PREPARATION AND BIOLOGICAL PROPERTIES OF *p*-ALKOXYCINNAMIC ACIDS; USE OF QUANTITATIVE RELATIONSHIPS BETWEEN STRUCTURE AND ACTIVITY

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A series of *p*-alkoxycinnamic acids and their *m*-chloro derivatives was prepared by Wittig reaction of substituted benzaldehydes with ethoxycarbonylmethylene phosphorane or with α-ethoxycarbonylethylidene phosphorane. Their activity in stabilizing the membrane of rat erythrocytes against hypotonic hemolysis and their antiinflammatory action in the kaolin edema test were evaluated. *p*-Alkoxycinnamic acids were prepared on the basis of conclusions reached by analysis of the quantitative relationship between structure and stabilizing activity of a series of substituted cinnamic acids, the synthesis and biological properties of which were described elsewhere.

Recently[1] we described the synthesis and the biological properties of a series of cinnamic acids substituted at the aromatic ring and branched at the α-position toward the carboxyl.* Together with their antiinflammatory action we determined their efficiency in stabilizing rat erythrocytes toward hypotonic hemolysis which is of importance as one of the orientation criteria of the antiinflammatory effect[2]. The stabilizing activity was now expressed as the function of physico-chemical properties of the above acids. Biological activity was correlated with chemical structure as proposed by Hansch and coworkers[3-5], using constants which characterize the contribution of the substituents to the physico-chemical properties of the whole molecule. The equation which simultaneously analyzes the contributions of substituents of electronic, steric and hydrophobic nature to biological activity is as follows:

$$\log(1/C) = a\pi - b\pi^2 + c\sigma + dE_S + e\,, \qquad (1)$$

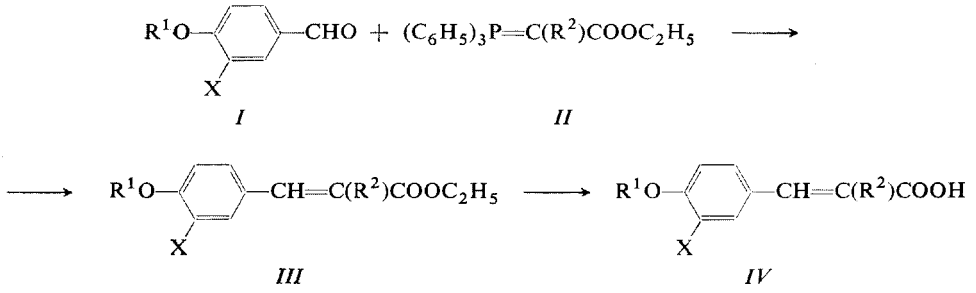
where C is the concentration which brings about the same biological effect, σ are the Hammett polar constants and E_S are steric parameters. With dissociating compounds, the polar constant σ can be replaced with pK. Parameter π is defined[3] as

$$\pi = \log P_X - \log p_H\,, \qquad (2)$$

* In this communication they are designated with arabic numerals *1—34*, to contrast them from the newly synthesized *p*-alkoxycinnamic acids and their *m*-chloro derivatives *IVa—IVm*.

where P_X and P_H are the partition coefficients in a system of *n*-octanol–water for the substituted and unsubstituted compound, respectively, and which represents the contribution of the substituent to the general lipophilic character of the molecule.

It was the aim of the present study to predict the optimally active compounds in the series of cinnamic acids studied. To check this prediction, we synthesized suitably substituted derivatives and determined their stabilization activity and antiinflammatory effect. We prepared cinnamic acids substituted at the aromatic ring with an alkoxy group in the *para* position in combination with a halogen in the *meta* position. For their preparation we made use of the Wittig reaction[6,7] of substituted benzaldehydes *I* with the corresponding α-ethoxycarbonylalkylidene phosphoranes *II*. Subsequent hydrolysis of the ethyl ester *III* led to the cinnamic acids *IV* (Scheme 1).



SCHEME 1

EXPERIMENTAL

Methods

The IR spectra of *p*-alkoxycinnamic acids *IV* were recorded in the range from 400 to 4000 cm^{-1} in KBr pellets using a UR 20 (Zeiss, Jena) spectrophotometer. The p*K* values of the original series of cinnamic acids were determined conductometrically[8] in water using a OK-102/1 conductometer (Radelkis, Hungary) in the concentration range of $2 \cdot 10^{-4}$—$1 \cdot 10^{-3}$M. In view of the low solubility of cinnamic acids in water, the determination was done with only 12 substances (*3*, *5*, *7*, *12*, *14*, *15*, *18*, *20*, *21*, *25*, *31*, *33*). From the experimental data obtained the following equations were derived:

	n	*s*	*r*	*F*	
$pK = -0{\cdot}4752\sigma - 1{\cdot}0172\sigma^* + 4{\cdot}9637$	12	0·0704	0·9768	93·68,	(*3*)
$pK = -0{\cdot}4583\sigma - 0{\cdot}4138E_s + 4{\cdot}9664$	12	0·0513	0·9878	100·39,	(*4*)

where σ are the Hammett constants of the aromatic substituents, (Table I), σ^* are the Taft constants of the α-substituents, E_S are the steric parameters of the α-substituents (Table II), *n* is the number of compounds, *r* the correlation coefficient, *s* the standard deviation and *F* the Fischer–Snedecor criterion.

Because of the close relationship between the steric parameters E_S and the σ^* constants in the given series of α-substituents* it is impossible to decide unequivocally the character of the effect of α-substituents on dissociation. Eq. (*4*) was used for computing the values of pK used in biological correlations and, together with the experimental values, they are shown in Table III. The variables ΔpK were calculated as $\Delta pK = 4{\cdot}96 - pK$, the value of 4·96 being the pK of α-methylcinnamic acid. The pK values of the alkoxycinnamic acid *IV* were determinated in 80% methyl cellosolve using a Titrigraph potentiometer (Radiometer, Copenhagen). The melting points were determined in a Böetius M block and are not corrected.

For substituents at the benzene ring, the π parameters were used as derived for benzoic acids[9], the alkyl values at the C_α atom being computed from the increments $\Delta\pi = 0{\cdot}5$ for CH_2 and $\Delta\pi = -0{\cdot}2$ for branching[10]. The value of parameter π for *p*-dimethylamino group was obtained

TABLE I

Parameters of Aromatic Substituents

Substituent	σ^a	π^b	Substituent	σ^a	π^b
p-NO_2	0·78	0·02	*m*-CH_3O	0·11	0·14
m-NO_2	0·71	−0·05	H	0	0
m-Br	0·39	0·99	*p*-$(CH_3)_2CH$	−0·15	1·40
m-Cl	0·37	0·83	*p*-CH_3	−0·17	0·45
p-Br	0·27	0·98	*p*-CH_3O	−0·27	0·08
p-Cl	0·23	0·87	*p*-$(CH_3)_2N$	−0·83	−0·28[c]
p-I	0·18	1.14			

[a] Values from ref.[12]; [b] values from ref.[9]; [c] parameter π calculated from $\pi = f(R_M, pK)$; see ref.[11].

TABLE II

Parameters of α-Substituents

Substituent	σ^{*a}	E_s^a	$\Delta\pi^b$	D^c
H	0·49	1·24	0	0
CH_3	0	0	0·3	3
C_2H_5	−0·10	−0·07	0·8	2
n-C_3H_7	−0·11	−0·36	1·3	2

[a] Values from ref.[13]; [b] values from ref.[10]; [c] formal variables corresponding to the number of hydrogen atoms at the carbon linked to a double bond.

* This relationship is expressed by $E_S = 2{\cdot}4629\sigma^* + 0{\cdot}0301$ ($n = 4$, $s = 0{\cdot}1351$, $r = 0{\cdot}9878$, $F = 80{\cdot}65$).

from the relation between π and R_M of partition chromatography[11]. Together with the values of polar constants and of steric parameters they appear in Table II.

The coefficients in the regression equations were computed from the experimental data by a multiple regression analysis using the least-square method. The statistical significance of the individual parameters was checked by the *F*-test at a selected level of significance, gradually increasing the number of parameters used. With all the equations derived here the statistical significance of regression lies at the 99% level. When working with multi-parameter equations there is the risk of accidental correlations unless there are sufficient experimental data available. The analysis carried out in ref.[14] showed that if at least 5 experimental values are available per variable, the probability of occurrence of accidental correlations is very low. This prerequisite has been met for all the equations shown here.

Determination of the stabilizing efficiency for rat erythrocyte membranes was done according to Kalbhen and coworkers[15], modified by using whole rat blood[1]. The efficiency was expressed as concentration in mol/litre, bringing about 50% inhibition of hemolysis (see column 5 of Table III). The determination of the antiinflammatory effect in the kaolin edema test was done according to Selye[16] using female rats of Wistar strain, weighing 130—150 g. Each compound was evaluated on a group of seven animals and the results were subjected to the t-test at a 95% level of significance. The effect is expressed as % inhibition of inflammation as compared with the untreated group.

Compounds Used

p-*Alkoxybenzaldehydes* I were prepared by alkylation of *p*-hydroxybenzaldehyde[17] and isolated by vacuum distillation. *p*-Isopropoxybenzaldehyde: b.p. 68—69°C/0·5 Torr (ref.[18] reports a b.p. of 135—136°C/16 Torr); *p*-allyloxybenzaldehyde b.p. 110—112°C/2·8 Torr (ref.[19] reports a b.p.

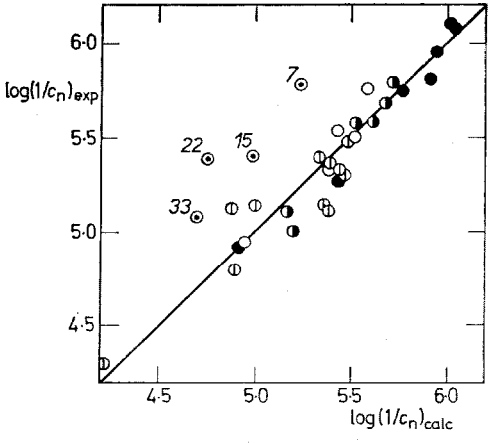


FIG. 1

Stabilizing Activity of Cinnamic Acids in Neutral Form

log $(1/C_n)_{calc}$ calculated from Eq. (*10*); ● α-unsubstituted, ◑ α-methyl, ○ α-ethyl, ⓘ α-n-propyl; ⊙ *p*-methoxy derivatives.

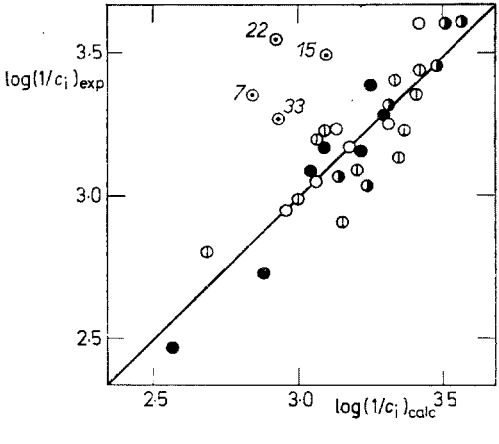


FIG. 2

Stabilizing Activity of Cinnamic Acids in Ionic Form

log $(1/C_i)_{calc}$ calculated from Eq. (*11*), ● α-unsubstituted, ◑ α-methyl, ○ α-ethyl, ⓘ α-n-propyl; ⊙ *p*-methoxy derivatives.

of 106°C/2·0 Torr); *p*-isobutoxybenzaldehyde: b.p. 105–106°C/1·4 Torr (ref.[20] reports a b.p. of 258°C); *p*-cyclohexyloxybenzaldehyde: b.p. 126–128°C/0·5 Torr, m.p. 91–93°C, for $C_{13}H_{16}O_2$ (204·3) calculated: 76·44% C, 7·90% H; found: 76·70% C, 8·15% H; *p*-benzyloxybenzaldehyde: b.p. 143–145°C/0·1 Torr, m.p. 69–70°C (ref.[20] reports m.p. of 72°C).

m-*Chloro*-p-*alkoxybenzaldehydes* I were prepared by oxidation of the corresponding benzyl chlorides by urotropin[21] and isolated by vacuum distillation. *m*-Chloro-*p*-methoxybenzaldehyde: b.p. 122–124°C/4·1 Torr, m.p. 48°C; *m*-chloro-*p*-allyloxybenzaldehyde: b.p. 116–118°C/0·4 Torr, for $C_{10}H_9ClO_2$ (196·6) calculated: 61·08% C, 4·58% H, 18·03% Cl; found: 61·35% C, 4·37% H, 17·70% Cl; *m*-chloro-*p*-isopropoxybenzaldehyde: b.p. 98–100°C/0·5 Torr, for $C_{10}H_{11}ClO_2$ (198·65) calculated: 60·46% C, 5·58% H, 17·85% Cl; found: 60·82% C, 5·65% H, 17·48% Cl; *m*-chloro-*p*-isobutoxybenzaldehyde: b.p. 108–110°C/1·0 Torr, for $C_{11}H_{13}ClO_2$ (212·7) calculated: 62·12% C, 6·16% H, 16·67% Cl; found: 62·18% C, 6·32% H, 16·58% Cl.

Triphenylethoxycarbonylmethylene phosphorane (IIa) was prepared according to Denney and Ross[22], triphenyl-α-ethoxycarbonylethylidene phosphorane (*IIb*) was prepared according to Isler and coworkers[23], m.p. 165–167°C (ref.[23] reports a m.p. of 156–157°C). For $C_{23}H_{23}PO_2$ (362·3) calculated: 76·15% C, 6·39% H, 8·54% P; found: 76·38% C, 6·38% H, 8·37% P.

p-*Alkoxycinnamic acids and their* m-*chloro derivatives* IV: a solution of 0·05M phosphorane *IIa* in 250 ml was added at 20°C to 0·05M substituted benzaldehyde *II* in 50 ml benzene. The mixture was heated under stirring in an atmosphere of nitrogen for 10 h, on the following day it was concentrated *in vacuo* and the residue was extracted with 150 ml of a 3 : 1 mixture of light petroleum (b.p. 60–65°C) and ether. After separation of the insoluble fraction, the filtrate was evaporated *in vacuo* and the crude ethyl ester of cinnamic acid (*III*) thus obtained was combined with 200 ml aqueous ethanol (1 : 1) containing 20 g potassium hydroxide. The mixture was boiled for 3 h and then evaporated to half its volume. After dilution with 150 ml water, the solution was filtered at 80°C with charcoal. After cooling to 0°C, the crude product was filtered, washed with water and crystallized from methanol (*IVb, c, d, e, f, g, i*) or from a 2 : 1 mixture of methanol and water (*IVa, h*). In the same way, *p*-alkoxy-α-methylcinnamic acids were prepared from phosphorane *IIb* and substituted benzaldehydes *I*; the acids were purified by crystallization from aqueous methanol (2 : 1). Their properties are shown in Table II.

N-*Methylpiperazinium salts of cinnamic acids* V: A solution of 22·0 mmol N-methylpiperazine in 20 ml acetone was added at 20°C to a solution of 15·0 mmol cinnamic acid in 150 ml acetone. The precipitate formed was filtered after 12 h in the refrigerator, washed with acetone and characterized by its melting point and elementary analysis (Table V).

RESULTS AND DISCUSSION

The values of stabilizing efficiency of the original series of cinnamic acids are shown in Table III. Since the acids studied are partly dissociated under the conditions of estimation of biological activity the expressions derived by Fujita[24,25] were used for expressing the efficiency of the undissociated and the dissociated form:

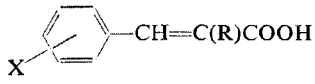
$$\log(1/C_n) = \log(1/C) + \log\{([H^+] + K_a)/[H^+]\}, \quad (5)$$

$$\log(1/C_i) = \log(1/C) + \log\{([H^+] + K_a)/K_a\}, \quad (6)$$

where C_n is the concentration of acid in the undissociated form, C_i in the anionic

TABLE III

Stabilization of Erythrocyte Membranes by Cinnamic Acids



Number	X	pK^a	ΔpK	C^b M . 10^3	log (1/C)	log(1/C_n)	log(1/C_i)
α-Unsubstituted R=H							
1	*p*-NO_2	4·08	0·88	0·672	3·173	6·093	3·174
2	*m*-NO_2	4·12	0·84	0·828	3·082	5·963	3·083
3	*m*-Cl	4·27 (4·33)	0·69	0·405	3·392	6·123	3·393
4	*p*-Cl	4·34	0·62	0·720	3·148	5·809	3·149
5	H	4·45 (4·40)	0·51	1·890	2·724	5·272	2·725
6	*p*-$(CH_3)_2CH$	4·52	0·44	0·525	3·280	5·761	3·281
7	*p*-CH_3O	4·57 (4·57)	0·39	0·449	3·348	5·780	3·350
8	*p*-$(CH_3)_2N$	4·83	0·13	3·455	2·462	4·635	2·465
α-Methyl-substituted R=CH_3							
9	*m*-NO_2	4·63	0·33	0·483	3·316	5·688	3·318
10	*m*-Cl	4·79	0·17	0·254	3·595	5·808	3·598
11	*p*-Cl	4·86	0·10	0·712	3·447	5·590	3·450
12	H	4·96 (4·94)	0	0·862	3·069	5·113	3·072
13	*p*-$(CH_3)_2CH$	5·03	−0·07	0·245	3·611	5·586	3·616
14	*p*-CH_3	5·04 (5·12)	−0·08	0·936	3·028	4·993	3·033
15	*p*-CH_3O	5·09 (5·13)	−0·13	0·326	3·486	5·401	3·491
α-Ethyl-substituted R=C_2H_5							
16	*p*-NO_2	4·66	0·30	0·678	3·169	5·511	3·171
17	*m*-NO_2	4·70	0·26	0·588	3·231	5·533	3·233
18	*m*-Cl	4·85 (4·85)	0·11	0·238	3·623	5·771	3·626
19	*p*-Cl	4·92	0·04	0·570	3·244	5·328	3·248
20	H	5·03 (4·93)	−0·07	1·135	2·945	4·920	2·950
21	*p*-CH_3	5·11 (5·08)	−0·15	0·880	3·055	4·951	3·061
22	*p*-CH_3O	5·15	−0·19	0·291	3·536	5·392	3·542
α-n-Propyl-substituted R = n-C_3H_7							
23	*p*-NO_2	4·74	0·22	0·851	3·070	5·332	3·072
24	*m*NO_2	4·77	0·19	1·277	2·894	5·127	2·897
25	*m*-Br	4·92 (4·91)	0·04	0·372	3·430	5·514	3·434
26	*m*-Cl	4·92	0·04	0·578	3·238	5·322	3·242
27	*p*-Br	5·00	−0·04	0·743	3·129	5·133	3·133
28	*p*-Cl	5·00	−0·04	0·401	3·397	5·401	3·401

TABLE III
(*Continued*)

29	*p*-I	5·02	−0·06	0·442	3·355	5·340	3·360
30	*m*-CH_3O	5·05	−0·09	0·635	3·197	5·152	3·202
31	H	5·10 (5·10)	−0·14	0·598	3·223	5·128	3·228
32	*p*-CH_3	5·18	−0·22	1·055	2·977	4·804	2·984
33	*p*-CH_3O	5·23 (5·24)	−0·27	0·499	3·302	5·079	3·309
34	*p*-$(CH_3)_2N$	5·49	−0·53	1·588	2·799	4·322	2·812

[a] In parentheses are shown pK values determined conductometrically. [b] Concentration causing 50% inhibition of hemolysis.

form, C being the total concentration, K_a the dissociation constant of the acid and $[H^+]$ the concentration of hydrogen ions in the external medium, *i.e.* 10^{-7}M in our experiments. Relationships between the stabilizing efficiency of the cinnamic acids in the undissociated form and the physico-chemical quantities are described by equations (*7*)–(*10*) (Table VI). In all the correlation equations, the *p*-methoxy derivatives have been omitted since they deviate clearly from the correlation relationships. Eq. (*7*) expresses the efficiency as a function of ΔK which replaces the polar effects of the substituents at the aromatic ring, as well as the inductive and steric effects of the substituents in α-position with respect to the carboxyl. By taking into account the hydrophobic effects of these substituents (introducing the sum of the appropriate π parameters) we obtained Eq. (*8*). In Eq. (*9*) a further improvement of correlation was achieved by introducing formal variables D which correspond to the number of hydrogen atoms at the carbon linked by a double bond. Finally, Eq. (*10*) which expresses best the above relationship, includes also the steric parameters of the α-substituents.*

Using the same approach, we analyzed the stabilizing effects of cinnamic acids in the anionic form and obtained Eq. (*11*). Its statistical criteria are less demanding than with Eq. (*10*) but a role is played here by the narrower range of values of corrected activity log $(1/C_i)$. Even if both Eq. (*10*) and (*11*) express the relationship between the structure of cinnamic acid and their stabilizing activity, the available data do not permit to decide[24] whether the cinnamic acids are active in both the undissociated and in the anionic form. However, for both forms the dependence on the physico-chemical parameters taken into account is rather similar. The efficiency of cinnamic acids not corrected for their dissociation is expressed as a function of physico-chemical parameters by Eq. (*12*). A graphical representation of Eq. (*10*) and (*11*) is

* In view of the mentioned relationship between E_S and σ^* one can obtain an equation with similar statistical criteria by introducing the polar constants σ^* instead of the parameters E_S.

shown in Figs 1 and 2. One may observe the deviation of the *p*-methoxy derivatives (compounds *7*, *15*, *22*, *33*) toward greater activity than would correspond to the physico-chemical properties considered in the regression equations.

The dependence of stabilizing activity on the formal variables *D* of the substituents at the C_α atom documents the different effect of α-substitution on dissociation and stabilizing activity of cinnamic acids. The same is demonstrated by the statistical

TABLE IV

Properties of *p*-Alkoxycinnamic Acids *IV*

Number	X	M.p., °C	pK^a	ν(KBr)^b	Calculated/Found		
R^1	R^2	yield, %			% C	% H	% Cl
IVa	H	154·5—155·5	6·65	1 620	69·88	6·84	—
i-C_3H_7	H	72·0		1 695	69·59	6·65	—
IVb	H	159·5—160·5	6·55	1 610	70·57	5·92	—
CH_2=$CHCH_2$	H	84·2		1 690	70·71	5·81	—
IVc	H	167—168[c]	6·42	1 610	70·88	7·32	—
i-C_4H_9	H	62·8		1 690	70·59	7·58	—
IVd	H	206—207·5	6·50	1 620	73·15	7·37	—
cyclo-C_6H_{11}	H	58·5		1 690	73·17	7·46	—
IVe	H	204—205[d]	6·65	1 625	75·57	5·55	—
$C_6H_5CH_2$	H	75·2		1 670	75·32	5·37	—
IVf	Cl	262—264[e]	6·15	1 615	56·48	4·26	16·67
CH_3	H	68·3		1 690	56·44	4·39	16·59
IVg	Cl	150—151	6·30	1 620	59·88	5·44	14·73
i-C_3H_7	H	72·3		1 690	60·27	5·62	14·82
IVh	Cl	185—187	6·30	1 620	60·38	4·65	14·52
CH_2=$CHCH_2$	H	67·8		1 690	60·40	4·61	14·31
IVi	Cl	154—155	6·10	1 615	61·30	5·94	13·92
i-C_4H_9	H	61·8		1 690	61·62	6·10	13·55
IVk	H	107—108	6·92	1 610	70·88	7·32	—
i-C_3H_7	CH_3	70·6		1 675	71·12	7·52	—
IVl	H	108—109	6·85	1 605	71·54	6·47	—
CH_2=$CHCH_2$	CH_3	65·1		1 675	71·26	6·47	
IVm	H	163—164	7·00	1 600	76·10	6·01	—
$C_6H_5CH_2$	CH_3	72·8		1 670	76·29	5·91	

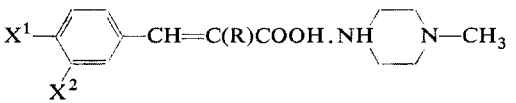
[a] p*K* values determined potentiometrically in 80% methyl cellosolve; [b] the first peak corresponds to C=C, the second to C=O of carboxyl; [c] ref.[19] reports a m.p. of 159°C; [d] ref.[19] reports a m.p. of 199°C; [e] ref.[20] reports a m.p. of 251°C.

significance of the steric parameters E_S together with ΔpK values in Eq. (*10*) and (*11*). One may thus conclude that the interaction of cinnamic acids with the biomacromolecule at the site of action is not affected merely by their acidity.

An important aspect of the correlation between efficiency and structure is the supply of data for predicting the maximally effective compound in a given series. In the case of cinnamic acids one may judge on the basis of correlation according to

TABLE V

Stabilization of Erythrocyte Membranes by N-Methylpiperazinium Salts *V*



Number R	X^1 X^2	M.p. °C	Calculated/Found, %				C^a M/1.10^3
			C	H	N	Cl	
—	CH_3O	149	64·72	7·97	10·07	—	0·411
H	H		64·56	7·84	10·12		
—	H	132—134	59·46	6·77	9·91	12·54	0·426
H	Cl		59·71	6·85	10·05	12·74	
—	H	102—103	60·70	7·13	9·44	11·95	0·380
CH_3	Cl		60·86	7·15	9·28	12·04	
Va	i-C_3H_7O	131—133	66·63	8·55	9·14	—	0·183
H	H		66·65	8·50	8·97		
Vb	CH_2=$CHCH_2O$	125—127	67·08	7·95	9·21	—	0·184
H	H		67·34	8·06	9·37		
Vc	i-C_4H_9O	135—137	67·47	8·81	8·74	—	0·125
H	H		67·66	9·10	8·73		
Vg	i-C_3H_7O	104—106	59·90	7·39	8·22	10·40	0·132
H	Cl		59·70	7·50	8·21	10·52	
Vh	CH_2=$CHCH_2O$	124—125	60·26	5·43	8·27	10·47	0·088
H	Cl		60·22	5·40	8·12	10·16	
Vi	i-C_4H_9O	115—117	60·93	7·67	7·90	9·99	0·062
H	Cl		61·18	7·47	7·56	9·89	
Vk	i-C_3H_7O	97—99	67·47	8·81	8·74	—	0·165
CH_3	H		67·38	8·95	8·62		
Vl	CH_2=$CHCH_2O$	91—92·5	67·89	8·23	8·80	—	0·144
CH_3	H		67·60	8·33	8·66		

[a] Concentration causing 50% inhibition of hemolysis.

TABLE VI

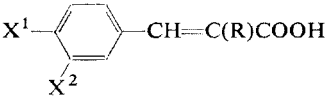
Coefficients in Regression Equations of the Type $\log(1/C_n) = a + b \cdot \Delta pK + c \cdot \sum\pi + d \cdot D + e \cdot E_S$ (for $n = 30$)

Equation	*a*	*b*	*c*	*d*	*e*	*s*	*r*	F
7	5·2410	1·0420	—	—	—	0·2647	0·7885	46·01
8	4·8718	1·3555	0·2583	—	—	0·2097	0·8781	45·47
9	4·5304	1·7319	0·2447	0·1770	—	0·1581	0·9353	60·50
10	4·2327	1·5656	0·3259	0·2801	0·3149	0·1412	0·9507	58·50
11[a]	2·1934	0·5652	0·3054	0·2807	0·3154	0·1411	0·8642	18·45
12[b]	2·2026	0·5920	0·3223	0·2757	0·2942	0·1363	0·8716	19·75

[a] Equation for stabilizing activity of cinnamic acids in the anionic form; [b] equation for stabilizing activity without correction for dissociation.

TABLE VII

Antiinflammatory Effect of Cinnamic Acids



Inhibition of kaolin edema; compounds applied at a dose of 100 mg/kg)

Number	X^1	X^2	R	Effect, %[a]
1	NO_2	H	H	14
10	H	Cl	CH_3	17
15	CH_3O	H	CH_3	15
19	Cl	H	C_2H_5	17
IVa	i-C_3H_7O	H	H	26
IVc	i-C_4H_9O	H	H	32
IVd	cyclo-$C_6H_{11}O$	H	H	37
IVb	CH_2=$CHCH_2O$	H	H	30
IVe	$C_6H_5CH_2O$	H	H	20
IVf	CH_3O	Cl	H	20
IVi	i-C_4H_9O	Cl	H	10
IVm	$C_6H_5CH_2O$	H	CH_3	33
IVl	CH_2=$CHCH_2O$	H	CH_3	38

[a] 3-Chloro-4-allylphenylacetic acid (Mervan) was used as reference standard (41% effect).

Eq. (*10*) and (*11*) that their stabilizing effect is directly proportional to the positive electronic effects of the substituents as well as to their lipophilicity. The quantity *D*, irrespective of its physico-chemical significance, defines the optimum α-substituent as methyl. However, its steric effect and its negative inductive effect weaken its positive effect on the stabilizing efficiency. Another criterion is the deviation of the *p*-methoxy derivatives (compounds *7*, *15*, *22*, *23*) always toward higher activity. Hence the conclusion was reached that the optimum stabilizing activity may be expected with cinnamic acids substituted in the *para* position of the aromatic ring by a higher alkoxy group in combination with a halogen in the *meta* position which increases the overall acidity, or with their α-methyl derivatives.

The compounds thus „computed" were then synthesized. To determine their stabilizing activity they were converted to N-methylpiperazinium salts to increase their solubility under the test conditions. The results are summarized in Table V where, for the sake of comparison, the stabilizing efficiencies of the N-methylpiperazinium salts of three cinnamic acids of the original series (*3*, *7*, *10*) are included. The experimental results bore out the predictions. Introduction of lipophilic substituents (isobutyloxy, isopropyloxy and allyloxy groups) to replace the methoxy group in *para* position, just as the subsequent substitution with chlorine in the *meta* position, significantly increased the stabilizing efficiency. Due to their poor solubility, it was not possible to determine the stabilizing efficiency of acids substituted with more lipophilic groups, such as benzyloxy, cyclohexyloxy and *n*-hexyloxy. The α-methyl derivatives *Vk*, *Vl* display a slight increase of activity as compared with the salts of α-unsubstituted acids (*Va*, *Vb*) which demonstrates the dominant role of *D* for the stabilizing efficiency as compared with the steric and electronic effects of the α-substituents.

The *p*-alkoxycinnamic acids *IV* were evaluated from the point of view of their antiinflammatory effect in the kaolin edema test. The results, together with the most effective compounds of the original series of cinnamic acids, are summarized in Table VII. It is readily appreciated that the application of deductions from an *in vitro* efficiency to *in vivo* effects is accompanied by gross inaccuracies[26]. Thus the results of the antiinflammatory action correspond only partly to stabilizing efficiency. It is clear that the introduction of the lipophilic alkoxy group into the *para* position, just as the substitution with a methyl group in the α-position toward the carboxyl, increased the antiinflammatory effect as compared with the original series of cinnamic acids. Substitution with chlorine in the *meta* position had a positive effect only with the *p*-methoxy derivative (compounds *15* and *IVf*) while with the more lipophilic isobutoxy derivative the introduction of chlorine into the molecule (compounds *IVc* and *IVi*) led to a reduction of the antiinflammatory effect. The lipophilicity appears to be increased here above the optimum value which results in a reduced transport rate toward the site of action.

In conclusion one may say that the analysis of regression equations expressing the

relationship between stabilizing activity of the cinnamic acids and their physico-chemical parameters led to the prediction and synthesis of significantly more potent compounds as compared with the parent series which was the subject of correlation. The stabilizing activity in the series studied is a suitable qualitative indicator of anti-inflammatory activity; in quantitative studies one must take into account the fact that the dominant role of transport in biological systems *in vivo* may substantially alter the relationship between physico-chemical properties and activity.

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