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THE FIBRINOLYTIC ACTIVITY OF N-ARYLANTHRANILATES

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Abstract—A new class of fibrinolytic agents is described. Out of twenty tested N-substituted anthranilates, only N-aryl-anthranilates dissolve human plasma clots. The most active being N-(4-fluorophenyl)-anthranilate, N-(2,3-dimethylphenyl)-anthranilate and N-(3-trifluoromethylphenyl)-anthranilate. Structure–activity relationship within the group of anthranilates is shortly discussed.

INTRODUCTION

CERTAIN organic anions when dissolved and incubated with human plasma clots are able to induce the process of fibrinolysis.[1–5] Surprisingly, there exists only a narrow range of concentration within which a compound may exert its activity. Above and below these limits no fibrinolysis occurs.[3] The most general requirement for the configuration of an active molecule seems to be: a large asymmetric anion* carrying a chain or a ring which is substituted with hydrophobic and hydrophilic radicals. To obtain the optimal activity, these radicals should be mixed in an appropriate proportion. Anions containing two phenyl rings were found to be highly active.[1]

The mechanism of action is obscure but clearly different from those of streptokinase, urokinase and other enzymatic fibrinolytics.[3] von Kaulla supposes that an active anion retracts the antiactivator of plasminogen from antiactivator–activator complex.[3, 5]

Any further progress in the field of chemically-induced fibrinolysis, depends on the discovery of more active compounds, suitable for use in human thrombolytic therapy. So far, known synthetic fibrinolytics are required in rather high concentrations to dissolve the clot *in vitro*. The most active of them discovered by von Kaulla[3–5] produce their biological action in following range of molarity: 4-iodobenzoate—30–80 mM/l. and *o*-thymotate—10–40 mM/l. Recently the same author found 5-benzyloxy-salicylate and 5-(2-chlorobenzyloxy)-salicylate to be still more active. The lowest fibrinolytic concentrations required were 8 mM/l. and 4 mM/l. respectively.[2]

In a previous paper[1], one of us described two active fibrinolytic agents, namely biphenyl-2-butyrate and N-phenylanthranilate, which were active in nearly the same range of concentrations 7–17 mM/l. In this work a number of N-substituted anthranilates was synthesized and their fibrinolytic activity determined. It is noteworthy that

* The organic acids are tested for their fibrinolytic activity usually in the form of sodium salts, hence since now the names of anions are referred to the corresponding sodium salts.

some of N-arylanthranilates possess anti-inflammatory, anti-bradykinin and analgetic properties.[6] At least two of them, N-(3-trifluoromethylphenyl)-anthranilate (Arlef®) and N-(2,3-dimethylphenyl)-anthranilate (Ponstan®) were submitted to clinical trial.[6] Both of them were tested for their fibrinolytic activity together with other N-substituted anthranilates.

MATERIALS AND METHODS

The dissolution of human plasma clots was used as the screening test of fibrinolytic activity. The procedure of von Kaulla[4] was employed. The following changes were introduced: sodium salts of investigated compounds were dissolved in the mixture containing 0·2M TRIS buffer pH 7·40 instead of the barbital-acetate buffer. The time of incubation of the standard clot with the tested solution was confined to an 18-hr period. The pH of the incubation mixture was tested and adjusted so as to be as low as possible. When the alkalinity exceeded pH 8·00 the incubation mixture had been rejected. The control incubation of clots in buffer solutions, pH 7·50 and 8·00 was carried out. Each anthranilate was tested at least three times in twenty concentrations within the range 1–50 mM/l. The pooled blood from different sources was used for a single estimation.

The results are shown in Fig. 1.

Two factors were measured to determine the fibrinolytic activity: (1) the lowest active concentration of the compound at the level of two pulses (Fig. 1) and (2) the range of the lowest and the highest active concentrations at the same level.

Out of twenty tested anthranilates, fourteen had been synthesized in our laboratory according to Ullmann's procedure.[7–9] The recorded melting points of certain synthesized N-substituted derivatives of anthranilic acid did not agree with those reported in original papers,[8, 9] and so these compounds underwent elementary analysis (Table 1). All compounds were crystallized from ethanol.

Three tested compounds: N-(2,6-dichloro-3-mtehylphenyl)-anthranilic acid, N-(2,3-dimethylphenyl)-anthranilic acid (mefenamic acid, Ponston®)[10] and N-(3-trifluoromethyl-phenyl)-anthranilic acid (flufenamic acid, Arlef®) were kindly supplied by Park, Davis and Co. Three others, namely anthranilic acid, N-acetylanthranilic acid and N-phenylanthranilic acid were obtained from usual commercial sources.

RESULTS

The results are presented in Table 1 and in Fig. 2.

It is evident that only N-arylanthranilates proved to be active fibrinolytic agents. Amongst them, the most active in the term of lowest active concentration are: N-(trifluoromethylphenyl)-anthranilate and N-(4-bromophenyl)-anthranilate (compounds Nos. 14 and 9 from Table 1). The least active are: N-(2-methoxyphenyl)-anthranilate, N-(4-methoxyphenyl)-anthranilate and N-phenyl-anthranilate itself (compounds Nos. 2, 1 and 15 respectively).

In terms of the widest range of activity the most active are: N-(4-fluorophenyl)-anthranilate, N-(4-methoxyphenyl)-anthranilate and N-phenylanthranilate (compounds Nos. 5, 1 and 15 respectively). The least active are: N-(2,5-dichloro-3-methylphenyl)-anthranilate and all five chloro-substituted and bromo-substituted derivatives of N-phenylanthranilate (compounds No. 13 and Nos. 6–10 respectively).

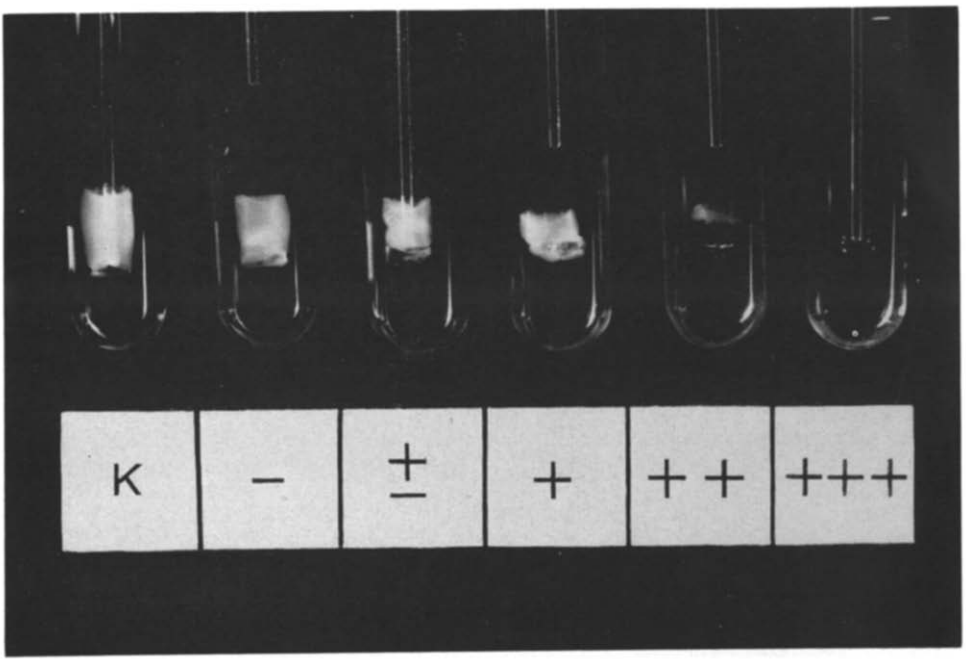
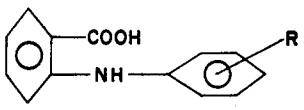


FIG. 1. The natural scale for evaluation of fibrinolytic activity. K = control clot; − = no fibrinolytic activity; ± = faint fibrinolytic activity (half of the clot is dissolved); + = moderate fibrinolytic activity (more than a half of the clot is dissolved); ++ = prominent fibrinolytic activity (only a trace of the clot remains); +++ = complete fibrinolysis.

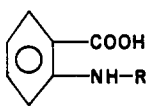
DISCUSSION

It was found that only N-arylanthranilates possess the structure endowed with the specific fibrinolytic activity. When the phenyl ring is substituted for condensed rings, acyl or alkyl groups, the compounds so obtained do not dissolve the clots. Biphenyl-2-carboxylate is the chemical compound very similar to N-phenylanthranilate, but deprived of -NH- joining isosteric group. Biphenyl-2-carboxylate was found to be inactive as the fibrinolytic agent.*

TABLE 1. THE DERIVATIVES OF ANTHRANILIC ACID TESTED FOR FIBRINOLYTIC ACTIVITY *in vitro*



No.	Radical *R*	m.p.°C	Formula	Calcd. % C	Calcd. % H	Found % C	Found % H	The range of fibrinolytic conc. (mg/100ml)
1	4-OCH_3	185–187	$C_{14}H_{13}O_3N$	69·12	5·38	68·79	5·43	169–506
2*	2-OCH_3	177–178	$C_{14}H_{13}O_3N$	69·12	5·38	69·22	5·60	193–458
3*	4-OC_2H_5	210–212	$C_{15}H_{15}O_3N$	70·02	5·87	70·39	5·54	128–257
4	3-OC_2H_5	139	$C_{15}H_{15}O_3N$	70·02	5·87	70·28	5·38	128–257
5	4-F	203 decm.	$C_{13}H_{10}O_2NF$	67·52	4·36	67·71	4·30	115–416
6*	4-Cl	174–175	$C_{13}H_{10}O_2NCl$	63·04	4·07	62·70	4·29	74–130
7*	3-Cl	169–170	$C_{13}H_{10}O_2NCl$	63·04	4·07	63·28	4·40	74–130
8*	2-Cl	178–181	$C_{13}H_{10}O_2NCl$	63·04	4·07	63·63	4·48	99–130
9*	4-Br	180–186	$C_{13}H_{10}O_2NBr$	53·43	3·45	53·47	3·65	45–59
10	3-Br	174–175	$C_{13}H_{10}O_2NBr$	53·43	3·45	53·30	3·58	88–118
11	4-I	198–200	$C_{13}H_{10}O_2NI$	46·05	2·97	46·58	2·99	no activity
12	2,3-di-CH_3	†						72–169
13	2,6-di-Cl-3-CH_3	†						112–140
14	3-CF_3	†						38–81



15*	phenyl	§						158–384
16	H	§						no activity
17	acetyl	§						no activity
18*	1-naphtyl	211–212	$C_{17}H_{13}O_2N$	77·55	4·98	77·49	5·06	no activity
19*	2-naphtyl	214–215	$C_{17}H_{13}O_2N$	77·55	4·98	77·29	5·44	no activity
20	*n*-hexyl	60–61	$C_{13}H_{19}O_2N$	70·55	8·65	69·99	8·33	no activity

* These compounds were originally synthesized by Ullmann and co-workers.[7–9]

† These compounds were kindly offered by Dr Robert A. Scherrer from Park, Davis and Co.

§ These compounds were obtained as standard reagents from different firms.

When small organic substituents or halogen atoms are introduced into the phenyl ring of N-phenylanthranilate, usually the fibrinolytic activity is enhanced, i.e. the substituted derivatives dissolve the clots in lower concentrations than the original compound. This enhancement of fibrinolytic activity is to some extent proportional

* Unpublished data.

to the mol. wt. of substituent. However, a new and undesirable property simultaneously emerges. Proportionally to the mol. wt. of the substituent, the range of fibrinolytic concentrations becomes increasingly narrow (Fig. 2), thus diminishing the actual effective fibrinolytic potency of the substituted N-phenylanthranilates.

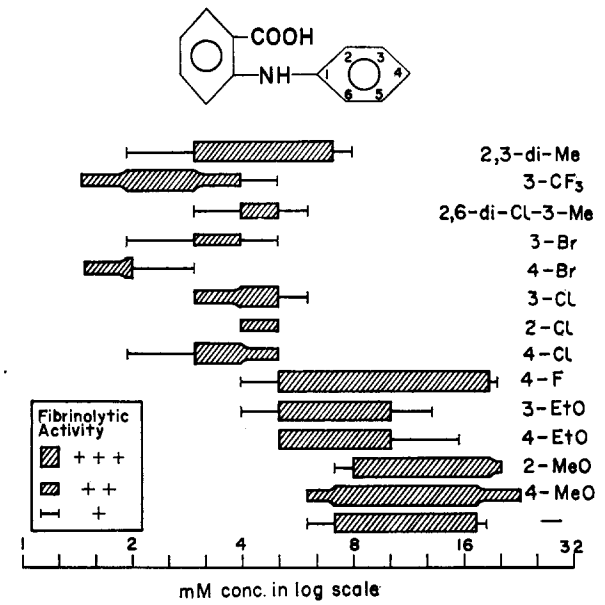


FIG. 2. The range of fibrinolytic activity of fourteen phenyl-substituted derivatives of N-phenylanthranilic acid. The concentrations of (mM) tested compounds are expressed in logarithmic scale. According to Fig. 1 three degrees (+, ++, +++) of fibrinolytic activity are taken into account and graphically deferentiated.

N-(4-fluorophenyl)-anthranilate is the only compound which induces the fibrinolysis in lower concentration than N-phenylanthranilate and in the same time it acts in wider range of active concentrations than N-phenylanthranilate itself.

The ratio of the highest to the lowest fibrinolytic concentrations for N-(2,3-dimethylphenyl)-anthranilate and N-(3-trifluoromethylphenyl)-anthranilate is nearly the same as for N-phenylanthranilate, but both compounds should be considered as the most potent fibrinolytic agents because of their extremely low fibrinolytic concentration still dissolving the clot (Fig. 2).

The above-described influence of the phenyl ring substitution on the fibrinolytic activity of N-aryl anthranilates may be clearly observed in the case of halogen-substituted derivatives. N-(4-fluorophenyl)-anthranilate produces the prominent fibrinolytic activity. Chloro-substituted and bromo-substituted N-phenylanthranilates dissolve the clots in lower concentration than fluoro-substituted derivatives, but because of their scanty range of activity and incomplete clot dissolution they are of little practical and experimental interest. The iodo-substituted derivative is inactive. The position in which the halogen atom is substituted in the phenyl ring seems to be of

no great importance, but the 4-substituted derivatives may display a slightly better profile of fibrinolytic activity (Fig. 2).

Summing up, the halogenation of N-phenylanthranilate with successively heavier halogen atoms diminishes the overall fibrinolytic potency of obtained derivatives. An opposite effect is observed when benzoic acid is halogenated. Benzoate is inactive, while 4-iodobenzoate is the most potent fibrinolytic agent in this homologous series.[4, 5]

The optimal fibrinolytic activity of a particular chemical structure is determined by appropriate proportions of hydrophobic and hydrophilic components distributed within the investigated chemical moiety. In the molecule of N-phenylanthranilate, this proportion is improved when an additional fluorine atom is introduced, but strongly overweighted on the hydrophobic side when chlorine or bromine atoms are incorporated into the phenyl ring. The iodination of the phenyl ring in the molecule of N-phenylanthranilate, results in too strong hydrophobic properties of the molecule and it is unable to induce the process of fibrinolysis.

The halogenated molecule of benzoic acid reaches its optimal balance between the hydrophilic and hydrophobic centres when the heaviest halogen is introduced into the molecule in the position 4. However, 4-iodobenzoate is still six times less active than N-(4-fluorophenyl)-anthranilate. Further iodination of 4-iodobenzoate renders the compound inactive as a fibrinolytic agent.[1] It is obvious that the structure of N-phenylanthranilate is more suitable to induce the fibrinolysis than the structure of the halogenated benzoic acid.

It is difficult to say anything about the mechanisms which are involved in the process of chemically-induced fibrinolysis *in vitro* and about the correspondence of this phenomenon to the experiments *in vivo*. The chemical specificity of fibrinolytic agents suggests that their activity must be connected with the enzymatic processes taking place in the incubating clot, which is equipped with all fibrinolytic proenzymes.

The maintenance of an appropriate hydrophobic–hydrophilic balance within the active molecule is unquestionable. It put the stress on the importance of the physical properties of the compounds rendering the fibrinolytic activity.

The lowest fibrinolytic concentration found in this work [N-(3-trifluoromethylphenyl)-anthranilate = 38 mg/100 ml] is still too high to be easily achieved in blood in experiments *in vivo*.

REFERENCES

1. R. GRYGLEWSKI, *Folia biol.* Kraków **14**, 3 (1966).
2. K. N. VON KAULLA, *Experientia, Basel* **21**, 439 (1965).
3. K. N. VON KAULLA, *Arzneimittel-Forsch.* **15**, 246 (1965).
4. K. N. VON KAULLA, *J. med. chem.* **8**, 164 (1965).
5. K. N. VON KAULLA, *Thromb. Diath. Haemorrh.* **7**, 404 (1962).
6. R. A. SCHERRER, C. V. WINDER and F. W. SHORTT, Ninth National Medicinal Chemistry Symposium of the American Chemical Society, University of Minnesota (1964).
7. F. ULLMANN and H. KIPPER, *Justus Liebigs Ann. Chem.* **355**, 343 (1907).
8. F. ULLMANN and G. RASETTI, *Justus Liebigs Ann, Chem.* **355**, 348 (1907).
9. F. ULLMANN and E. TEDESCO, *Justus Liebigs Ann. Chem.* **355**, 336 (1907).
10. C. V. WINDER, J. WAX, L. SCOTTI, R. A. SCHERRER, E. M. JONES and E. W. SHORT, *J. Pharmac.* **138**, 405 (1962).