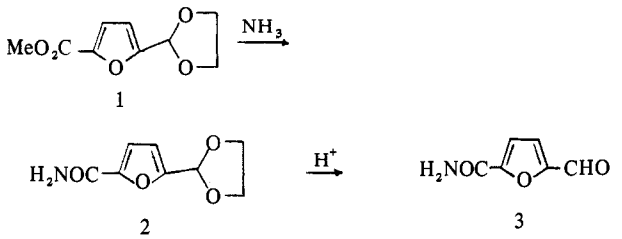
furaldehydes with semicarbazide, 3-amino-2-oxazolidinone,[6] and 1-aminohydantoin.[7] Treatment of the protected aldehyde **1** (Scheme I) with ammonia gave the amide **2** from

Scheme I



which the aldehyde **3** was liberated. Reaction of 5-iodo-2-furaldehyde[8] with $Cu_2(CN)_2$ in DMF gave 5-formyl-2-furonitrile (**4**). Hydrolysis of 2-dichloromethyl-5-furansulfonamide (**5**) gave 5-formyl-2-furansulfonamide (**6**). 5-Formyl-2-furoic acid[9] (**7**) with MeOH-HCl gave the ester **8**.

Pharmacology. The compounds in Table I were tested for antibacterial activity *in vitro* using a standard agar-dilution technique[10] against the following organisms: *Staphylococcus aureus* NCTC 7447; *Escherichia coli* NCTC 86; *Klebsiella pneumoniae* NCTC 7242; and *Salmonella typhi* NCTC 8383. None of the compounds were found to possess significant antibacterial activity. The results confirm the essential role of the nitro group in conferring activity.

Experimental Section

Melting points were determined on a Büchi apparatus and are uncorrected. Elemental analyses for the elements stated in Table I were within ±0.4% of the theoretical values. The structures of the compounds were verified from ir spectra on a Unicam SP 200 spectrophotometer and from nmr spectra on a Varian T-60 spectrophotometer.

Preparation of Azomethines. General Procedure. To an aqueous solution of the *N*-amino compound (or its hydrochloride) was added an equivalent weight of aldehyde dissolved in hot H_2O or hot EtOH. The product was collected by filtration and recrystallized from a suitable solvent (Table I). In some cases buffering to pH 4–5 using NaOAc improved the rate of reaction.

2-(5-Carbamoyl-2-furyl)-1,3-dioxolane (2). A mixture of 1[11] (22 g, 0.11 mol) and concentrated aqueous NH_3 solution was stirred for 20 hr and then evaporated to give **2** as an oil (19.4 g, 96%) which was subsequently hydrolyzed to **3** without purification.

5-Formyl-2-furamide (3). Concentrated HCl (1 ml) was added to a suspension of **2** (19.4 g, 0.105 mol) in H_2O (200 ml). The product was collected (11.1 g, 76%) and recrystallized from H_2O, mp 206–207°. *Anal.* ($C_6H_5NO_3$) C, H, N.

5-Formyl-2-furonitrile (4). A mixture of 5-iodo-2-furaldehyde (44.4 g, 0.2 mol), $Cu_2(CN)_2$ (23 g, 0.12 mol), and DMF (200 ml) was stirred and heated under reflux for 3 hr. After cooling, H_2O (400 ml) was added and the suspension filtered. Extraction of the filtrate with $CHCl_3$ and evaporation gave the product (19.6 g, 81%). Distillation gave pure **4**, bp 108–109° (20 mm), mp 19–20°. *Anal.* ($C_6H_3NO_2$) C, H, N.

5-Formyl-2-furansulfonamide (6). A suspension of **5** (23 g, 0.1 mol) in H_2O (250 ml) was heated at 70° for 15 min. Evaporation of the H_2O gave a gum (17.7 g, 94%) which crystallized on standing. Recrystallization from H_2O gave mp 107–108°. *Anal.* ($C_5H_5NO_4S$) C, H, N.

Methyl 5-Formyl-2-furoate (8). A solution of **7** (2.8 g, 0.02 mol) in MeOH-HCl (25 ml) was allowed to stand overnight. Evaporation of the solvent and recrystallization from cyclohexane gave 2.7 g (87%) of **8**, mp 92–93° (lit.[12] mp 93°). *Anal.* ($C_7H_6O_4$) C, H.

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References

(1) M. C. Dodd and W. B. Stillman, *J. Pharmacol. Exp. Ther.*, **82**, 11 (1944).
(2) K. Miura and H. K. Reckindorf, *Progr. Med. Chem.*, **5**, 320 (1967).
(3) R. E. Bambury, H. K. Yaktin, and K. K. Wycoff, *J. Heterocycl. Chem.*, **5**, 95 (1968).
(4) S. Somasekhara, G. K. Suthar, N. V. Upadhyaya, and S. L. Mukherjee, *Curr. Sci.*, **21**, 614 (1968).
(5) S. Toyoshima, K. Shimada, K. Kawabe, and T. Kanzawa, *Yakugaku Zasshi*, 89, 779 (1969).
(6) G. Gever, U. S. Patent 2,652,402 (1953).
(7) D. Jack, *J. Pharm. Pharmacol., Suppl. II*, 108T (1959).
(8) Z. N. Nazarova, *J. Gen. Chem. USSR*, **25**, 509 (1955).
(9) Ya. L. Goldfarb and B. I. Rosovik, USSR Patent 184,877 (1966); *Chem. Abstr.*, 66, 115,590 (1967).
(10) W. A. Vischer, *Arzneim.-Forsch.*, **18**, 1529 (1968).
(11) E. I. Du Pont de Nemours and Co., British Patent 705,950 (1951).
(12) O. Moldenhauer, G. Trautmann, W. Irion, R. Pfluger, H. Döser, D. Mastaglio, H. Marwitz, and R. Shulte, *Justus Liebigs Ann. Chem.*, **580**, 169 (1953).
(13) G. Jansen, J. Lei, and N. Clauson-Kaas, *Acta Chem. Scand.*, **25**, 340 (1971).

dl-α-[4-Isobutyl(cyclohexen-1-yl)]alkanoic Acids and Derivatives as Fibrinolytic and Thrombolytic Compounds

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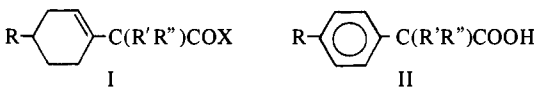
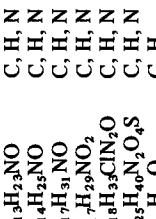
In a previous publication[1] we have described the synthesis and antiinflammatory properties of several *dl*-α-[4-cycloalkyl(cyclohexen-1-yl)]alkanoic acids and derivatives (I, R = cycloalkyl). Compound I (R = cyclohexyl; R′ = H; R″ = CH_3; X = OH) was the most active product in the uv erythema test, the carrageenan edema test, and the adjuvant-induced arthritis assay. Thus, partial saturation of the benzene nucleus of the well-known α-(phenyl)alkanoic acids (II, R = cyclohexyl; R′ and/or R″ = H or alkyl)[2] did not suppress antiinflammatory activity.



As compound II (R = isobutyl; R′ = H; R″ = CH_3) also showed high antiinflammatory activity,[3] we have synthesized a series of corresponding *dl*-α-[4-isobutyl(cyclohexen-1-yl)]alkanoic acids and derivatives (I, R = isobutyl).

None of the compounds (I, R = isobutyl) retained any antiinflammatory activity in the rat hind-paw carrageenan edema test[4] at doses as high as 200 mg/kg po. As antiinflammatory activity appears to correlate well with fibrinolytic activity within a group of acidic antiinflammatory drugs,[5] all compounds (I, R = cycloalkyl) previously described and I (R = isobutyl) were screened for fibrinolytic activity. Surprisingly, at 100 mg/kg po all compounds (I, R = cycloalkyl) were devoid of fibrinolytic activity (see Biological Testing, test 2) but several compounds (I, R = iso-

Table I



Compd	R′	R″	X	Method	Solvent	Formula	Analyses	Mp or bp (mm), °C	LD_{50} in mice, mg/kg po (±S.E.)	Doses, mg/kg po	ELT, test 2: % decrease of lysis, Time in min 30	60	90	180
1	H	CH_3	OC_2H_5	A		$C_{15}H_{26}O_2$	C, H	82–84 (0.02)		100	0	0	0	0
2	H	CH_3	OH	B		$C_{13}H_{22}O_2$	C, H	119–120 (0.04)	1450 (76)	25	0	0	0	0
										50	0	4^b	25^c	21^c
										100	46^d	60^d	40^c	0
3	H	CH_3	NHOH	D	Cyclohexane	$C_{13}H_{23}NO_2$	C, H, N	123–124		100	0	0	0	0
4	H	CH_3	NH_2	C	*n*-Hexane	$C_{13}H_{23}NO$	C, H, N	74–74.5		100	0	0	0	0
5	H	CH_3	$NHCH_3$	C		$C_{14}H_{25}NO$	C, H, N	154–158 (0.007)		100	0	0	0	0
6	H	CH_3	$N(C_2H_5)_2$	C		$C_{17}H_{31}NO$	C, H, N	118–120 (0.08)		100	0	0	0	0
7	H	CH_3	$c\text{-}N(CH_2CH_2)_2O$	C		$C_{17}H_{29}NO_2$	C, H, N	140–142 (0.01)		100	0	0	0	0
8	H	CH_3	$c\text{-}N(CH_2CH_2)_2N^+\text{-}HCH_3Cl^-$	C	*i*-PrOH–Et_2O	$C_{18}H_{33}ClN_2O$	C, H, N	230–232		100	0	0	0	0
9	H	CH_3	$c\text{-}N(CH_2CH_2)_2N^+\text{-}HCH_2C_6H_5CH_3SO_3^-$	C	*i*-PrOH–Et_2O	$C_{25}H_{40}N_2O_4S$	C, H, N	230–231		100	0	0	0	0
10	H	C_2H_5	OC_2H_5	A		$C_{16}H_{28}O_2$	C, H	100–102 (0.1)		100	0	0	0	0
11	H	C_2H_5	OH	B		$C_{14}H_{24}O_2$	C, H	119–120 (0.01)	2000 (193)	100	46^d	4^b	0	0
12	H	C_2H_5	NHOH	D	Cyclohexane	$C_{14}H_{25}NO_2$	C, H, N	128-129	>2000	100	7^b	40^d	10^b	0
13	H	C_2H_5	$c\text{-}N(CH_2CH_2)_2O$	C		$C_{18}H_{31}NO_2$	C, H, N	148–150 (0.01)	>2000	100	15^b	11^b	26^c	29^c
14	CH_3	CH_3	OC_2H_5	A		$C_{16}H_{28}O_2$	C, H	89–92 (0.04)	*a*					
15	CH_3	CH_3	OH	B		$C_{14}H_{24}O_2$	C, H	37–39	>2000	100	19^c	25^c	0	0

[a] Insufficient data to state potency. [b] Not significant. [c] $p \leq 0.02$. [d] $p \leq 0.01$.

butyl) were active. In the series of compounds (I), the anti-inflammatory activity seemed to depend on the 4-cycloalkyl moiety while the 4-isobutyl moiety would seem responsible for fibrinolytic activity. In this paper we describe derivatives (I, R = isobutyl) and their fibrinolytic properties.

Experimental Section

Chemistry. All derivatives were prepared following methods described previously.[1] A mixture of *cis*- and *trans*-ethyl *dl*-α-[4-isobutyl(1-hydroxycyclohexyl)]alkanoate was formed by a Reformatsky reaction between 4-(isobutyl)cyclohexanone[6] and an α-bromo ester (R′R″)C(Br)COOEt. The crude cis/trans mixture so obtained was dehydrated with P_2O_5 yielding a β-unsaturated ester I (R = isobutyl; X = OEt) (method A). The latter was either saponified (method B), furnishing an acid (I, R = isobutyl; X = OH), or treated with NH_2OH (method D) thus producing a hydroxamic acid (I, R = isobutyl; X = NHOH). Several amides were prepared from I (R = isobutyl, X = OH) by condensing the acid chloride I (R = isobutyl, X = Cl) with an amine (method C). The compounds synthetized (I, R = isobutyl) are listed in Table I.

Biological Testing. Material and Methods. The studies were carried out on the following animal species: Charles River CD1 strain male mice COBS; Charles River CD strain male rat COBS; common grey rabbit.

Test 1. Measurement of Thrombolytic Activity by the *in Vivo* Standard Hanging Plasmatic Clots Method.[7] Plasmatic clots were obtained by recalcification of human plasma from outdated blood bank bottles. Clots were suspended in tubes containing solutions of increasing concentrations of the product to be tested and incubated on a water bath at 37°. Lysis was observed after 24 hr.

As previously observed lysis of standard human plasma clots occurred only within a certain range of concentrations.[8,9] The

Table II. Platelet Stickiness (Test 3)

Compd 2 doses, mg/kg (route)	Time after administration	% decrease of stickiness
10 (iv)	5 min	51^a
10 (iv)	10 min	48^a
25 (po)	1 hr	23^a
50 (po)	1 hr	53^b
50 (po)	3 hr	30^a
100 (po)	1 hr	19^a
100 (po)	3 hr	53^b

[a] $p \leq 0.02$. [b] $p \leq 0.01$.

Table III. Platelet Aggregation (Test 4)

Species	Compd 2 doses, μg/ml	% of inhibition after induction by: ADP	Thrombin	Epinephrine
Human	500	43	46	
	1000	46	100	0
	1500	73		11
	2000	79		30
Rat	100	0	47	*a*
	250	5	100	
	500	25		
	700	100		
Rabbit	100	0	0	*a*
	500	10	17	
	1000	37	40	
	1500	92		

[a] Rabbit and rat plasma do not aggregate with epinephrine: P. Desnoyers, J. Labaume, M. Anstett, M. Herrera, J. Pesquet, and J. Sebastien, *Arzneim.-Forsch.*, **22** (10), 1691 (1972).

Table IV. Fibrin Monomer Level (Test 5)

Compd 2 doses, iv	Time after administration, min	% decrease
10	2	45^a
10	10	49^a
25	2	47^b
25	10	53^a

[a] $p \leq 0.01$. [b] $p \leq 0.001$.

Table V. Chronic Administration (Test 2)

Compd 2 doses, mg/kg/day po	% decrease of euglobulin lysis time after day				
	15	30	60	30 after cessation of treatment	30 after starting again
100	33	51	54	0	52
p values	$0.05 \leq p \leq 0.02$	$0.02 \leq p \leq 0.01$	$p \leq 0.001$	0	$0.02 \leq p \leq 0.01$

greater the activity of the compound, the narrower the active concentration range. However, this test could only be performed on water-soluble compounds. Like other authors, we have chosen *o*-thymotic acid (OTA) as reference compound.[9]

Test 2. Measurement of Systemic Fibrinolytic Activity by Determination of Plasma Euglobulin Lysis Time (ELT) after Oral Administration to Rat.[10] This method consisted of precipitating by means of a flow of carbon dioxide the euglobulin fraction of diluted plasma. The resultant precipitate was removed by centrifugation, redissolved in a buffer solution, and then coagulated by thrombin. The euglobulin clot was incubated at 37° and the time of complete lysis determined.

Surprisingly, OTA was found inefficient as an *in vivo* reference compound and, consequently, the studied compounds could not be compared to this known standard. When compounds of high activity were detected using this test, we have attempted to study their possible effects on thromboembolic diseases by means of the following techniques.

Test 3. Decrease or Inhibition of Rabbit Blood Platelet Adhesiveness on Glass Bead Column.[11] The per cent adhesiveness was calculated by determining the number of platelets before and after passage through the column. The platelet count was carried out in triplicate by means of an electronic particle counter.

Test 4. Decrease or Inhibition of the Platelet Aggregating Effect of ADP, Thrombin, and Epinephrine on Rat and Rabbit Plasma Measured by Means of a Photometric Technique.[12] The compounds were incubated in platelet-rich plasma for 5 min before addition of the aggregating agent. The results were expressed as the percentage inhibition of aggregation induced by the compound as measured by determination of the planimetric relationship of the surfaces obtained with the aggregating agent alone on the one hand, and the compound and aggregating agent on the other, at the different concentrations tested.

Test 5. Decrease of Fibrin Monomer Level in the Rabbit after Intravenous Perfusion.[13] In addition, the acute toxicity (LD_{50} in mice) of active compounds was assessed by the Probit method[14] up to 2000 mg/kg po.

Results and Discussion

Only two compounds were sufficiently soluble under our experimental conditions (aqueous buffered solution at pH 7) to be tested against standard hanging clots (test 1). Compound **2** was active from 0.02 to 0.007 mol/l. while OTA was active at 0.02-0.008 mol/l. On the other hand, compound **8** although soluble was inactive.

Five compounds (**2**, **11–13**, and **15**) out of the 14 tested possessed marked activity in test 2. The toxicities of active compounds and the results of test 2 are listed in Table I.

Tests 3, 4, and 5 were performed on the most active compound **2** which induced a marked decrease in platelet stickiness (*cf.* Table II), a pronounced inhibition of platelet aggregation (*cf.* Table III) induced by ADP, thrombin, or epinephrine in rabbit, rat, and human plasma, and a decrease of fibrin monomer levels (*cf.* Table IV) after iv injection. Contrary to nicotinic acid,[15] this compound was able to decrease the ELT in the rat after daily administration over a period of 2 months (*cf.* Table V). No tachyphylatic phenomena were apparent.

Although only 14 derivatives have been tested, some features of a structure-activity relationship were apparent from the *in vivo* results (test 2). Most of the compounds containing an asymmetrical anion (**2**, **11**, **12**, and **15**) were active. This fact supports a recent hypothesis concerning the presence of such an anion in a series of benzoic acid derivatives found to be active *in vitro*.† Furthermore, most of the nonanionic compounds (**1**, **4–6**, **8–10**) were inactive.

The reason why the hydroxamic acid derivative **3** and the morpholid **7** (both with $R' = H$; $R'' = CH_3$) were inactive while the corresponding compounds **12** and **13** (both with $R' = H$; $R'' = C_2H_5$) were active is not clear. This could be due to the higher lipid solubility of **12** and **13**.

References

(1) M. Vincent, G. Remond and J. C. Poignant, *J. Med. Chem.*, 15, 75 (1972).
(2) T. Y. Shen, *Annu. Rep. Med. Chem.*, 217 (1967).
(3) S. S. Adams, K. F. Mc Cullough, and J. S. Nicholson, *Arch. Int. Pharmacodyn.*, 178, 115 (1969).
(4) C. A. Winter E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, 111, 544 (1962).
(5) R. J. Gryglewski in "Chemical Control of Fibrinolysis-Thrombolysis," 1st ed, J. M. Schor, Ed., Wiley-Interscience, New York, N. Y., 1970, Chapter 2.
(6) A. R. Pinder, *J. Chem. Soc.*, 1577 (1956).
(7) K. N. von Kaulla, and C. Taylor, *Thromb. Diath. Haemorrh.*, 5, 489 (1961).
(8) K. N. von Kaulla, *J. Med. Chem.*, 8, 164 (1965).
(9) Z. Roubal and O. Nemecek, *ibid.*, 9, 840 (1966).
(10) K. N. von Kaulla and R. L. Schultz, *Amer. J. Clin. Pathol.*, **29**, 104 (1958).
(11) E. W. Salzman, *Thromb. Diath. Haemorrh., Suppl.*, **26**, 305 (1967).
(12) G. V. R. Born and M. J. Cross, *J. Physiol.*, 168, 178 (1963).
(13) B. Lipinski and K. Worowski, *Thromb. Diath. Haemorrh.*, **20**, 44 (1968).
(14) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, 57, 261 (1944).
(15) P. De Nicola, A. Gibelli, and G. Turazza, *Thromb. Diath. Haemorrh.*, 15 (1-2), 231 (1966).

†K. N. von Kaulla, personal communication.

Synthesis of 5-Trifluoromethyl-2′-deoxyuridine 5′-Phosphate and 5-Trifluoromethyl-2′-deoxyuridine 5′-Triphosphate†

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This laboratory has been interested in the biochemistry of 5-trifluoromethyl-2′-deoxyuridine (F_3dThd‡), a fluorinated analog of thymidine with clinical antitumor and antiviral activity.[1] The nucleotide, F_3dThd-5′-P, is a powerful inhibitor[2,3] of thymidylate synthetase, the enzyme responsible for metabolic conversion of deoxyuridylic acid to thymidylic acid. F_3dThd also is incorporated into DNA,[4] presumably after conversion *in vivo* to the 5′-triphosphate (F_3dThd-5′-PPP). To facilitate our studies on the mech-

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‡Approved IUPAC abbreviation.