolated which were characterized by ^{1}H-NMR spectra and the purity of which was checked by gas chromatography. Using this method we synthetized the following acids (number, substituent X, m.p. in °C after crystallization from benzene–light petroleum 1 : 1, m.p. °C from literature): *IVh*, 3-C_6H_5CO, 92—94, lit.[29] 94; *IVj*, 4-C_6H_5O, 68—69, lit.[30] 69—70; *IVk*, 4-iso-C_4H_9, 75—76, lit.[19] 75—77; *IVm*, 4-iso-C_5H_{11}, 125—127°C/66 Pa, lit.[30] 139—140/106 Pa; and acid *IVn* given in Table I.

Method A3: 8·1 g (0·045 mol) of *IVb* were refluxed with 45 ml of 52% hydrobromic acid for 15 h. After cooling to —5°C the precipitate separated was filtered off under suction and washed with 1,2-dichloroethane. Yield, 5·5 g of crude 2-(4-hydroxyphenyl)propionic acid, m.p. 128—129°C (lit.[31] gives m.p. 129°C). The crude product was refluxed in 50 ml of 10% methanolic hydrogen chloride for 5 h. After evaporation the residue was mixed with 50 ml of water and 200 ml of ether. The ethereal layer was separated, dried over magnesium sulphate and evaporated to yield 6·0 g of methyl 2-(4-hydroxyphenyl)propionate (*IX*).

30·3 g (0·1 mol) of malonate *VII* (prepared from ethyl 3-chloro-4-methoxyphenylacetate according to ref.[19]) was refluxed with 50 ml of 52% hydrobromic acid for 12 h under addition after 4 and 8 h of an additional 25 ml of hydrobromic acid. The mixture was poured into 500 g of ice and the precipitated oil was extracted with three 100 ml portions of ether. From the ethe-

TABLE I
Characterization of 2-Arylpropionic Acid *IV*

Number	Method yield, %[a]	B.p. (m.p.) °C/kPa	Calculated/Found		
			% C	% H	% Cl
IVg	A3	134—136/0·05	59·38	6·23	14·61
	53·8		59·64	5·95	14·50
IVl	A3	134—136/0·04	71·15	8·53	—
	38·6		71·38	8·71	
IVn	A2	85—86[b]	77·55	8·68	—
	16·0		77·29	8·93	
IVo	A3	167/0·12	62·10	7·07	13·10
	55·7		62·45	6·83	12·94
IVp	A3	72—74[c]	64·75	7·13	11·95
	45·0		64·83	7·31	11·84
IVr	A3	164—166/0·12	63·26	7·43	12·45
	55·0		63·07	7·23	12·30

[a] In the case of method A2 the yield was calculated with reference to 4-cyclohexylphenylacetic acetic acid as starting compound, in the case of method A3 per the starting ester *IX* or *X*, respectively; [b] crystallized from 60% methanol, lit.[2]; gives m.p. 110—112°C; [c] crystallized from 50% acetic acid.

real solution the product was extracted with 5% NaOH (2 × 500 ml) and the alkaline solution was filtered with charcoal and acidified with 10% H_2SO_4. The separated oil was extracted with ether and the extract dried and concentrated to afford 2-(3-chloro-4-hydroxyphenyl)-2-methylmalonic acid (oil, 21·5 g) which was decarboxylated by heating at 200°C for 15 min. The 2-(3-chloro-4-hydroxyphenyl)propionic acid was purified *via* its sodium salt and isolated in the form of an oil (17·5 g). It was identified on the basis of its ^{1}H-NMR spectrum. Its esterification gave methyl 2-(3-chloro-4-hydroxyphenyl)propionate (*X*, 12·5 g) which was isolated in pure state by distillation, b.p. 88—90°C/16 Pa. It was identified by ^{1}H-NMR spectra and its purity (97·6%) was determined by gas chromatography.

Alkylation of esters *IX* and *X* and subsequent hydrolysis according to ref.[20] gave acids *IVg,l,o,p,r* the physical-chemical constants of which are presented in Table I.

3-Arylpropionic Acids *V*

Method B1: This represents an analogy of the procedure used[10] for the preparation of 2-methyl-3-arylpropionic acids *II*, with the difference that ethyl 2-ethoxycarbonyl-3-arylpropionate

TABLE II

Characterization of 3-Arylpropionic Acids *V*

Number	Method yield, %[a]	M.p., °C solvent[b]	Calculated/Found		
			% C	% H	% Cl
Vb	B2	64—65	65·48	7·60	—
	81·0	25% M	65·38	7·85	
Vc	B3	75—76	69·20	7·75	—
	56·6	B-P 1 : 1	59·29	8·04	
Vd	B3	44—45	59·39	6·23	14·61
	63·4	60% M	59·56	6·37	14·91
Vf	B2	72—74[c]	64·40	8·78	—
	71·7	60% M	64·44	9·05	
Vg	B2	60—62	75·68	8·78	—
	72·3	50% M	75·77	8·71	
Vi	B3	118—119	66·09	5·20	12·20
	49·6	B-P 1 : 1	66·21	5·33	11·98
Vl	B3	123—125	64·75	7·13	11·94
	41·6	70% M	65·11	7·11	12·21
Vm	B3	52—54	63·26	7·43	12·45
	36·0	75% M	63·49	7·42	12·57

[a] For method B2 the yield was calculated with reference to the starting ester of cinnamic acid, in method B2 per starting ester *XI* or *XII*, respectively; [b] solvents used: M methanol, B benzene, P light petroleum; [c] crystallizes as monohydrate.

obtained on reaction of substituted benzyl chloride with ethyl malonate was hydrolyzed and decarboxylated. Using this method we prepared the following acids (number, substituent X, yield in % referred to the starting substituted benzyl chloride, m.p. in °C, solvent, m.p. from literature in °C): *Ve*, 4-iso-C_3H_7, 48·5, 73—74, 50% methanol, lit.[31,32] 74—75; *Vj*, 4 cyclo-C_6H_{11}, 32·4, 123—125, 60% methanol, lit.[33] 125.

Method B2: hydrogenation of cinnamic acids was carried out according to ref.[10]. Acid *Va* was prepared in this manner in a 61·2% yield (m.p. 101—102°C from 20% methanol, ref.[34] gives m.p. 107°C), as well as the acids *Vb,f,g* (Table II).

Method B3: demethylation of acid *Va* (in the same manner as *IVb* in method A3) gave 3-(4-hydroxyphenyl)propionic acid with m.p. 124—126°C (lit.[35] gives m.p. 127—128°C) the esterification of which by boiling with 10% methanolic hydrogen chloride gave methyl 3-(hydroxyphenyl)propionate (*XI*). It was isolated by distillation. b.p. 90°C/8·5 Pa, and identified by ^{1}H-NMR spectrometry. Its purity (99·8%) was checked by gas chromatography.

30·3 g (0·1 mol) of malonate *VIII* (prepared according to ref.[10] by condensation of 3-chloro-4-methoxybenzyl chloride with ethyl malonate) were worked up in the same manner as in the preparation of ester *X* from malonate *VII*. 3-(3-Chloro-4-hydroxyphenyl)propionic acid with m.p. 76—78°C was isolated as intermediate and it was characterized by its ^{1}H-NMR spectrum. When esterified with methanol methyl 3-(3-chloro-4-hydroxyphenyl)propionate was obtained (*XII*, 12·2 g), b.p. 99—102°C/40 Pa and m.p. 59—62°C, the purity of which (100%) was tested by gas chromatography.

Alkylation of esters *XI* and *XII* and subsequent hydrolysis (using the procedure described in ref.[20]) afforded the following acids (number, substituent X, yield in % per starting ester *XI*, m.p. in °C, lit. m.p. in °C): *Vh*, 4-$C_6H_5CH_2O$, 65·7, 120—121, benzene–light petroleum 1 : 1, lit.[36] 123—124; *Vk*, 4-n-$C_6H_{13}O$, 59·0, 74—75, light petroleum, lit.[37] 73—74; and acids *Vc.d,i,l,m* (Table II).

RESULTS AND DISCUSSION

The experimental results of the activation of fibrinolysis and the inhibition of the denaturation of serum albumin in the series of 2-arylpropionic acids *IV* are surveyed in Table III. Their processing by regression analysis gave equations (*4*) and (*5*) which express the relationship of both activities to lipophilic parameters π of aromatic substituents.

	n	*r*	*s*	*F*	
$\log(1/C^F) = 0{\cdot}603\sum\pi + 0{\cdot}906$	13	0·991	0·066	613	(*4*)
$\log(1/C^I) = 0{\cdot}486\sum\pi + 2{\cdot}984$	15	0·992	0·061	766	(*5*)

4-Phenoxy derivative *IVj* deviates distinctly from the regression equations (*4*) and (*5*): it is conspicuously more active in the activation of fibrinolysis than would correspond to its lipophilicity. In both activities the statistical significance of the regression equations does not increase by introduction of polar constants σ of substituents X. We arrived at the same result in the series of 3-arylpropionic acids as well (Table IV), as evident from the regression equations (*6*) and (*7*)

TABLE III

Physico-chemical and Biological Properties of α-Arylpropionic Acids *IV*

Number X	pK	Σσ Σπ	Activation of fibrinolysis C^F mol l^{-1} . 10^3	Activation of fibrinolysis $\log(1/C^F)_{exp}$ $\log(1/C^F)_{calc}$ [a]	Inhibition of the denaturation of BSA C^I mol l^{-1} . 10^5	Inhibition of the denaturation of BSA $\log(1/C^I)_{exp}$ $\log(1/C^I)_{calc}$ [b]
IVa	6·85	0	100	1·000	120·0	2·921
H		0		0·960		2·984
IVb	7·05	—0·27	>100 [c,d]	<1·000	107·0	2·971
4-CH_3O		0·01		0·996		2·988
IVc	6·96 [e]	—0·17	60	1·222	53·9	3·268
4-CH_3		0·45		1·178		3·202
IVd	6·65	0·23	60	1·222	52·7	3·278
4-Cl		0·70		1·329		3·324
VIe	6·66	0·23	40	1·398	37·9	3·446
4-Br		0·90		1·450		3·421
IVf	6·93	—0·12	30	1·523	37·9	3·446
4-C_2H_5		0·90		1·450		3·421
IVg	6·83	—0·02	25	1·602	23·0	3·633
3-Cl-4-i-C_3H_7O		1·26		1·667		3·596
IVh	6·56	0·38	20	1·698	26·0	3·585
3-C_6H_5CO		1·32		1·703		3·625
IVi	6·95	—0·15	20	1·698	19·8	3·703
4-i-C_3H_7		1·40		1·751		3·664
IVj	6·86	—0·03	6 [d]	2·222	10·5 [d]	3·979
4-C_6H_5O		1·60		1·871		3·761
IVk	6·95	—0·12	10	2·000	9·9	4·004
4-i-C_4H_9		1·90		2·053		3·907
IVl	7·10	—0·34	6	2·222	9·2	4·036
4-n-$C_5H_{11}O$		2·01		2·119		3·961
IVm	6·92	—0·12	5	2·301	8·0	4·097
4-i-C_5H_{11}		2·40		2·264		4·150
IVn	7·00 [e]	—0·22	4	2·398	6·5	4·187
4-c-C_6H_{11}		2·46		2·391		4·179
IVo	6·81	0·03	4	2·398	7·2	4·143
3-Cl-4-n-$C_5H_{11}O$		2·46		2·391		4·179
IVp	6·82	0·03	>100 [c,d]	<1·000	4·8	4·319
3-Cl-4-$C_6H_{11}CH_2O$		2·92		2·667		4·403
IVr	6·81	0·01	> 10 [c,d]	<2·000	— [f]	—
3-Cl-4-n-$C_6H_{13}O$		2·96		2·691		—

[a] The values were calculated using equation (*4*); [b] the values were calculated using equation (*5*); [c] insoluble at higher concentration; [d] the compound was not included in the regression analysis; [e] the values were calculated using equation (*2*); [f] insoluble under the conditions of the test.

TABLE IV

Physico-chemical and Biological Properties of β-Arylpropionic Acids *V*

Number X	pK^a	$\Sigma\sigma$ $\Sigma\pi$	Activation of fibrinolysis		Inhibition of the denaturation of BSA	
			C^F mol l^{-1} . 10^3	$\log(1/C^F)_{exp}$ [b] $\log(1/C^F)_{calc}$	C^I mol l^{-1} . 10^5	$\log(1/C^I)_{exp}$ $\log(1/C^I)_{calc}$ [c]
Va	7·00	—0·27	120	0·921	100·0	3·000
4-CH_3O		0·01		0·793		2·972
Vb	6·98	—0·27	>100 [d,e]	<1·000	100·0	3·000
3-CH_3O-i-C_3H_7O		0·41 [f]		1·039		3·179
Vc	7·01	—0·39	80	1·097	34·0	3·469
4-iso-C_3H_7O		0·81		1·286		3·386
Vd	6·88	—0·02	30	1·523	22·0	3·658
3-Cl-4-i-C_3H_7O		1·26		1·563		3·620
Ve	6·97	—0·15	25	1·602	21·3	3·762
4-i-C_3H_7		1·40		1·649		3·692
Vf	6·99	—0·24	8	2·097	9·5	4·022
3-CH_3O-4-n-$C_6H_{13}O$		1·80 [f]		1·945		3·937
Vg	6·96	—0·12	10	2·000	10·0	4·000
4-i-C_4H_9		1·90		1·957		3·951
Vh	7·02	—0·35	— [e,g]	—	11·8	3·928
4-$C_6H_5CH_2O$		1·91		—		3·957
Vi	6·89	0·02	5	2·301	7·0	4·155
3-Cl-4-C_6H_5.CH_2O		2·36		2·240		4·190
Vj	6·99	—0·22	6	2·222	[e,g]	—
4-c-C_6H_{11}		2·46		2·301		—
Vk	7·00	—0·36	> $10^{d,e}$	<2·000	6·5	4·187
4-n-$C_6H_{13}O$		2·51		2·333		4·267
Vl	6·90	0·03	>100 [d,e]	<1·000	[e,g]	—
3-Cl-4-C_6H_{11}.CH_2O		2·92		2·586		—
Vm	6·91	0·01	>100 [d,e]	<1·000	[e,g]	—
3-Cl-4-n-$C_6H_{13}O$		2·96		2·610		—

[a] In the derivation of the regression equation (*3*) the pK values of 3-phenylpropionic acid (pK = = 6·93, $\sigma = 0$) and 3,4-methylenedioxy derivative (pK = 6·97, σ = —0·16) were also used; [b] the values were calculated using equation (*6*); [c] the values were calculated using equation (*7*); [d] insoluble at higher concentration; [e] the compound was not included in the regression analysis; [f] the $\Sigma\pi$ values were obtained by partition chromatography[25]; [g] insoluble under the conditions of the test.

TABLE V

Physico-chemical and Biological Properties of Cinnamic Acids *VI*

Number R	X	$\Sigma\pi$[a]	Activation of fibrinolysis		Inhibition of the denaturation of BSA	
			C^F mol l^{-1} . 10^3	$\log(1/C^F)_{exp}$ [b] $\log(1/C^F)_{calc}$	C^I mol l^{-1} . 10^5	$\log(1/C^I)_{exp}$ [c] $\log(1/C^I)_{calc}$
VIa	4-Cl	0·87	30	1·523	27·0	3·569
H				1·553		3·605
VIb	4-i-C_3H_7O	0·88	30	1·523	28·0	3·553
H				1·559		3·610
VIc	3-Cl-4-i-	1·43	15	1·824	16·2	3·791
H	-C_3H_7O			1·891		3·903
VId	3-CH_3O-	2·26[d]	4	2·398	5·3	4·276
H	-4-n-$C_6H_{13}O$			2·392		4·344
VIe	4-NO_2	0·32	50	1·301	39·0	3·409
CH_3				1·221		3·313
VIf	3-CH_3O	0·44	60	1·222	44·9	3·348
CH_3				1·294		3·377
VIg	3-CH_3O-	0·52[d]	40	1·398	36·5	3·438
CH_3	-4-i-C_3H_7O			1·342		3·420
VIh	3-Cl	1·13	20	1·698	16·2	3·791
CH_3				1·710		3·743
VIi	4-Br	1·28	20	1·698	15·7	3·804
CH_3				1·801		3·823
VIj	3-Br	1·29	15	1·824	16·1	3·793
CH_3				1·807		3·828
VIk	4-i-C_3H_7	1·70	6	2·222	6·9	4·161
CH_3				2·054		4·046
VIl	4-t-C_4H_9	2·00	5·5	2·260	5·6	4·252
CH_3				2·235		4·206
VIm	3-Cl	1·63	8	2·097	8·2	4·086
C_2H_5				2·012		4·009
VIn	4-Cl	1·67	8	2·097	7·5	4·125
C_2H_5				2·036		4·030
VIo	H	1·30	9·5	2·022	11·2	3·951
n-C_3H_7				1·813		3·834
VIp	3-Cl	2·13	5	2·301	4·3	4·367
n-C_2H_7				2·313		4·275
VIr	4-Cl	2·17	6	2·222	6·3	4·201
n-C_3H_7				2·338		4·296
VIs	4-Br	2·28	4	2·398	5·2	4·284
n-C_3H_7				2·404		4·354
VIt	3-Br	2·29	4	2·398	5·0	4·301
n-C_3H_7				2·410		4·360
VIu	3-I	2·58	3	2·523	2·9	4·548
n-C_3H_7				2·585		4·514

	n	r	s	F	
$\log(1/C^F) = 0{\cdot}616\sum\pi + 0{\cdot}787$	8	0·978	0·117	131	(6)
$\log(1/C^I) = 0{\cdot}518\sum\pi + 2{\cdot}966$	10	0·983	0·087	222	(7)

From the regression equations (*4*)–(*7*) the correct conclusion may be made that even in the series *IV* and *V* the activities under investigation are affected by lipophilicity exclusively.

Acids *IVp*, *IVl* and *Vm*, characterized by the lipophilicity of aromatic substituents $\sum\pi = 2{\cdot}92-2{\cdot}96$, are completely inactive because their $\log(1/C^F) < 1{\cdot}000$. If linear dependences on lipophilicity did apply even for these acids, expressed by corresponding equations (*4*) and (*6*), their activity would correspond to the value $\log . (1/C^F) > 2{\cdot}6$ (Tables III and IV). In the series of acids *IV* derivatives *IVm*, *IVn* with the lipophilicity of the aromatic substituents $\sum\pi = 2{\cdot}46$ are most active, and in the series of acids *V* derivatives *Vi* and *Vj* with the lipophilicity $\sum\pi = 2{\cdot}36$ or 2·46, respectively, are the most active. Hence, in both series the clear decrease of fibrinolytic capacity is observed within the narrow range of lipophilicity $\sum\pi = 0{\cdot}5$. The same result was also observed[12] in the preceding series of acids *III*.

In the series of acids *VI*, prepared earlier[15,16], the results of the evaluation of the activation of fibrinolysis were also worked up by the method of regression analysis. We obtained equation (*8*) in which $\sum\pi$ is the sum of the lipophilic parameters π of the substituents X on the aromatic nucleus and of alkyls R in the position α to the carboxyl group. A similar equation (*9*) expresses the dependence of the inhibition of the denaturation of serum albumin on total lipophilicity of variable substituents in the same series of acids *VI*. The experimental results are summarized in Table V.

	n	r	s	F	
$\log(1/C^F) = 0{\cdot}603\sum\pi + 1{\cdot}028$	20	0·976	0·090	265	(8)
$\log(1/C^I) = 0{\cdot}532\sum\pi + 3{\cdot}143$	20	0·974	0·084	328	(9)

If characterizing lipophilicity of substituents X and R separately, by corresponding parameters π_X or π_R, respectively, a regression equation (*10*) is obtained for the activation of fibrinolysis. The close values of the slopes for both parameters indicate that the hydrophobic bond of the aromatic substituents and of the connecting chain between the carboxyl and the aromatic ring have a similar character at the site

[a] The $\Sigma\pi$ values were calculated as the sum of the parameters π of the substituents X and R; [b] the values were calculated using equation (*8*); [c] the values were calculated using equation (*9*); [d] the values were obtained by partition chromatography[25] of the acids *VI*.

of the activation of fibrinolysis. From equation (*11*) it is evident that the same conclusion may be made for the inhibition of the denaturation of serum albumin as well.

	n	*r*	*s*	*F*	
$\log(1/C^{F}) = 0{\cdot}587\pi_X + 0{\cdot}621\pi_R + 1{\cdot}033$	20	0·977	0·091	178	(*10*)
$\log(1/C^{I}) = 0{\cdot}504\pi_X + 0{\cdot}561\pi_R + 3{\cdot}151$	20	0·976	0·083	170	(*11*)

Earlier[11] we tried to check the effect of the connecting chain between the carboxyl group and the aromatic ring on the activation of fibrinolysis. For this purpose we made use of regression analysis of some acids *I*–*VI*. Now we applied regression analysis on the same group of arylaliphatic acids, extended by further derivatives of acids *III*–*VI*. In this case we characterized lipophilicity by the logarithm of the partition coefficient log *P* (in the system 1-octanol–water). The values of log P_H of unsubstituted acids, necessary for the computation of log *P* of substituted derivatives according to equation (*1*), are given in Table VI. The dependence of fibrinolytic capacity of arylaliphatic acids *I*–*VI* on their total lipophilicity is expressed by equation (*12*). Using two variables characterizing lipophilicity of aromatic substituents ($\sum\pi_X$) and the connecting chain ($\Delta\pi$) we obtained equation (*13*).

	n	*r*	*s*	*F*	
$\log(1/C^{F}) = 0{\cdot}620 \log P - 0{\cdot}324$	95	0·960	0·131	1 082	(*12*)
$\log(1/C^{F}) = 0{\cdot}639 \sum\pi_X + 0{\cdot}545\,\Delta\pi - 0{\cdot}315$	95	0·963	0·126	594	(*13*)

TABLE VI

Basic Parameters of the Arylaliphatic Acids *I*—*VI*

Series of acids	log P_H [a]	$\Delta\pi$ [b]	log P_{opt}	C^{F}_{min} mol l^{-1} . 10^3
Arylacetic	1·45	0·50	4·4—4·9	4
3-Arylbutyric	2·15	1·20	4·5	4
2-Methyl-3-arylpropionic	2·15[c]	1·20	4·6	5[d]
2-Arylpropionic	1·80	0·85	4·3—4·8	4
3-Arylpropionic	1·91	0·96	4·3—4·8	5
3-Aryl-2-propenoic	2·13	1·18	4·7	3

[a] Experimental values were taken from lit.[22]; [b] the $\Delta\pi$ values were calculated from the increment of the methylene group ($\Delta\pi = 0{\cdot}5$) in arylacetic acids and from the values of log P_H; [c] the value was taken from isomeric 3-arylbutyric acids; [d] activity of 2-methyl-3-(4-cyclohexylphenyl)propionic acid (log $P = 4{\cdot}61$), not given in the original paper[10].

The introduction of the values pK (determined in 80% methyl cellosolve) into equation (*12*) did not increase its statistical significance. From the relationships mentioned it may be considered that the change of the connecting chain between the carboxyl and the aromatic ring affects the fibrinolytic capacity of these acids probably exclusively by the corresponding change in lipophilicity. From the close values of the slopes for both parameters π_X and $\Delta\pi$ it is evident that the character of the hydrophobic bond of the connecting chain and the aromatic substituents at the site of the activation of fibrinolysis is very similar. As shown by equations (*14*) and (*15*), derived for the inhibition of the denaturation of serum albumin in the same group of acids, the same conclusion is also valid for the binding of acids *I* – *VI* onto the serum albumin.

	n	r	s	F	
$\log(1/C^I) = 0{\cdot}532 \log P + 1{\cdot}991$	95	0·983	0·071	2 706	(*14*)
$\log(1/C^I) = 0{\cdot}529 \sum\pi_X + 0{\cdot}549 \sum\pi + 2{\cdot}482$	95	0·983	0·071	1 340	(*15*)

As already said before, the fibrinolytic capacity increases in individual series of acids linearly with lipophilicity; after the achievement of the maximum, however, a steep decrease in activity takes place. In the series of acids *III* – *V* the optimum of lipophilicity was determined within a narrow range, since even the lipophilic derivatives are well soluble under the conditions of the test. From the data collected in Table VI, expressed by means of log P_{opt}, the maximum of fibrinolytic capacity is attained when the total lipophilicity log P_{opt} is within the 4·3 – 4·9 range. This maximum of activity is characterized by the concentration C^F_{min} $4-5 \cdot 10^{-3}$ mol l^{-1}. In the series of acids *I* and *II* it was impossible to determine the optimum of lipophilicity, owing to the restricted solubility of more lipophilic derivatives. In spite of this, even in these series the results indicate that the maximum of activity, C^F_{min}, is achieved at total lipophilicity log $P_{opt} > 4{\cdot}5$. Finally, in acids *VI*, the maximum activity ($C^F_{min} = 3 \cdot 10^{-3}$ mol l^{-1}) of acid *VIu* is connected with the value of the lipophilicity log P = 4·61. In this series the more lipophilic derivatives are completely insoluble.

From the results obtained the following conclusion may be drawn:

1) The bond of arylaliphatic acids at the site of activation of fibrinolysis has a distinctly hydrophobic character and it is similar to their bond to the serum albumin. This conclusion is in agreement with the experimental results of von Kaulla[38] who found that under the same conditions, but in the presence of serum albumin, a higher concentration of fibrinolytically active acids is necessary for the dissolution of the coagulum.

2) In individual series of acids and in the whole group of arylaliphatic acids *I* – *VI* the slopes of the dependence of both activities on lipophilic parameters range between

0·5 and 0·65. From these values it may be assumed[39,40] that the interaction of acids *I – VI* with the protein at the site of the activation of fibrinolysis and with serum albumin takes place on the surface of the biomacromolecule. This conclusion agrees with the hypothesis of von Kaulla[41] concerning the mechanism of activation of fibrinolysis in the *in vitro* system which assumes that the critical step of activation takes place on the surface of fibrin*.

3) From the summarizing regression equations (*12*) and (*13*) it is evident that the hydrophobic bond of all the acids *I – VI* at the site of activation of fibrinolysis is effected in the same manner without regard to the type of the connecting chain. The character of this binding is the same for the connecting chain and for the substituents on the aromatic ring. Hence, it is very probable that an extensive hydrophobic region exists near the cationic centre in the site of activation of fibrinolysis. The rapid decrease of the fibrinolytic capacity with increasing lipophilicity in individual series of arylaliphatic acids indicates that the capacity of the hydrophobic bond is limited in this region. From the comparison of the optimal lipophilicities log P_{opt} in Table VI it follows that the limit hydrophobic bond is probably the same in all series of arylaliphatic acids.

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* The assumed mechanism of activation of fibrinolysis in the *in vitro* systems is described in ref.[42].

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