(0.5 mmole) of *p*-toluenesulfonic acid, and 6.4 ml (73 mmoles) of morpholine. The mixture was stirred at 130° for 5 hr and cooled to room temperature, and 50 ml of cold 50% MeOH was added. The mixture was stirred in the cold 1 hr, and the yellow solid was collected by filtration and washed with water to afford 5.1 g (97%). This material was used in the next step. An analytical sample, mp 95–100°, was obtained by recrystallization from methanol.

Anal. Calcd for $C_{41}H_{51}N_3O_6S_3$: C, 63.3; H, 6.61; S, 12.4. Found: C, 63.3; H, 6.37; S, 11.8.

2,6-Bis(2-methoxy-5-carboxymethyl)-4-carboxymethylanisole (VIII). To 5.1 g of the thioamide VII was added 60 ml of 2-methoxyethanol and 60 ml of 20% KOH. The mixture was refluxed for 24 hr and filtered, and the filtrate was evaporated *in vacuo* to near dryness. The brown syrup was taken up in water, chilled, and acidified (pH 1–2) with HCl. The mixture was warmed on a steam bath while stirring for a few minutes, and the supernatant was decanted from the gummy material. The brown gum was crystallized from water, collected by filtration, and taken up in EtOAc. The solution, left in a refrigerator for 2 days, gave a tan crystalline material (1.22 g) which was triturated in fresh EtOAc to give 0.66 g, mp 175–181°. An analytical sample, mp 183–185°, was obtained by recrystallization from methyl ethyl ketone.

Anal. Calcd for $C_{29}H_{36}O_8$: C, 66.6; H, 5.79. Found: C, 66.5; H, 5.79.

Tris-N-methylamide of 2,6-Bis(2-methoxy-5-carboxymethylbenzyl)-4-carboxymethylanisole (IX).—A mixture of 0.70 g of VIII and 15 ml of α,α-dichloromethyl methyl ether was refluxed 15 min and evaporated *in vacuo*. Benzene (20 ml) was added and evaporated *in vacuo*. The residual gum was dissolved in 15 ml of dry CH_2Cl_2 and treated with a slow stream of gaseous methylamine for 10 min. The flask was stoppered and kept at room temperature for 20 hr. The solvent was removed *in vacuo* and the residue was stirred with water for 15 min. The tan solid was collected by filtration, washed with 1.5 *N* NH_4OH and water, and dried to leave 0.69 g (92%) of tan crystals, mp 215–220°. A small portion was recrystallized from ethanol for analysis: mp 221–223°.

Anal. Calcd for $C_{32}H_{39}N_3O_6$: C, 68.4; H, 7.00; N, 7.48. Found: C, 67.9; H, 6.91; N, 7.37.

Reduction of this amide with lithium aluminum hydride in hot tetrahydrofuran gave a negligible amount of amine, while treatment with diborane in tetrahydrofuran gave a syrupy amine whose picrate or hydrochloride could not be made to solidify.

Tris-N,N-dimethylamide of 2,6-Bis(2-methoxy-5-carboxymethylbenzyl)-4-carboxymethylanisole (X).—The acid chloride from 0.60 g (1.1 mmoles) of VIII was taken up in 10 ml of anhydrous CH_2Cl_2, chilled, and treated with a mixture of 3 ml (45 mmole) of anhydrous dimethylamine in 5 ml of anhydrous CH_2Cl_2. The mixture was stirred briefly, then the flask was stoppered and allowed to stand at room temperature for 21 hr. The mixture was evaporated to dryness *in vacuo*, and the brown material was extracted with EtOAc. The EtOAc extract was washed with water, dried over $MgSO_4$, and evaporated *in vacuo* giving 0.64 g (93%) of brown gum, λ_{max}^{nujol} 6.05 μ (C=O of amide). This material did not crystallize and was considered to be of sufficient purity for the next step.

2,6-Bis(2-methoxy-5,β-dimethylaminoethylbenzyl)-4,β-dimethylaminoethylanisole (Ib) Tripicrate.—To a chilled slurry of 0.43 g (11 mmoles) of lithium aluminum hydride in 25 ml of anhydrous tetrahydrofuran was added dropwise a mixture of 0.61 g (1 mmole) of the dimethyl amide (X) in 20 ml of anhydrous tetrahydrofuran. The mixture was refluxed 5.5 hr, and excess hydride was decomposed by the careful addition of absolute EtOH. The reaction mixture was then treated with water, stirred briefly, and evaporated *in vacuo* to near dryness. The pasty material was extracted with ether, and the ethereal extract was dried over $MgSO_4$ and evaporated *in vacuo* to give 176 mg of clear syrup. A chilled solution of 126 mg (0.2 mmole) of the free amine in 10 ml of dry ether was treated with dry HCl, giving white, hygroscopic material. The ether was removed by decantation, and the hydrochloride salt was taken up in absolute EtOH and added to a saturated solution of 0.17 g (0.7 mmole) of picric acid in water. The mixture was left at room temperature for 15 hr. The alcohol–water mixture was decanted, and the gummy material was crystallized from water to yield 215 mg. The yellow solid was taken up in a warm ethanol–2-methoxyethanol mixture and the solution was allowed to stand at room temperature for 15 hr. The alcohol mixture was decanted from the gummy material, and 2-propanol was added, giving yellow crystals, mp 90–100°. A 140-mg portion was twice recrystallized from 80% 2-methoxyethanol to give 70 mg of yellow crystals, mp 171–174°.

Anal. Calcd for $C_{53}H_{61}N_{15}O_{21}$: C, 51.0; H, 4.84; N, 13.4. Found: C, 51.1; H, 5.05; N, 13.3.

Antiinflammatory Compounds Exhibiting Fibrinolytic Activity

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A group of diphenyldioxopyrazolidine derivatives possessing antiinflammatory activity was subjected to the von Kaulla test for fibrinolytic activity. The compounds which had exhibited antithrombotic effectiveness in the clinic showed also a marked fibrinolytic activity. These findings allow the assumption of a parallelity between the antithrombotic properties of some antiinflammatory compounds and their capability of an active participation in the fibrinolytic process.

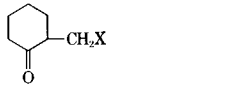
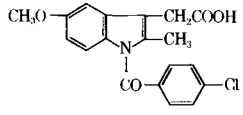
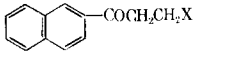
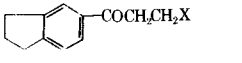
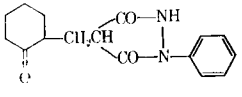
The clinical application of antiphlogistic drugs is based on a broad pharmacodynamical spectrum of antiphlogistic, analgetic, antipyretic, and uricosuric activities; a more general classification differentiates their antirheumatic and antithrombotic effectiveness. As to the chemical structure, the principal representatives of antiphlogistic drugs are salicylates, corticoids, derivatives of diphenyldioxopyrazolidine, antimalarial drugs, and indole derivatives. In phlebitis and thrombosis, the application of diphenyldioxopyrazolidine derivatives has been constantly spreading in recent time. Neither the principle nor the mechanism of the action of this group, however, have been fully elucidated yet.

Recently von Kaulla[1] elaborated a test for screening the fibrinolytic activity of synthetic organic compounds. This *in vitro* test consists essentially of the formation of clots from recalcified citrated human plasma and the incubation of the clots in media containing the compound to be tested in a number of graded concentrations. The fibrinolysis-inducing capacity is measured and expressed by the specific optimal concentration of the compound studied at which a complete lysis occurs. In a series of papers,[1-3] von Kaulla studied the de-

(1) K. N. von Kaulla, *J. Med. Chem.*, **8**, 164 (1965).
(2) K. N. von Kaulla, *Experientia*, **21**, 439 (1965).
(3) K. N. von Kaulla, *Thromb. Diath. Haemorrhag.*, **7**, 404 (1962).

TABLE I

RESULTS[a] OF THE SCREENING EVALUATION OF FIBRINOLYTIC ACTIVITIES OF ANTIINFLAMMATORY COMPOUNDS

No.	Compd[b]	Incubation, hr	Concn, M																		
			0.1	0.09	0.08	0.07	0.06	0.05	0.04	0.03	0.02	0.01	0.009	0.008	0.007	0.006	0.005	0.004	0.003	0.002	0.001
1	$(CH_3)_3CCOCH_2CH_2X$	24																(+)	(+)		
		48																+	+	+	
2	$C_6H_5COCH_2CH_2X$	24													(+)	+	(+)	(+)			
	(benzopyrazone)	48													(+)	+	+	+			
3	o-$OHC_6H_4COCH_2CH_2X$	24											(+)	(+)	(+)						
		48										(+)	(+)	+	+						
4	$(CH_3)_2CHCH_2COCH_2CH_2X$	24												(+)							
		48										(+)	(+)	+	+	+					
5	$CH_3(CH_2)_3X$ (phenylbutazone)	24										(+)	(+)	+							
		48										+	+	+	(+)	(+)					
6		24									+										
		48							(+)	(+)	+	(+)									
7	$CH_3SCH_2CH_2X$	24							(+)	(+)	(+)										
		48							+	+	+										
8	$CH_3COCH_2CH_2X$ (ketophenyl-	24				+	+	+	+												
	butazone)	48				+	+	+	+	(+)											
9		24											(+)	(+)	+	(+)					
		48										(+)	(+)	+	+	+	(+)				
	(indomethacin)																				
10	p-IC_6H_4COOH	24	(+)	(+)	+	+	+	+	+												
		...																			
11	2-OH-5-$CH_3COC_6H_3COOH$	24	+	+	(+)																
		...																			
12	p-ClC_6H_4COOH	24	+																		
		...																			
13	3,5-I_2-4-$NH_2C_6H_2SO_3H$	24																			
		48	+																		
14		24																		(+)	
		48																		(+)	
15		24																			
		48																		(+)	
16	o-$FC_6H_4COCH_2CH_2X$	24																(+)			
		48														(+)	(+)	(+)			
17		24					(+)	(+)	(+)												
		48				(+)	(+)	(+)	(+)												

[a] + = complete lysis of plasma clot, (+) = partial lysis of plasma clot. [b] X = $-HC\begin{matrix}CONC_6H_5\\|\\CONC_6H_5\end{matrix}$

pendence of the fibrinolysis-inducing capacity of various compounds on their chemical structure; he was interested mainly in various derivatives of benzoic and salicylic acids.

This contribution presents the results of investigations of fibrinolytic properties performed in a series of derivatives which have been synthesized within the framework of our search for antiinflammatory drugs. The series comprises derivatives of 1,2-diphenyl-3,5-dioxopyrazolidine that have, in position 4 of the pyrazolidine nucleus, a substituent containing an oxoalkyl group. One of them, ketophenylbutazone[4] [1,2-diphenyl-3,5-dioxo-4-(3-ketobutyl)pyrazolidine], has been used clinically for several years as an uricosuric, antiphlogistic, and antirheumatic drug. Also, a number of related compounds exhibit a marked antiinflammatory activity, which in the case of benzopyrazone[5] [1,2-diphenyl-3,5-dioxo-4-(2-benzoylethyl)pyrazolidine], has already been clinically verified. The fibrinolysis-inducing capacity of the new compounds was compared with that of several well-known drugs used in the therapy of inflammatory and rheumatic diseases and also with that of some compounds studied previously by von Kaulla, whereby a good reproducibility of his method was confirmed.

Experimental Section

Principle.—The technical arrangement of the experiment was identical with that described by von Kaulla[1] in which human plasma clots were incubated at 37° in buffered solutions of the compounds to be tested. The fibrinolysis-inducing effect was apparent by a partial to complete dissolution of the clots suspended on glass rods in the incubation medium.

Apparatus and Chemicals.—The apparatus and glassware matched in quality and dimensions those described in the cited paper.[1] Of help was the etching of the lower ends of the glass rods which improved the adhesion of the plasma clots and thus facilitated the handling.

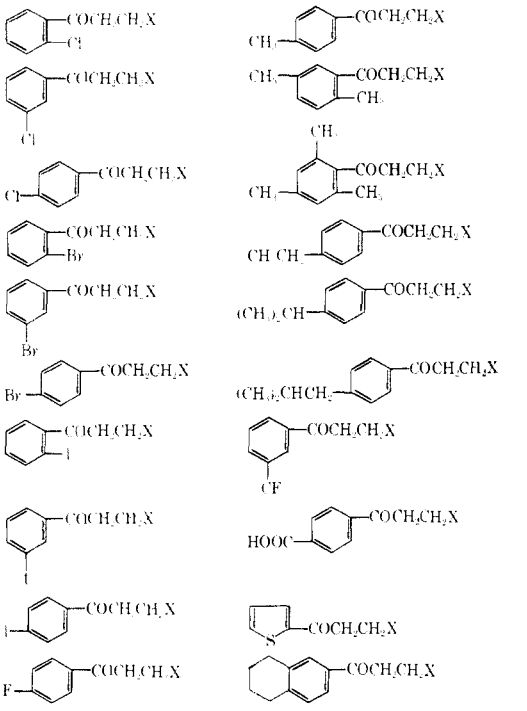
The compounds presented in Tables I and II were of analytical grade. Weighed samples of several compounds, insoluble in the barbital–acetate buffer solution, were first dissolved in 0.5–1 ml of dimethylacetamide, acetone, or ethanol, and the volume was made up to 10 ml with the buffer solution, the pH being immediately adjusted to 7.42. Parallel experiments with phenylbutazone (1,2-diphenyl-3,5-dioxo-4-*n*-butylpyrazolidine) and ketophenylbutazone proved that the solvents used did not interfere with the testing of the fibrinolysis. Each of the two compounds was dissolved at the same concentration either with the solvents added or in the buffer solution alone. Identical results were obtained proving that the solvents named above, when used in the amounts mentioned before, were inert and did not influence the testing of fibrinolytic activity.

The results were read after 24 hr. In some cases, where after 24 hr a distinct lytic effect could be observed but the clot had not dissolved completely, the incubation was prolonged for another 24 hr. In such cases the fibrinolysis progressed. Both time intervals are indicated in Tables I and II. In four cases even the 48-hr incubation was not sufficient for a complete dissolution of the plasma clots although after 24 hr a partial lytic effect was well apparent. In these exceptional cases a subsequent pharmacological test in animals proved that the antiinflammatory activity of the compounds in question was either very low or nonexistent.

The evaluation of results is presented in the Table I which comprises compounds possessing fibrinolysis-inducing capacity. Table II lists compounds in which the testing did not reveal any fibrinolytic activity.

TABLE II

COMPOUNDS[a] WITHOUT FIBRINOLYTIC ACTIVITY



[a] X = $-HC<\begin{matrix}CO-N-C_6H_5\\ |\\ CO-N-C_6H_5\end{matrix}$

Discussion

The experiments revealed in a group of diphenyldioxopyrazolidine derivatives a parallelity between their antiinflammatory effectiveness and their fibrinolysis-inducing capacity. This parallelity indicates that the latter capacity exhibited by various types of antiinflammatory drugs may be an important component of their effectiveness. Moreover, this activation is specific, since those compounds which exhibit a marked antithrombotic effectiveness in the clinical test show also fibrinolytic activity in the *in vitro* test described here, whereas some other well-known drugs, *e.g.*, acetylsalicylic acid, which fail to show any antithrombotic effectiveness in the clinic, do not reveal any fibrinolytic capacity in the test.

The important finding of a decreased fibrinolytic activity in patients suffering from rheumatoid arthritis,[6] in whom the fibrinolytic capacity was successfully normalized again by the administration of corticoids, justifies further the opinion of the authors[6] that a disturbance of the fibrinolytic capacity may be a factor contributing to the development of chronic rheumatic inflammatory conditions. Consequently, antiinflammatory drugs possessing fibrinolysis-inducing capacity may exert a beneficial influence also in this particular disease. These concepts are also connected with the investigations of Astrup[7] and Fearnley[8] of the imbalance between the coagulation and the fibrinolysis in an inflammatory condition, representing another region where two systems influence the deposition and renewed dissolution of fibrin.

(4) Z. Horáková, O. Němeček, V. Pujman, J. Mayer, J. Čvrtník, and Z. Votava, *Arzneimittel-Forsch.*, **8**, 229 (1958).

(5) V. Musil, B. Brůnová, and O. Němeček, *Collection Czech. Chem. Commun.*, **29**, 1669 (1964).

(6) G. R. Fearnley, R. Chakrabarti, and E. D. Hocking, *Lancet*, **No. I**, 91 (1965).

(7) T. Astrup, *ibid.*, **No. II**, 565 (1956).

(8) G. R. Fearnley, *ibid.*, **No. I**, 992 (1961).