QUANTITATIVE RELATIONS BETWEEN STRUCTURE AND ACTIVATION OF FIBRINOLYSIS IN SELECTED SERIES OF ARYLALIPHATIC ACIDS

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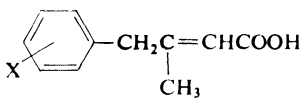
The paper describes the reactions of arylacetones *IX* with triethyl phosphonoacetate, producing esters of 4-aryl-3-methyl-2-butenoic acid, *X*, and 4-aryl-3-methyl-3-butenoic acid, *X'*. Their hydrolysis gave a mixture of the isomeric acids *I* and *I'*, whose composition was investigated by ^{1}H NMR spectra. Also prepared were 3-methyl-3-phenyl-2-propenoic acids, *II*, 2-aryl--2-hydroxypropanoic acids, *IV*, and substituted α-benzyloxyphenylacetic acids, *V*. These acids, along with aryloxoaliphatic acids *III*, were investigated for efficacy in activation of fibrinolysis. The lipophilicities of the acids studied were determined either through the partition coefficients (acids *Ia* and *IIa*), using a system n-octanol–water (pH 3·5) or by partition chromatography. The experimental values of log *P* were compared with those calculated from the fragmental constants *f* and the parameters π. With the acids *V* the decrease in lipophilicity was similar to that observed with arylalkoxyaliphatic acids. With the acids *I*, *I'* and *II* the fibrinolytic capacity was linearly proportional to lipophilicity. Although we evaluated fibrinolytic capacity of mixtures of the acids *I* and *I'*, the linear relation was in agreement with that derived previously for a group of arylaliphatic acids. The presence of a functional group on the connecting chain in the acids *III* and *IV* had a negative effect on the fibrinolytic capacity. The decrease in fibrinolytic capacity might be due to the functional groups being capable of forming hydrogen bonds.

The study of activation of fibrinolysis is a branch of research of the trombolytically active substances[1]. Some very strong activators of fibrinolysis were found among anti-inflammatory substances of acidic nature[2,3]. The experimental results, obtained in series of arylacetic[4], 3-arylbutanoic[5,6], 3-arylpropanoic[7] and 3-phenyl-2-propenoic[7,8] acids were evaluated by the method of regression analysis. The fibrinolytic capacity in the individual series and the whole group of the given arylaliphatic acids was found to depend exclusively on lipophilicity. The linear relation between activation of fibrinolysis and lipophilicity in the whole group of arylaliphatic acids is expressed[9] by equation (*1*).

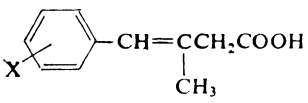
		n	*r*	*s*	*F*	
$\log(1/C^F) = 0{\cdot}620 \log P - 0{\cdot}324$		95	0·960	0·131	1 082	(*1*)

This linear relation is valid up to a certain optimum value of lipophilicity, beyond which the fibrinolytic capacity rapidly decreases. The optimum of lipophilicity, log P_{opt}, occurs in a narrow range of 4·4 to 4·9. The corresponding maximum fibrinolytic activity, expressed by the minimum concentration dissolving the plasmatic coagulum, $C^{F}_{min} = 4 \cdot 10^{-3}$ to $3 \cdot 10^{-3}$ mol l^{-1}.

The results obtained allowed us to draw the conclusion[7,9] that the binding of an arylaliphatic acid to the site of fibrinolysis is of a strongly hydrophobic nature, like its binding to serum albumin. This interaction probably affects the surface of the biomacromolecule, the cationic centre on the site of fibrinolysis being surrounded by a vast hydrophobic region with a limited capacity of hydrophobic binding. In the present paper we verify the thresholds of validity of the regression equation (*1*) by evaluating the activation of fibrinolysis in series of 4-aryl-3-methylbutenoic acids *I* and *I'*, 3-methyl-3-phenyl-2-propenoic acids *II*, aryloxoaliphatic acids *III*, 2-aryl-2-hydroxypropanoic acids *IV* and substituted α-benzyloxyphenylacetic acids *V*.



I



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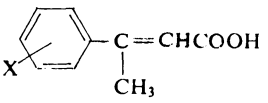

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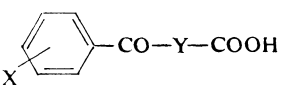

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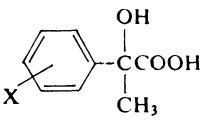
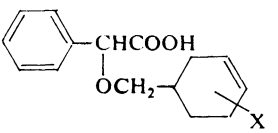


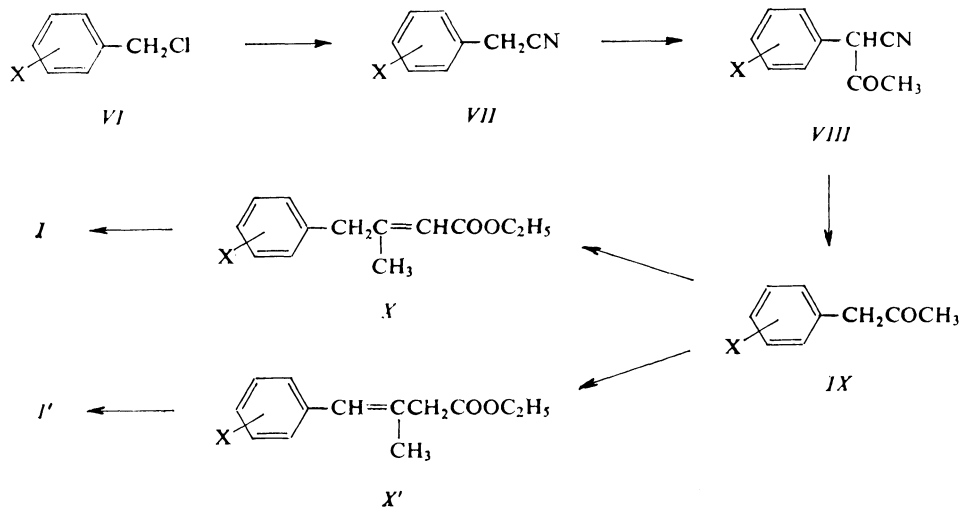
IV



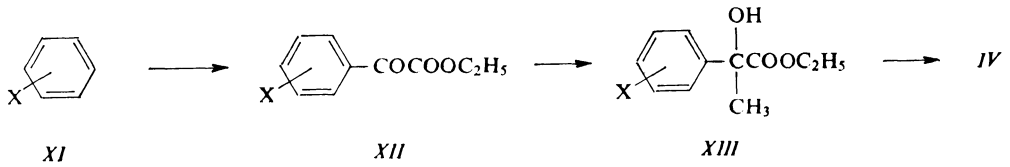
V

The acids *I* and *I'* were isolated in the form of mixtures, prepared according to Scheme 1, in which a mixture of the esters *X* and *X'* was obtained by the Horner–Wittig reaction[10] of the corresponding arylacetone *IX* with triethyl phosphonoacetate, followed by hydrolysis. The acids *II* were obtained from the corresponding substituted acetophenones by the Reformatski reaction with ethyl bromoacetate[11], followed by dehydration with phosphoryl trichloride in benzene and by hydrolysis. To prepare the aryloxoaliphatic acids *III* we used the Friedel–Crafts reaction of anhydrides of dicarboxylic acids with the appropriately substituted aromatic compounds. The preparative procedure and the physico-chemical properties of these acids will be described elsewhere[12]. The acids *IV* were obtained from substituted aromatic compounds according to Scheme 2, which is part of one of the preparative ways[13,14] leading to 2-arylpropanoic acids. The acids *V* were obtained by reaction of 2-phenyl-

-2-hydroxyacetate with the corresponding benzyl chlorides in the presence of sodium hydride, followed by hydrolysis.



SCHEME 1



SCHEME 2

EXPERIMENTAL

Methods

The ^{1}H NMR spectra of the acids *I*—*V* and intermediates *VII*—*X*, *XIII* were measured in 6% solutions in deuteriochloroform, with tetramethylsilane as internal standard, employing a spectrometer BS 487s—80 MHz (Tesla, Czechoslovakia). Purity of the compounds *VI*—*X*, *XII*, *XIII* and of triethyl phosphonoacetate was tested by gas chromatography in a chromatograph Fractometer F 7 (Perkin–Elmer), using a stainless steel column (I.D. 3 mm, length 2 m), packed with Gas-Chrom Q 125—150 μm moistened with 3% polyethyleneglycol (mol. mass *c.* 20 000).

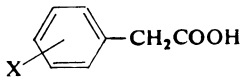
The partition coefficients of the acids *Ia* and *IIa* were determined by a described shake-flask technique[15] in a system n-octanol–aqueous acetate buffer, pH 3·5. The concentrations of an acid in the two phases were measured spectrophotometrically in a spectrophotometer Unicam SP8000; the partition coefficients were calculated as the ratios of concentrations in the octanol and the

aqueous phases, $P = C_0/C_w$. For chromatographic testing of the acids *IVa—IVe*, *Va—Ve* and of the arylacetic acids (Table I) we used a thin layer of silanized silica gel (Kieselgel 60, F_{254}, silanisiert, Merck, F.R.G.) impregnated with silicone oil (Lukoil 100). The mobile phase was a mixture of acetone and a citrate buffer (pH 3·40) in a ratio of 1 : 1. The plates were impregnated with a 5% solution of silicone oil in diethyl ether and dried at 20°C for 16 h.

The lipophilicities of the acids *I—V* were characterized by their partition coefficients (log *P*) in the system n-octanol–aqueous acetate buffer. The values of log *P* of the acids *I—III* were calculated from the equation $\log P = \log P_H + \Sigma\pi$, where P_H are the experimentally determined partition coefficints of the corresponding non-substituted acids and $\Sigma\pi$ the sum of parameters of the substituents on the aromatic ring. With the acids *I* and *I'* we used the π parameters derived[16] for arylacetic acids, with the acids *II* and *III* the π parameters derived[16] for benzoic acids. With the 3,4-disubstituted derivatives we bore in mind the decrease in lipophilicity associated with intramolecular interactions of the two substituents at the *ortho*-position. In agreement with the results of partition chromatography of 3,4-disubstituted derivatives of arylaliphatic acids[4,17–19], the sum of the π parameters was reduced by 0·23. In the series of acids *IV* and *V* the values of log *P* were obtained by substituting the experimental values of R_M into equation (*2*), describing the relation between log *P* and experimental values of R_M of arylacetic acids.

TABLE I

Chromatography of arylacetic acids



X	R_F	R_M	log P^a
H	0·755	−0·49	1·45
3-Cl-4-CH_3O	0·70	−0·37	1·91
4-Cl	0·68	−0·33	2·15
4-C_2H_5	0·60	−0·18	2·43
3-Cl-4-CH_2=$CHCH_2O$	0·58	−0·14	2·61
3-Cl-4-i-C_3H_7O	0·487	0·025	2·71
4-i-C_4H_9O	0·46	0·07	2·76
4-i-C_3H_7	0·503	−0·01	2·85
4-t-C_4H_9	0·428	0·125	3·13
4-n-$C_5H_{11}O$	0·367	0·235	3·46
4-i-C_4H_9	0·40	0·18	3·35
3-Cl-4-n-$C_5H_{11}O$	0·32	0·33	3·91
4-n-$C_6H_{13}O$	0·25	0·47	3·96

[a] Values calculated from logarithm of experimental partition coefficient of phenylacetic acid[15,20] and parameters π derived in the series of arylacetic acids[16].

	n	r	s	F	
$\log P = 2{\cdot}563R_M + 2{\cdot}838$	13	0·983	0·144	308	(*2*)

The values of log *P* of the acids *IV*—*IVe* (Table V) obtained from equation (*2*) are close to those calculated by the fragmental method (for the acid *IVa*), with the use of the parameters π (for the derivatives *IVb*—*IVe*). With the acids *V* the values of log *P* thus calculated (Table V) were considerably higher than those obtained from equation (*2*). Similar differences in lipophilicity were previously observed with arylalkoxy derivatives of arylaliphatic acids[4,18,20].

The regression coefficients in equations (*1*)—(*8*) were calculated from experimental data by multiple regression analysis. The statistical significance of equations (*1*)—(*8*) was evaluated by the correlation coefficient *r*, the standard deviation *s* and the Fischer–Snedecor criterion *F*. The individual parameters in the multi-parameter equations were evaluated by the Student t-test on a statistical significance level of $\alpha \leqq 0{\cdot}005$.

Activation of fibrinolysis was assessed by the method of hanging clot[21], prepared from human plasma and suspended in the solution to be tested. The activation was expressed by the minimum molar concentration C^F that dissolves the clot after 24-hour incubation at 37°C.

4-Aryl-3-methyl-2(or 3)-butenoic Acids (*I* and *I'*)

Benzyl chlorides *VI* were obtained by chloromethylation as previously described[22]. 4-Chlorobenzyl chloride was obtained from 4-chlorobenzyl alcohol by its reaction with thionyl chloride[23], followed by distillation; b.p. 96—98°C/2·1 kPa (reported[24] b.p. 114°C (2·85 kPa). Arylacetonitriles *VII* were prepared by reaction of *VI* with sodium cyanide in dimethyl sulphoxide; the yields and b.p. are given in Table II. 2-Acetylarylacetonitriles *VIII* were obtained by reaction of *VII* with ethyl acetate in the presence of sodium ethoxide[25]; the m.p. of the crude products are given in Table II. Boiling of *VIII* in dilute sulphuric acid[26] gave arylacetones *IX* (for b.p. and yields see Table II). A solution of 0·1 mol of sodium methoxide in 25 ml of methanol was added to a mixture of 0·1 mol of triethyl phosphonoacetate and 0·1 mol of IX dissolved in 120 ml of dimethylformamide. The mixture was stirred 4 h at 20°C and 8 h at 110°C. After cooling it was poured into ice-cold water and the product was extracted into ether. The combined ethereal extracts were washed with 5% sodium hydroxide and water, then dried with magnesium sulphate. The ether was distilled off and the mixture of crude exters *X* and *XI'* was hydrolysed by boiling in 200 ml of 10% potassium hydroxide in 50% ethanol. Crystallization from suitable solvents gave mixtures of the acids *I* and *I'*, whose analytical data are given in Table III. The ratios of the acids *I* and *I'* in the mixtures were estimated from the ^{1}H NMR spectra (Table IV).

3-Methyl-3-phenyl-2-propenoic Acids (*II*)

These were prepared from substituted acetophenones by the Reformatski reaction as previously described[11]. The analytical data and yields are given in Table V.

2-Aryl-2-hydroxypropanoic Acids (*IV*)

A mixture of *XI* (0·27 mol) and an ester-chloride of oxalic acid (0·2 mol) in 150 ml of 1,2-dichloroethane was cooled to 10°C, then aluminium chloride (0·28 mol) was added while the temperature was not allowed to exceed 15°C. The mixture was stirred 1 h at 10°C, 4 h at 20°C, then it was poured into 400 ml of water and 100 ml of conc. hydrochloric acid. The product was extracted into 1,2-dichloroethane. The combined extracts were washed with a mixture of aqueous saturated sodium chloride and 10% hydrochloric acid (1 : 1). After drying and removal

TABLE II

Characterization of intermediates *VII*—*IX*

Substituent	*VII*		*VIII*		*IX*		
	b.p., °C/kPa Rep. b.p., °C/kPa	(ref.)	Yield[a] %	M.p., °C	b.p., °C/kPa Rep. b.p., °C/kPa	(ref.)	Yield[b] %
H	—		—	86—87[c]	99/2·00 109—112/3·20	(26)	56·0
4-CH_3O	99—102/0·05 94—97/0·04	(27)	85·0	75—76	76—78/0·05 117—122/0·70	(28)	28·0
3-Cl-4-CH_3O	49—51·5[d]		92·5	147—148	108—110/0·05 100—102/0·03	(29)	64·4
4-Cl	143—145/1·75 137—139/1·60	(30)	87·4	126—127	48—50/0·02 85—86/0·13	(31)	40·0
4-C_2H_5	135—137/2·00 127—130/1 : 86	(32)	72·4	62—65	134—136/2·90 —		21·6
4-i-C_3H_7	139—141/2·00 134—135/1·33	(33)	88·6	73—77	130—134/2·90 137/2·90	(34)	19·6
4-i-C_4H_9	62—64/0·02 113/0·27	(35)	91·0	53—55	132—135/2·25 —		23·7

[a] Yield referred to the substituted benzyl chloride *VI*; [b] yield referred to arylacetonitrile *VII*; [c] rep.[25] m.p. 87—89°C; [d] m.p. of the crude product.

TABLE III

Physico-chemical properties and fibrinolytic capacity of mixtures of acids *I* and *I'*

Number Substituent	M.p., °C Solvent	Yield %	Formula (m.mass)	Calculated/Found % C	 % H	log P^a C^F, mol l^{-1} . 10^3	log $(1/C^F)_{exp}$ log $(1/C^F)_{calc}$ [c]
Ia	99—101	73·0	$C_{11}H_{12}O_2$	74·97	6·87	2·50	1·097
H	70% E		(176·2)	75·06	6·80	80	1·219
Ib + *Ib'*	106—109	60·5	$C_{12}H_{14}O_3$	69·88	6·84	2·51	1·222
4-CH_3O	50% A		(206·2)	69·64	6·95	60	1·226
Ic + *Ic'*	83—85	50·5	$C_{12}H_{13}ClO_3$	59·88	5·44[d]	2·96	1·523
3-Cl-4-CH_3O	30% M		(240·7)	59·96	5·42	30	1·507
Id + *Id'*	85—87	40·0	$C_{11}H_{11}ClO_2$	62·75	5·26[e]	3·20	1·699
4-Cl	H		(210·7)	63·01	5·26	20	1·657
Ie + *Ie'*	120—123/8·5[f]	69·5	$C_{13}H_{16}O_2$	76·44	7·89	3·48	1·699
4-C_2H_5			(204·3)	76·17	7·98	20	1·832
If + *If'*	125—128/10·8[f]	53·0	$C_{14}H_{18}O\delta$	77·03	8·31	3·90	2·222
4-i-C_3H_7			(218·3)	76·85	8·40	6	2·095
Ig + *Ig'*	124—127/8·5[f]	53·6	$C_{15}H_{20}O_2$	77·55	8·68	4·40	2·301
4-i-C_4H_9			(232·3)	77·28	8·79	5	2·407

[a] Values calculated from logarithm of experimental partition coefficient of acid *Ia* (2·50) and parameters π for arylacetic acids[16]; [b] solvents employed: E ethanol, M methanol, A acetic acid, H n-hexane; [c] values calculated from equation (*6*); [d] calculated: 14·43% Cl, found: 14·66% Cl; [e] calculated: 16·83% Cl, found: 16·79% Cl; [f] b.p. (°C/Pa).

of the solvent the arylglyoxylate *XII* was isolated either by distillation (D) or by chromatography on a column of silica gel (Silpearl, Kavalier,Czechoslovakia) using a mixture of benzene and n-hexane as eluent (CC). Further given are; number, substituent, mode of isolation, b.p. or m.p., yield, purity by gas chromatography: XII, H, D, 103–105°C/0·27 kPa, rep.[36] b.p. 130°C/1·33 kPa, 98·0%, 99·6%; *XIIb*, 4-OCH_3, D, 133–135°C/0·2 kPa, 66·5%, 96·5%; *XIIc*, 3-Cl-4-OCH_3, D, 125–127°C/0·1 kPa, 56·2%, 98·5% *XIId*, 4-C_6H_5, CC, 35—36°C, ref.[37] m.p. 38–39°C, 51·5%, 99·4%; *XIIe* 4-i-C_4H_9, D,130–132°C/0·2 kPa, ref.[38] b.p. 138–141°C/0·4 kPa, 79·4%, 97·0%.

To 0·2 mol of *XII* dissolved in 50 ml of ether, cooled to −5°C, was added a solution of methyl magnesium iodide, prepared from 0·255 mol of methyl iodide and 0·278 gramatoms of magnesium; the temperature did not exceed 0°C. The mixture was stirred 4 h at 0°C and 3 h at 20°C, then poured into 900 ml of water and 70 ml of conc. sulphuric acid. The ester *XIII* was extracted into ether and the combined ethereal extracts were washed with a solution of sodium pyrosulphite and water. After drying and evaporation of ether the oily residue was purified on a column of silica gel. The ester *XIII* thus obtained, purity 96–98% (gas chromatography), was hydrolysed by boiling for 4 h in a solution 25 g of potassium carbonate in 200 ml of 50% ethanol. The ethanol was distilled off and the alkaline solution was made up to 200 ml with water, filtered with activated carbon and acidified with sulphuric acid. The product was crystallized from a suitable solvent (Table VI).

2-Aryl-2-arylmethoxyacetic Acids (*V*)

To a suspension of sodium hydride (0·125 mol in the form of an 80% dispersion in paraffin oil) in 50 ml of dimethylformamide was added 0·1 mol of methyl 2-phenyl-2-hydroxyacetate dissolved in 50 ml of dimethylformamide. The mixture was heated to 45°C for 10 min, cooled to 15°C

TABLE IV

^{1}H NMR spectra of mixtures of acids *I* and *I'*

Number	δ_H (bs)	δ_{CH_2} (s)	δ_{CH_2} (d) $J = 1·0$ Hz	% *I* % *I'*
Ia	6·45	3·25	2·00	100
Ib + *Ib'*	6·45, 6·32, 5·78, 5·69	3·35, 3·22, 3·18	2·10, 1·95, 1·80	89·9 21·1
Ic + *Ic'*	6·38, 6·25, 5·78, 5·65	3·31, 3·15	2·10, 1·90, 1·78	49·4 50·6
Id + *Id'*	6·41, 6·30, 5·77, 5·63	3·91, 3·36, 3·13	2·04, 1·92, 1·89, 1·75	76·1 23·9
Ie + *Ie'*	6·48, 6·38, 5·78, 5·70	3·97, 3·40, 3·21, 3·18	2·10, 1·95, 1·79	66·1 33·9
If + *If'*	6·48, 6·38, 5·77, 5·70	3·96, 3·39, 3·21, 3·14	2·19, 1·92, 1·78	59·4 40·8
Ig + *Ig'*	6·48, 6·38, 5·78, 5·70	3·96, 3·38, 3·23, 3·17	2·10, 1·95, 1·79	59·7 40·3

TABLE V

Physico-chemical properties and fibrinolytic capacity of acids *II*

Number Substituent	M.p., °C solvent	Yield[a] %	Formula (m.mass)	Calculated/Found % C	Calculated/Found H H	log P^b C^F, mol l^{-1} . 10^3	log $(1/C^F)_{exp}$ log $(1/Cv)_{calc}$[c]
IIa	95—96[d]	52·2	$C_{10}H_{10}O_2$	74·75	6·22	2·42	1·097
H	50% methanol		(162·2)	74·24	6·08	80	1·169
IIb	150—152[e]	30·0	$C_{11}H_{12}O_3$	68·73	6·30	2·50	1·155
4-CH_3O	60% methanol		(192·2)	68·99	6·48	70	1·219
IIc	130—131[f]	47·3	$C_{11}H_{12}O_2$	74·97	6·86	2·90	1·398
4-CH_3	70% methanol		(176·2)	74·97	6·77	40	1·469
IId	148—150	39·2	$C_{10}H_9BrO_2$	49·81	3·76[g]	3·41	1·699
3-Br	90% methanol		(241·1)	49·90	3·60	20	1·788
IIe	87—89	42·0	$C_{13}H_{16}O_2$	76·44	76·44	3·82	2·222
4-i-C_3H_7	70% methanol		(204·3)	76·67	7·99	6	2·045
IIf	111—113	49·0	$C_{14}H_{18}O_2$	77·03	8·31	4·32	2·301
4-i-C_4H_9	90% methanol		(218·3)	66·29	8·21	5	2·357

[a] Yield referred to acetophenone; [b] values calculated from logarithm of experimental partition coefficient of acid *IIa* (2·42) and parameters for benzoic acids[16]; [c] values calculated from equation (*6*); [d] rep.[11] m.p. 95°C; [e] rep.[11] m.p. 152°C; [f] rep.[11] m.p. 133°C; [g] calculated: 33·15% Br; found 33·20% Br.

TABLE VI

Physico-chemical properties and fibrinolytic capacity of acids *IV* and *V*

Number Substituent	M.p., °C solvent[c]	Yield[a] %	Formula (m.mass)	Calculated/Found % C	 % H	R^F R_M	log P_{exp}[b] log P_{calc}[d]	C^F mol l^{-1} . 10^3	log $(1/C^F)_{exp}$ log $(1/C^F)_{calc}$[e]
IVa	91—92[f,g]	84·0	$C_9H_{20}O_3$	61·70	6·33	0·86	0·82	100	1·000
H	T–H 2 : 1	42·0	(166·2)	61·84	6·08	−0·79	0·91		—
IVb	121—123[h]	66·5	$C_{10}H_{12}O_4$	61·21	6·17	0·84	0·99	100	1·000
4-CH_3O	T	82·4	(196·2)	61·34	6·14	−0·72	0·92		—
IVc	121—123[f]	56·2	$C_{10}H_{11}ClO_4$	50·11	5·05[i]	0·805	1·25	100	1·000
3-Cl-4-CH_3O	T	69·5	(230·7)	50·38	4·86	−0·62	1·37		—
IVd	106—107[j]	66·5	$C_{13}H_{18}O_3$	70·24	8·16	0·50	2·84	100	1·000
4-i-C_4H_9	B–H 1 : 2	86·0	(222·3)	70·47	8·03	0	2·81		1·437
IV	171—173[k]	51·5	$C_{15}H_{14}O_3$	74·36	5·82	0·533	2·71	100	1·000
4-C_6H_5	T	56·8	(276·7)	74·18	5·79	−0·05	2·81		1·356
Va	157—159	34·4	$C_{15}H_{14}O_3$	74·36	5·82	0·685	1·97	40	1·398
H	B–H 1 : 4		(276·7)	74·56	5·71	−0·34	2·71		1·356
Vb	132—134	24·0	$C_{16}H_{15}ClO_4$	62·65	4·93[l]	0·605	2·36	20	1·699
3-Cl-4-CH_3O	B–H 1 : 4		(306·7)	63·09	4·07	−0·185	3·32		1·734
Vc	204—206	65·0	$C_{15}H_{13}ClO_3$	65·10	4·74[m]	0·568	2·57	10	2·000
4-Cl	B–E–H 2 : 1 : 3		(276·7)	65·27	5·01	−0·11	3·57		1·889

[a] With acids *IV* the first number denotes the yield of ester *XII*, the second the yield of acid *IV* referred to the starting *XII*; [b] values of log *P* calculated from equation (*2*) by substituting experimental values of R_M; [c] solvents employed: T toluene, H n-hexane, B benzene, E ethyl acetate; [d] values of log *P* calculated by the fragmentation method[41] (for acids *IVa*, *Ca*), using parameters for arylacetic acids and/or for benzyl alcohol[16]; [e] values calculated from equation (*1*); [f] crystallizes as hemihydrate; [g] rep.[39] m.p. 88—90°C; [h] rep.[40] m.p. 128—139°C; [i] calculated: 14·96% Cl; found: 14·80% Cl; [j] rep.[38] m.p. 107—106·5°C; [k] rep.[36,37] m.p. 168—169°C; [l] calculated: 11·56% Cl; found: 11·49% Cl; [m] calculated: 12·81% Cl; found: 12·60% Cl.

and a solution of 0·11 mol of the corresponding benzyl chloride was added. After stirring for 16 h at 20°C the mixture was poured into 1 200 ml of water and brought to pH 3 with hydrochloric acid. The separated oil was extracted into ether. The combined ethereal extracts were washed with water, dried with magnesium sulphate and distilled. The oily residue (the crude ester) was stirred in a mixture of 200 ml of 2M-NaOH and 100 ml of ethanol at 20°C for 34 h, then at 50°C for 1 h. The ethanol was distilled off, the residue was filtered with activated carbon and the filtrate was acidified with dilute sulphuric acid (1 : 1) at 5°C. The separated crude product *V* was crystallized from a suitable solvent (Table VI).

RESULTS AND DISCUSSION

The reactions of arylacetones *IX* with triethyl phosphonoacetate gave mixtures of esters *X* and *X'*, whose hydrolysis resulted in mixtures of 4-aryl-3-methyl-2-butenoic acids (*I*) and 4-aryl-3-methyl-3-butenoic acids (*I'*). The fact that the product was not a single compound was demonstrated by the multiple signals in the ^{1}H NMR spectra for the substituents (H, CH_2, CH_3) on the double bond. The signals corresponding to the substituents are given in Table IV. The elemental composition (described by the molecular formula of the acids *I*) and the nature of the ^{1}H NMR spectra suggest a mixture of the isomeric acids *I* and *I'*. The analogous character of the ^{1}H NMR spectra of crude esters *X* and *X'* proves that even in the reaction of an arylacetone *IX* with triethyl phosphonoacetate there occurs isomerization *in situ*, giving rise to the esters *X* and *X'*.

To distinguish quantitatively between the structures *I* and *I'* we used the signals δ_H for hydrogen on the double bond. In the acids *I* the hydrogen atom is at geminal position to the carboxyl group. Compared to the olefinic hydrogen in the isomers *I'*, its signal is shifted, as a result of strong shielding, to lower values of the field. The bulky substituent —CH_2COOH at the *cis* position of acids *I'* prevents the aromatic ring and the double bond from being coplanar. Consequently, the ring is in a twisted conformation toward the double bond and the olefinic hydrogen at the geminal position is strongly shielded by the aromatic ring. Besides, the individual signals of olefinic hydrogens in the two structures *I* and *I'* are doubled as a result of isomerism on the double bond. In view of the too small differences in δ the individual isomers cannot be determined quantitatively. The ratios of *I* : *I'* were calculated from the sums of isomers *Z* and *E* of the individual structures; the values are given in the last column of Table IV.

The non-substituted derivative was the only substance obtained after crystallization as a pure compound, its ^{1}H NMR spectrum corresponded to 4-phenyl-3-methyl-2-butenoic acid (*Ia*). The other derivatives were assessed in activation of fibrinolysis in the form of mixtures of acids *I* and *I'*. To characterize the lipophilicity of these acids we used log *P* of *Ia* as the first approximation, assuming that the lipophilicities of the isomers *I* and *I'* are not much different. This assumption seems plausible, since the values of log *P* calculated by the fragmental method[41] from equations (*3*) and (*4*) are very close.

$$\log P(Ia) = f(C_6H_5) + f(CH_3) + f(CH) + f(CH_2) + F_{=} + 4F_b + \\ + F_{cBr} + f(COOH)^{1/3\varnothing} = 1{\cdot}90 + 0{\cdot}89 + 0{\cdot}43 + 1{\cdot}32 - 0{\cdot}55 - 0{\cdot}48 - \\ - 0{\cdot}13 - 0{\cdot}75 = 2{\cdot}63 \qquad (3)$$

$$\log P(Ia') = f(C_6H_5) + f(CH_3) + f(CH) + 2f(CH_2) + F_{=}^{\varnothing} + 4F_b + \\ + F_{cBr} + f(COOH)^{1R} = 1{\cdot}90 + 0{\cdot}89 + 0{\cdot}43 + 1{\cdot}32 - 0{\cdot}42 - 0{\cdot}48 - \\ - 0{\cdot}13 - 1{\cdot}03 = 2{\cdot}48 \qquad (4)$$

Experimental results of activation of fibrinolysis in the series of acids *I* and *II* were processed by regression analysis. We obtained equation (*5*), whose slope and intercept are very close to those in equation (*1*), derived[7,9] from the six preceding series of arylaliphatic acids. The overall regression analysis, including the acids *I* and *II*, led to equation (*6*)

	n	*r*	*s*	*F*	
$\log(1/C^F) = 0{\cdot}655 \log P - 0{\cdot}471$	13	0·980	0·097	266·7	(*5*)
$\log(1/C^F) = 0{\cdot}625 \log P - 0{\cdot}348$	108	0·962	0·127	1 307·4	(*6*)

The fibrinolytic capacities of the acids *I* and *I'* are probably independent of position of the double bond in the chain between the carboxyl group and the aromatic ring. From comparing the capacities of the acids *II* on the one hand and 2-methyl-3-phenyl-2-propenoic acids[4] on the other, it is also obvious that the fibrinolytic capacity is not influenced by the position of the methyl group on the double bond. It appears that the acids *I*, *I'* and *II* activate fibrinolysis by a mechanism similar to that observed with the series of arylaliphatic acids studied previously. This mechanism does not seem to be of general character, since the fibrinolytic capacities of the acids *III* an *IV* do not accord with the previously derived equation (*1*). Regression analysis of the experimental results of acids *III* (Table VII) afforded equations (*7*) and (*8*). The fibrinolytic capacities of the acids *III* depended on lipophilicity only, but the dependence was parabolic, in contrast to the results with the arylaliphatic acids studied previously. The region of linear dependence on lipophilicity was shifted to a higher efficacy, but at the lipophilicity corresponding to $\log P > 4$ the acids *III* were already weaker in fibrinolytic capacity than arylaliphatic acids of the same lipophilicities.

	n	*r*	*s*	*F*	
$\log(1/C^F) = 0{\cdot}453 \log P + 0{\cdot}398$	10	0·946	0·143	68·6	(*7*)
$\log(1/C^F) = 1{\cdot}709 \log P - 0{\cdot}193 (\log P)^2 - 1{\cdot}505$	10	0·982	0·089	84·5	(*8*)

In the series of acids *IV* the presence of a hydroxyl group in the connecting chain is also obviously responsible for a decrease in fibrinolytic capacity. With the lipophilic derivatives *IVd* and *IVe* fibrinolysis was not activated even at a concentration of 0·1 mol l^{-1}, although the fibrinolytic capacity $\log (1/C^F)_{calc}$ calculated for the given lipophilicity from equation (*1*) is markedly higher. The replacement of the hydroxyl by a benzyloxy group in the acids *V* manifested itself by an increase in fibrinolytic capacity, so that the experimental data were comparable with the values calculated from equation (*1*). To calculate $\log (1/C^F)_{calc}$ (Table VI) we employed the values

TABLE VII
Fibrinolytic capacity of acids *III*

Number	Y X	log P^a	C^F mol l^{-1} . 10^3	$\log (1/C^F)_{exp}$ $\log (1/C^F)_{calc}$ [b]
IIIa	CH_2CH_2 4-i-C_3H_7O	2·11	40	1·398 1·242
IIIb	CH_2CH_2 4-Br	2·21	50	1·301 1·331
IIIc	CH_2CH_2 4-i-C_3H_7	2·63	30	1·523 1·654
IIId	CH_2CH_2 3-Cl-4-i-C_3H_7O	2·71	20	1·699 1·709
IIIe	CH_2CH_2 4-i-C_4H_9	3·13	10	2·000 1·953
IIIf	CH_2CH_2 3-Cl-4-c-C_6H_{11}	4·52	6	2·222 2·276
IIIg	$CH_2CH(CH_3)$ 4-c-C_6H_{11}	41·9	5	2·301 2·267
IIIh	$CH_2CH_2CH_2$ 3-Cl-4-CH_2O	2·18	60	1·222 1·303
IIIi	$CH_2CH_2CH_2$ 4-i-C_4H_9	3·40	8	2·097 2·075
IIIj	$CH_2CH(CH_3)CH_2$ 4-C_6H_5	3·85	6	2·222 2·214

[a] Values calculated from experimental partition coefficients of the corresponding non-substituted acids (P_H) and parameters π derived for benzoic acids[16]. Employed values of log P_H were: 1·23 (ref.[12]) for acids *IIIa—f*; 1·73 for *IIIg*; 1·50 for *IIIh*, *i*; 1·95 for *IIIj*. [b] Values calculated from equation (*8*).

of log P_{calc} obtained from the tabulated parameters of lipophilicity, as we had done in the case of arylalkoxyaryliphatic acids[4,18,20]. These values prove to be in better accordance with the experimental capacity log $(1/C^F)_{exp}$ than the values of log P_{exp}, which include the decrease in lipophilicity caused by interaction of the aromatic rings. This results is in agreement with the previous conclusions[7,9,42], demonstrating that the acids bind to the surface of the biomacromolecule on the site of activation of fibrinolysis, so that interaction of the aromatic rings is not likely.

To conclude it can be stated that the presence of a functional group in the chain linking the carboxyl and the aromatic ring adversely affects the fibrinolytic capacity. It cannot be ruled out that the decrease in fibrinolytic capacity is associated with the power of functional group to form hydrogen bonds, this is strongest in the hydroxyl group, whereas in the benzyloxy group it is much weaker[43,44].

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