QUANTITATIVE RELATIONSHIPS BETWEEN STRUCTURE AND FIBRINOLYTIC ACTIVITY IN THE SERIES OF α-METHYL-β-ARYLPROPIONIC ACIDS

Miroslav KUCHAŘ, Václav REJHOLEC, Zdeněk ROUBAL and Oldřich NĚMEČEK

Research Institute for Pharmacy and Biochemistry, 130 60 Prague 3

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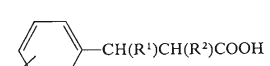
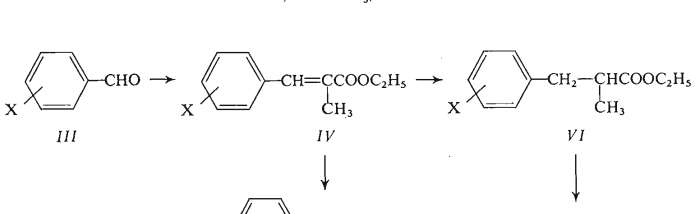
A series of α-methyl-β-arylpropionic acids *I* was prepared both by hydrogenation of corresponding α-methylcinnamic esters and by decarboxylation of α-carboxy-α-methyl-β-arylpropionic acids. The substances were tested in the form of cyclohexylammonium salts as activators of fibrinolysis and inhibitors of heat denaturation of serum albumin. Similarly as in the series of isomeric β-aryl-n-butyric acids correlation analysis indicated that both activities are mainly affected by the lipophilic character of the aromatic substituents and that the electronic effects are statistically insignificant. From the comparison of the regression relationships in both series of acids it is evident that neither activity is affected by a change in the structure of the side chain. For the estimation of the lipophilicity of 3,4-dialkoxy derivatives the parameters π were used which were obtained by reverse phase partition chromatography on silica gel thin layer.

Recently we described the synthesis of β-aryl-n-butyric acids *II* and their effect on the activation of fibrinolysis[1,2]. On the basis of quantitative relationships between the structure and the activity we established that the fibrinolytic activity of these acids is affected primarily by their lipophilicity, similarly as the inhibition of serum albumin that serves as a suitable criterion of the binding to this protein. An important difference in the relation of both mentioned activities to lipophilicity appeared in the case of acids *II* in the region of higher lipophilicity[3] where the originally linear dependence changes to a parabolic one in the case of fibrinolysis activation, with the optimum of activity at $\pi_{opt} = 2{\cdot}46$. Now we have prepared a series of α-methyl-β--arylpropionic acids *I* in order to check the effect of the position of the methyl group in relation to the carboxyl group on both activities.

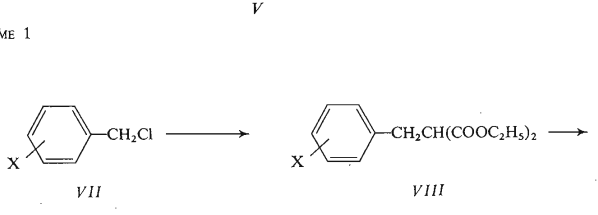
Acids *I* which do not contain halogen on the aromatic nucleus were prepared by hydrogenation of corresponding cinnamic acids *IV* or their ethyl esters *V* obtained[4,5] on reaction of substituted benzaldehydes *III* with triphenyl-α-ethoxycarbonylethylidenephosphorane (Scheme 1). For the preparation of acids *I* substituted with halogen we made use of the reaction of substituted benzyl chlorides *VII* with diethyl malonate; the benzyl malonates formed (*VIII*) were converted to methyl derivatives *IX* the hydrolysis and decarboxylation of which gave the required acids *I* (Scheme 2). In view of the fact that some of the acids *I* are oily substances the evaluation of which by biological tests is difficult, they were converted to cyclohexylammonium salts *XI*.



I; $R^1 = H$, $R^2 = CH_3$
II; $R^1 = CH_3$, $R^2 = H$



SCHEME 1



SCHEME 2

We determined the activity of the salts *XI* with respect to fibrinolysis activation and the test of denaturation of serum albumin, and we evaluated the relationships of these activities to the physico-chemical parameters of salts *XI* using the regression analysis method[6,7]. The regression equations obtained were compared with similar relationships derived[1-3] in the series of β-aryl-n-butyric acids *II*. For the evaluation of lipophilicity we used the parameters[8,9] π.

EXPERIMENTAL

Methods

The IR spectra of acids *I* and of their cyclohexylammonium salts *XI* were measured in the 400 to 4000 cm^{-1} region, using a 5% solution in chloroform and a UR-20 (Zeiss, Jena) spectrophotometer. The ^{1}H-NMR spectra were measured on a BS 487 C—80 MHz (Tesla, Czechoslovakia) spectrometer in 6% solution in deuteriochloroform. Tetramethylsilane was used as internal standard. Gas chromatography of compounds *IV*, *VIII* and *IX* was carried out on a gas chromatograph Fractometer (Perkin–Elmer F7), using a stainless steel column of 3 mm diameter, 2 m long, packed with Gas-Chrom Q 125—150 μm wetted with 3% of polyethylene glycol (m.w. about 20000). Chromatography of cyclohexylammonium salts *XI* was carried out[9] on a thin layer of silica gel impregnated with 7·0 wt.% of silicone oil (in the form of a 5% solution in dioxane), using 50% acetone as mobile phase. The p*K* values of acids *I* were determined in 80% methylcellosolve at 45°C on a Titrigraph potentiometer (Radiometer, Copenhagen), type SBR-2c. The p*K* values of compounds *Ia—Il* and *Ip* correlated with the σ constants according to equation (*1*).

		n	*s*	*r*	*F*	
$pK = -0{\cdot}416\ (\pm 0{\cdot}115)\ \sigma + 6{\cdot}959\ (\pm 0{\cdot}028)$;		13	0·027	0·991	163·8	(*1*)

in which the symbols *n*, *s*, *r* and *F* indicate the number of compounds, standard deviation, multiple correlation coefficient, and Fischer–Snedecor criterion, respectively. The values in brackets are the limits of confidence determined by the t-test at the level of statistical significance $\alpha = 0{\cdot}005$. The melting points were determined on a Boetius M melting point block and they are not corrected.

For the expression of the lipophilicity of aromatic substituents the parameters π were used which were derived for arylacetic acids[8]. For the calculation of the parameters π of higher alkyls of alkoxy groups the following increments[10] were used: $\Delta\pi(CH_2) = 0{\cdot}5$, $\Delta\pi$ (branching) $= -0{\cdot}2$. In the calculation of $\sum\pi$ of 3,4-disubstituted derivatives the difference between the lipophilicity of the aromatic fragments[11] —C_6H_4— and —C_6H_3= was taken into consideration, which is in agreement with the lipophilicity of hydrogen[12] $\pi_H = 0{\cdot}23$. This value was substracted from the sum of the parameters $\sum\pi$ of both substituents, as it follows from the calculation of $\sum\pi$ for 3-chloro-4-isopropoxy derivative *XII*:

$$\sum\pi = \pi_{3-Cl} + \pi_{4-i-C_3H_7O} - \pi_H = 0{\cdot}68 + 0{\cdot}81 - 0{\cdot}23 = 1{\cdot}26$$

For 3,4-dialkoxy derivatives *XIp* and *XIr* for which the additivity of the parameters π does not apply[13] the values $\sum\pi$ 0·41 or 1·70, respectively, were used which were obtained by the evaluation of the lipophilicity by partition chromatography.

For the estimation of electronic effects of aromatic substituents we used polar constants σ, taken from ref.[14]. For the substituents *p*-n-$C_6H_{13}O$ and *p*-i-C_4H_9O the constants were calculated by putting the experimental p*K* values of the acids *Im* and *Ir* into equation (*1*). The constants σ for *p*-n-$C_6H_{13}O$ (−0·24) and *p*-i-C_4H_9O (−0·37) obtained in this manner are in good agreement with the values that were obtained in a similar manner[13] in the series of cinnamic and α-methylcinnamic acids (−0·22 or −0·36, respectively).

The regression coefficients were calculated from experimental data by multiple regression analysis on a Hewlett–Packard calculator 9820. The statistical significance of the equations was evaluated on the basis of the standard deviation *s*, correlation coefficient *r* and Fischer–Snedecor criterion *F*. Individual parameters were estimated statistically using Student's t-test; in all equations the level of statistical significance α is minimally 0·005.

Biochemical Evaluation

The inhibition of heat denaturation of bovine serum albumin was determined according to Mizushima[15], as described in ref.[1]. The efficiency was expressed by the molar concentration causing a 50% inhibition. The activation of fibrinolysis was estimated using the "hanging clot" method[16] prepared from human plasma and suspended in a solution of the tested compound[1]. The efficiency was expressed by the minimum molar concentration that dissolves the coagulum after 24 h incubation at 37°C.

Esters of Cinnamic Acids *IV*

These were prepared from substituted benzaldehydes and triphenyl-α-ethoxycarbonylethylidenephosphorane; the method is described in refs[4,5]. Pure esters were isolated by distillation; further the following data are given: number, substituent *X*, yield (%), b.p. (°C/Torr): *IVd*, 4-i-C_3H_7O, 87·0, 112/0·9; *IVi*, 4-t-C_4H_9, 63·8, 120/0·7; *IVj*, 4-i-C_4H_9, 86·2, 122—126/1·1; *IVp*, 3-CH_3O-4-i-C_3H_7O, 126—128/0·8; *IVr*, 3-CH_3O-4-n-$C_6H_{13}O$, 89·6, 168—169/0·15.

Cinnamic Acids *V*

Esters *IV* were hydrolyzed in crude state by boiling with 10% aqueous-ethanolic potassium hydroxide. Further the following data are given: number, substituent *X*, yield (%), m.p. (°C), solvent, literature m.p. (°C) or elemental analysis: *Va*, H, 76·0, 79—80·5, methanol–water 1 : 1, lit.[4] m.p. 79—80; *Vb*, 3-CH_3O, 65·5, 90—91, methanol–water 1 : 1, for $C_{11}H_{12}O_3$ (192·2) calculated: 68·78% C, 6·30% H; found: 68·88% C, 6·48% H; *Vc*, 4-CH_3O, 73·0, 157—158, methanol, lit.[4] m.p. 158—159; *Vh*, 4-i-C_3H_7, 62·5, 89—90, methanol–water 2 : 1, lit.[4] m.p. 89·5—90·5; *Vo*, 4-2′-ethylhexyl, 51·3, 106—108, methanol, for $C_{17}H_{24}O_2$ (260·4) calculated: 78·42% C, 9·29% H; found: 77·85% C, 9·62% H.

Hydrogenation of Cinnamic Acids and Its Esters

Cinnamic acid *V* or its ester *IV* (40·0 mmol) was dissolved in 100 ml of methanol, and 0·5 g of active charcoal and 1·0 g of palladium chloride (in the form of a 40% solution in concentrated hydrochloric acid) were added to the solution. The apparatus was rinsed with nitrogen and then kept under hydrogen under stirring and normal pressure until the consumption of hydrogen ceased. In all instances it corresponded to the theory. After filtration of the catalyst methanol was evaporated and the residue distilled in a vacuum (in the case of the starting acid *V*) affording the acids *Ia*, *b*,*c*,*h*, or purified by column chromatography (*Io*). When the starting ester *IV* was used the residue was hydrolyzed in refluxing solution of 10 g of potassium hydroxide in 100 ml of an ethanol–water mixture 1 : 1 for 5 h. Then the mixture was evaporated to half its volume, 50 ml of water were added and the solution filtered after addition of charcoal. Crude acid *I* precipitated after acidification of the filtrate with 50% sulfuric acid, and it was then extracted with ether. The extract was dried over magnesium sulfate and evaporated to dryness. If the product (*Id*, *j*) was oily it was purified by vacuum distilaltion; the crystalline crude products were purified by crystallization (*Ii*, *p*) from a suitable solvent (Table II) or by column chromatography on silica gel (*Ir*).

Substituted Benzyl Malonates *VIII*

Diethyl malonate (0·155 mol) followed by substituted benzyl chloride *VII* (0·15 mol) was added at 20°C into a solution of sodium ethoxide prepared by dissolution of 3·45 g of sodium in 85 ml

of ethanol and the reaction mixture was refluxed for 8 h. After evaporation of ethanol the oily residue was diluted with 200 ml of ether, 100 ml of water was added to it and the ethereal layer was separated and washed with three 100 ml portions of water. After drying over magnesium sulfate ether was evaporated and the benzyl malonate *VIII* isolated by distillation. The yields and the boiling points are given in Table I.

α-Benzyl-α-methylmalonates *IX*

Benzylmalonate *VIII* (0·06 mol) was added to a solution of sodium ethoxide prepared on dissolution of 1·45 g of sodium in 30 ml of ethanol; the mixture was cooled and methyl iodide (0·072 mol) added to it at less than 10°C and then the mixture was refluxed for 5 h. After evaporation 100 ml of water was added to the residue and the separated oil was extracted with 200 ml of ether. The ethereal extract was washed with two 100 ml portions of water, dried over magnesium sulfate, filtered and evaporated. The intermediate *IX* was isolated by distillation. The yield and the b.p. are given in Table I.

Hydrolysis and Decarboxylation

Ester *IX* (0·04 ml) was refluxed with a solution of 9 g of potassium hydroxide and 90 ml of 50% ethanol for 5 g. After evaporation the residue was heated with 20 ml of 50% potassium hydroxide at 100°C for 2 h. After dilution with 50 ml of water the solution was filtered with active charcoal. Acidification of the filtrate with 50% sulfuric acid caused precipitation and the precipitate was extracted with 100 ml of ether. After drying and evaporation corresponding malonic acid *X* was isolated. After identification by ^{1}H-NMR spectrum acid *X* was decarboxylated in crude state by heating at 200°C. The development of carbon dioxide ceased after 20 min and the melt was cooled to 20°C. After dissolution in 60 ml of 1M-NaOH the solution was filtered with charcoal. Acidification of the filtrate with 50% sulfuric acid caused separation of product *I* which was extracted with 100 ml of ether, the extract was dried and evaporated. From the residue acid *I* was isolated by distillation or crystallization (for yield and method of isolation see Table II).

TABLE I
Characterization of the Intermediates *VIII* and *IX*

Intermediate	*VIII*		*IX*	
	yield, %	b.p., °C/Torr	yield, %	b.p., °C/Torr
e	40·9	111—113/0·1	76·0	98—100/0·15
f	43·6	110—111/0·2	90·0	110—112/0·3
g	37·2	129—132/0·35	72·9	118—120/0·3
k	45·5	136—137/0·1	82·5	135/0·15
l	39·8	146—148/0·2	83·2	140—142/0·25
m	39·5	155—157/0·25	88·7	139—142/0·12
n	39·0	138—140/0·2	69·0	120—122/0·1

TABLE II
Characterization of Acids *I*

Acid Substituent	Method (yield[a], %)	pK^b	B.p. (m.p.) °C/Torr	Calculated/Found % C	% H	% Cl (Br)
Ia H	*A* (47·5)	6·97	120—121/1·5[c]	73·14 73·02	7·37 7·48	— —
Ib 3-CH_3O	*A* (56·5)	6·91	129—131/0·5	68·02 68·16	7·27 7·35	— —
Ic 4-CH_3O	*A* (57·5)	7·05	105—110/0·8[d]	68·02 67·91	7·27 7·45	— —
Id 4-i-C_3H_7O	*A* (54·5)	7·15	132—135/0·45	70·18 70·42	8·15 8·28	— —
Ie 3-Cl	*B* (54·8)	6·81	107—109/0·2	60·50 60·79	5·58 5·66	17·87 17·67
If 4-Cl	*B* (75·4)	6·85	51·5—53[e]	60·50 60·23	5·58 5·51	17·87 17·99
Ig 4-Br	*B* (79·4)	6·86	70—71·5[e]	49·44 49·46	4·56 4·62	32·88 32·96
Ih 4-i-C_3H_7	*A* (61·0)	7·03	134—135/0·5	75·69 75·45	8·80 8·94	— —
Ii 4-t-C_4H_9	*A* (43·8)	7·03	110—111·5[f]	76·38 76·62	9·09 9·30	— —
Ij 4-i-C_4H_9	*A* (52·0)	7·02	142—143/1·1	76·38 76·29	9·09 9·12	— —
Ik 3-Cl, V-CH_3O	*B* (85·4)	6·90	73—74[f]	57·75 57·82	5·72 5·85	15·51 15·59
Il 3-Cl, 4-i-C_3H_7O	*B* (5·64)	7·02	136—138/0·44	60·78 60·90	6·67 6·56	13·80 14·00
Im 3-Cl, 4-i-C_4H_9O	*B* (66·7)	6·95	82—83[e]	62·05 62·17	7·07 7·11	13·10 13·06
In 3-Br, 4-i-C_3H_7[g]	*B* (57·4)					
Io 4-2′-ethylhexyl[g]	*A* (47·3)					
Ip 3-CH_3O, 4-i-C_3H_7O[h]	*A* (64·4)	7·09	57—58·5[f]	62·20 62·38	8·17 8·06	— —
Ir 3-CH_3O, 4-n-$C_6H_{13}O$	*A* (33·5)	7·02	42—43·5[i]	69·38 69·59	8·90 8·63	— —

Cyclohexylammonium Salts *XI*

Cyclohexylamine (0·023 mol) in 10 ml of ether was added to a filtered solution of acid *I* (0·015 mol) in 30 ml of ether and the mixture was allowed to stand at 5°C for 2 h. The precipitated product was filtered and washed with ether. The salt *XI* was thus obtained in an about 95% yield (the melting points are in Table III).

RESULTS AND DISCUSSION

Applying regression analysis of experimental results of the inhibition of serum albumin denaturation (Table IV) we obtained equation (*2*) expressing a linear dependence of this activity on the lipophilicity of aromatic substituents. With the introduction of polar constants σ, the same as the square of π, the statistical significance of the regression relationship decreased. From a comparison with the similar equation (*3*) which was derived[2,3] for the inhibition of serum albumin denaturation in the series of β-aryl-n-butyric acids *II*, the similarity of the slopes of the lipophilic parameters π and the constant terms in both equations is evident. The structural change in the side chain of both series of acids did not practically affect the dependence of the inhibition of serum albumin denaturation on lipophilicity. In equally substituted derivatives the level of the inhibition, expressed in $\log(1/C)$, is almost identical.

	n	s	r	F	
$\log(1/C) = 0{\cdot}584(\pm 0{\cdot}116)\,\pi + 3{\cdot}132(\pm 0{\cdot}134)$	14	0·082	0·995	294·6	(*2*)
$\log(1/C) = 0{\cdot}503(\pm 0{\cdot}070)\,\pi + 3{\cdot}147(\pm 0{\cdot}113)$	19	0·076	0·985	552·0	(*3*)

In a similar manner we also evaluated experimental results of the activation of fibrinolysis (Table IV) and obtained a regression equation (*4*) the statistical significance of which did not increase on introduction of polar constants σ or the square of π. The linear dependence is in agreement with a similar equation (*5*), derived[3] for the activation of fibrinolysis in the series of β-aryl-n-butyric acids *II*. In regression analysis[3] of the fibrinolytic activity of acids *II* we have found that in a broad range of lipophilicity the originally linear dependence on lipophilicity changes to a quadratic one with an optimum of lipophilicity of aromatic substituents $\pi_{opt} = 2{\cdot}48$. In the

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[a] In acids *Ia,b,c,h,o* the yield of hydrogenation is meant, in acids *Id,i,j,p,r* the yield of hydrogenation and hydrolysis, in acids *Ie,f,g,k,l,m,n* the yield of hydrolysis and decarboxylation; [b] the p*K* values were determined in 80% methylcellosolve at 25°C; [c] lit.[17] gives b.p. 160°C/12 Torr; [d] lit.[18] gives b.p. 308°C; [e] crystallized from methanol–water 2 : 1; [f] crystallized from methanol–water 1 : 1; [g] the acid was isolated in the form of an oil and identified by IR and ^{1}H-NMR spectra; [h] crystallizes as monohydrate; [i] purified by column chromatography on silica gel with n-hexane–benzene 1 : 1.

TABLE III

Characterization of Cyclohexylammonium Salts *XI*

Salt	M.p., °C	Calculated/Found			
		% C	% H	% N	% Cl(Br)
XIa	119—121	73·02	9·57	5·32	—
		73·31	9·39	5·42	—
XIb	98—99·5	69·62	9·28	4·73	—
		69·74	9·52	4·76	—
XIc	130—131	69·62	9·28	4·73	—
		69·70	9·39	4·74	—
XId	126—128	71·03	9·68	4·36	—
		71·05	9·96	4·36	—
XIe	119—121	64·55	8·17	4·71	11·92
		64·69	8·12	4·76	11·95
XIf	155—156	64·55	8·17	4·71	11·92
		64·42	8·30	4·68	11·92
XIg	147—149	56·05	7·07	4·09	23·37
		56·19	7·34	4·32	23·69
XIh	126—127	74·75	10·24	4·60	—
		74·89	10·49	4·77	—
XIi	131—133	75·35	10·42	4·38	—
		75·65	10·57	4·23	—
XIj	126—128	75·35	10·42	4·38	—
		75·31	10·66	4·38	—
XIk	128—130	62·38	8·00	4·28	10·82
		62·37	8·01	4·16	10·63
XIl	95—97	64·15	8·51	3·94	9·97
		64·30	8·44	4·15	10·17
XIm	117·5—118·5	65·00	8·73	3·79	9·59
		65·01	8·71	3·71	9·83
XIn	108—109	59·38	7·86	3·64	20·80
		59·66	8·11	3·82	20·62
XIo	106—108	76·74	11·00	3·73	—
		76·96	11·25	3·95	—
XIp	111—113	68·34	9·46	3·99	—
		68·62	9·55	3·99	—
XIr	77—79	70·23	9·98	3·56	—
		70·39	9·90	3·64	—

TABLE IV

Biological Activities of Cyclohexylammonium Salts *XI*

Compound *XI*	σ	π	R_M [a]	Inhibition of serum albumin denaturation		Activation of fibrinolysis	
				$\log(1/C)_{exp}$	$\log(1/C)_{calc}$ [b]	$\log(1/C)_{exp}$	$\log(1/C)_{calc}$ [c]
a	0	0	−0·10	3·192	3·132	0·921	0·841
b	0·12	0·04	−0·20	3·169	3·138	<1·000 [d]	—
c	−0·27	0·01	−0·19	3·169	3·155	0·921	0·834
d	−0·45	0·81	0·05	3·607	3·605	1·347	1·397
e	0·37	0·68	0·02	3·498	3·529	1·222	1·306
f	0·23	0·70	0	3·524	3·541	1·222	1·326
g	0·23	0·90	0·03	3·620	3·658	1·398	1·459
h	−0·15	1·40	0·28	3·932	3·949	1·824	1·806
i	−0·20	1·68	0·41	4·071	4·113	2·000	2·001
j	−0·12	1·90	0·45	4·292	4·241	2·222	2·153
k	0·10	0·46	−0·06	3·327	3·401	<1·000 [d]	—
l	−0·08	1·26	0·21	3·896	3·868	— [e]	—
m	0·02 [f]	1·76	0·41	4·201	4·160	— [e]	—
n	0·24	1·62	0·39	4·071	4·078	2·000	1·959
o	−0·15	3·75	—	— [e]	—	<2·000 [d]	3·324

[a] Thin-layer chromatography on silica gel impregnated with silicone oil[9]; [b] the values calculated from equation (*2*); [c] the values calculated from equation (*4*); [d] insoluble at higher concentrations, not included in the correlation; [e] insoluble within the whole range of the concentrations measured; [f] calculated from equation (*1*) and experimental values of p*K*.

TABLE V

Biological Activities of 3,4-Dialkoxy Derivatives

Number	R_M / σ	$\sum\pi_{tab}$ [a] / $\sum\pi_{calc}$ [b]	Inhibition of serumalbumin denaturation		Activation of fibrinolysis	
			$\log(1/C)_{exp}$	$\log(1/C)_{calc}$ [c]	$\log(1/C)_{exp}$	$\log(1/C)_{calc}$ [d]
XIp	−0·06	0·62	3·327	3·494 [e]	1·097	1·246 [e]
	−0·32	0·41		3·371 [f]		1·106 [f]
XIr	0·40	2·32	4·071	4·487 [e]	2·000	2·374 [e]
	−0·12 [g]	1·70		4·125 [f]		1·963 [f]

[a] The values from ref.[8] were used; [b] obtained by putting experimental R_M values into the regression equation expressing the relationship between R_M and parameters[9] π; [c] the values calculated from equation (*2*); [d] the values calculated from equation (*4*); [e] tabulated π values were used for calculation; [f] the values $\sum\pi$ obtained from partition chromatography were used for the calculation; [g] see note [f] in Table IV.

series of acids *I* it was impossible to check the parabolic dependence of fibrinolysis on the lipophilicity by regression analysis in view of the insolubility of more lipophilic derivatives under the conditions of the test. However, for 4-(2'-ethylhexyl) derivative *XIo* it was found that its fibrinolytic activity, expressed as $\log(1/C)$, is lower than 2. In the case of the validity of equation (*4*) even within a broader range of lipophilicity it should have the value 3·2. From the comparison of the slopes of parameters π and the values of the constant terms in equation (*4*) and (*5*) it follows that the activation of fibrinolysis is not affected by the position of the methyl group in relation to the carboxyl group in either isomeric series of acids *I* and *II*.

$$\log(1/C) = 0{\cdot}664(\pm 0{\cdot}230)\,\pi + 0{\cdot}834(\pm 0{\cdot}268) \quad 10 \quad 0{\cdot}121 \quad 0{\cdot}989 \quad 133{\cdot}7 \qquad (4)$$

$$\log(1/C) = 0{\cdot}563(\pm 0{\cdot}247)\,\pi + 0{\cdot}916(\pm 0{\cdot}269) \quad 11 \quad 0{\cdot}135 \quad 0{\cdot}942 \quad 70{\cdot}6 \qquad (5)$$

When tabulated parameters π are used for the expression of lipophilicity, 3,4-dialkoxy derivatives *XIp* and *XIr* deviate distinctly from the regression equations (*2*) and (*4*). The experimental values (Table V) of the inhibition of serum albumin denaturation and the activation of fibrinolysis are in both substances lower than the values calculated from equations (*2*) or (*4*). The reason of this anomaly evidently consists in a decrease of lipophilicity of 3,4-dialkoxy derivatives, observed also in the evaluation of lipophilicity of β-aryl-α-methylpropionic acids by means of partition chromatography[9,13]. It is probable that this decrease in lipophilicity is a consequence of the changes in the solvation of both alkoxy groups which are mutually in an *ortho* position[19,20].

Using thin-layer chromatography on reversed phases[9] we could determine corresponding parameters of $\sum\pi$ for the combination of methoxy and isopropoxy groups ($\sum\pi = 0{\cdot}41$) and methoxy and n-hexyloxy ($\sum\pi = 1{\cdot}70$) groups in positions 3, 4 from experimental R_M values of compounds *XIp* and *XIr*. Introducing these $\sum\pi$ values into equations (*2*) and (*4*) we calculated corresponding activities of compounds *XIp* and *XIr* (Table V). These values are in good agreement with experimental results.

The elemental analyses were carried out in the microanalytical department, Research Institute for Pharmacy and Biochemistry (head Dr J. Kŏrbl), the infrared spectra were measured by Mrs P. Vejdělková under the direction of Dr B. Kakáč, the ^{1}H-MNR *spectra were recorded by Dr J. Holubek. Gas chromatography was carried out by Mr S. Vaněček and thin layer chromatography by Mrs M. Jelínková under the direction of Dr V. Rábek. The* pH *values were determined by Mr E. Kraus.*

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