Method G. A solution of 0.05 mol of substituted acryloyl anilide and 0.8 g of ammonium chloride in 120 mL of 95% ethanol was cooled to 0 °C. The solution was saturated with ammonia gas, placed into a steel autoclave, and heated to 80 °C for 48 h. The solvent was evaporated, and the product was taken up in ether and extracted with 1 M hydrochloric acid. The aqueous phase was alkalinized to pH 11 with sodium hydroxide and the amine extracted with methylene chloride. The organic phase was dried and the solvent evaporated. Recrystallization of the base, or a salt, yielded pure β-amino anilide.

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Troponoids. 3. Synthesis and Antiallergy Activity of *N*-Troponyloxamic Acid Esters

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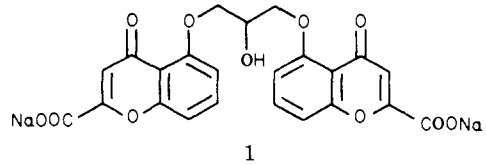
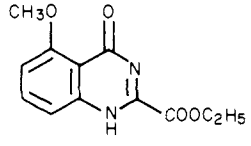
A number of oxamic acid derivatives of tropones and tropolones were synthesized and their antianaphylactic activity was determined in passive paw anaphylaxis (PPA). Several of these esters possessed oral activity. A comparison of the effect on the biological activity of the esters and the corresponding acid and its salt is reported. The experiments suggesting a relationship between the activity and the bioavailability of the ester **19** are also described. A study of the fate of ester **19** in serum on oral or intravenous administration to rats and dogs is reported. In vitro results of the effect of the compounds **19**, **45**, and **45a** on the activity of the guinea pig lung and beef heart phosphodiesterase are presented. The various factors that may contribute to the antiallergy activity of compounds of this series are discussed.

The discovery[1] of disodium cromoglycate (DSCG, **1**) has



1



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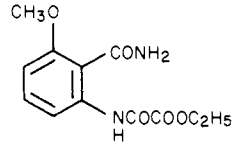

3

opened the way to the treatment of allergic rhinitis and asthma by the prophylactic action of chemotherapeutic agents. DSCG has been shown to exert its effect by inhibiting the liberation of mediators of the immediate-type hypersensitivity reaction initiated by an antigen–antibody interaction.[2] This reaction is usually studied in the passive cutaneous anaphylaxis (PCA) assay in rats, induced by reagenic antibody.[1] Recently, a method of inducing passive anaphylaxis in the hind paw (PPA) of rats was reported from our laboratories and its utility as a screening procedure for compounds of this type was demonstrated.[3]

Various heteroaromatic structures incorporating chromones,[4] xanthones,[5] and quinazolinones[6] have been reported to possess antiallergic activity of the type exhibited by DSCG. Many of these products were found to be orally active in the PCA assay.

It was found[6] that quinazolinecarboxylic acid esters of the type **2** were contaminated with an intermediate, which was found to have structure **3**. These oxamates were found

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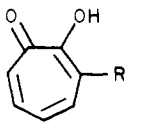
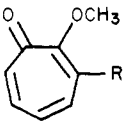
Table I. Proportion and TLC Behavior of C-3 and C-7 Regioisomers of Substituted 2-Methoxytropone



a



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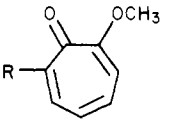


c

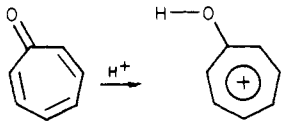
no.	R	yield, %	R_f	TLC solv[e]	yield, %	R_f
6	-Br[a]	24	0.7	E/H (1:1)	67	0.2
7	$-NHSO_2PhCH_3$[a]	7.7[d]	0.6	E/H (3:1)	27[d]	0.2
8	$-OCH_3$[a]	18	0.5	E/A (1:1)	67	0.2
9	-OPh[a]	18	0.8	ether	60	0.2
10	$-CH_2COOH$[b]	33[c]	0.6	B/A (7:3)	33[c]	0.4
11	$-CH_3$[b]	20	0.6	B/A (4:1)	48	0.3

[a] Dimethyl sulfate method. [b] Diazomethane method. [c] Yield and R_f are those of the methyl ester of **10b** and 10c. [d] N- and O-dimethylated products. [e] E = ethyl acetate; A = acetone; B = benzene; H = hexane.

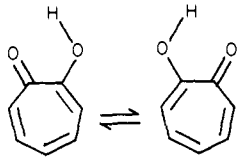
to be much more potent in the PCA assay than the corresponding parent quinazolinones. Since then another group has also reported antianaphylactic activity associated with oxamic acid ester derivatives.[35]

In view of the above results we considered the synthesis of the oxamates of tropone derivatives for biological evaluation as antianaphylactic agents.

Tropones and tropolones represent an interesting class of nonbenzenoid aromatic molecules. The aromaticity of tropone resides in its ability to generate a tropylium ion under acidic conditions, as shown in structure **4**. In



4



5a 5b

contrast, tropolones owe their stability to the highly dynamic tautomerism between structures **5a** and **5b**.

Bioisosterism of the catechol moiety with the α-hydroxyketo grouping of tropolone derivatives has already been demonstrated.[7] It was therefore of interest to explore the utility of this group of compounds in areas of medicinal interest.

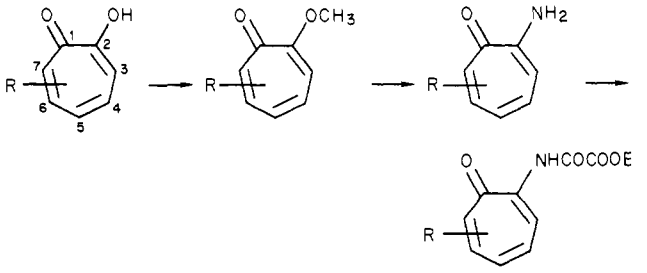
Chemistry. The oxamates were synthesized according to Scheme I.

Methylation of tropolone substituted at C-3 or C-4 leads to a mixture of regioisomeric methoxy derivatives. The unambiguous structure assignment of these regioisomers by spectroscopic methods, viz., infrared or ultraviolet spectroscopy or ^1H NMR, is not often possible. We have earlier reported[8] an analysis of ^{13}C NMR spectra of 2-substituted tropones. It was demonstrated that by using the ^{13}C NMR spectra it is possible to assign the structure of C-3, C-7 regioisomers with reasonable certainty.

In the course of our work, we have methylated a variety of 3-substituted tropolones. The experimental conditions employed (a) dimethyl sulfate–potassium carbonate–methyl ethyl ketone or (b) diazomethane. The results of these experiments are shown in Table I.

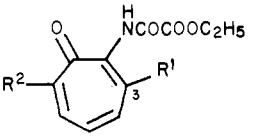
The structure of the bromo derivatives **6b** and **6c** were earlier assigned by the X-ray method.[10] We have confirmed[9] the structures of regioisomers **6b,c**, **8b,c**, and

Scheme I[a]



[a] R is one of the ring substituents shown in Table III.

Table II. C-3 Signals of ^{13}C NMR of Substituted 2-Oxamate Derivatives of Tropone



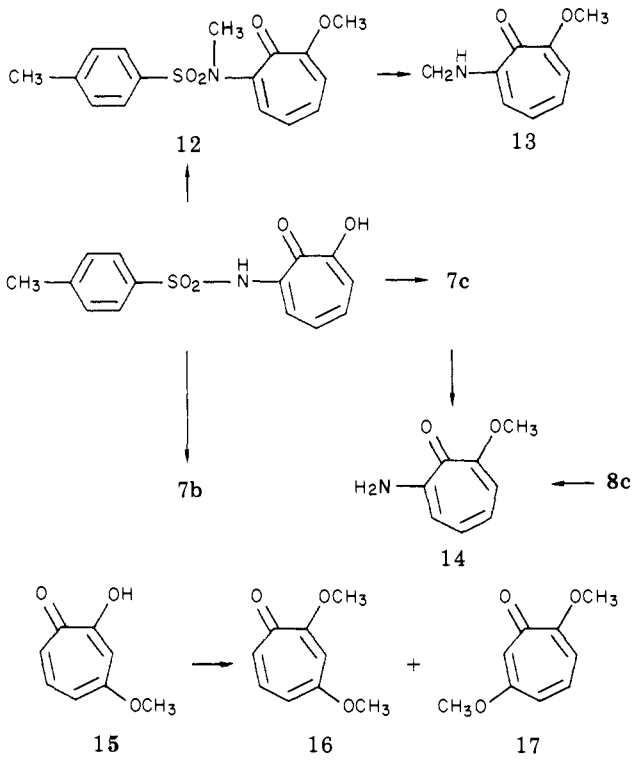
no.	R^1	R^2	C-3	solv
19	H	H	121.9	$CDCl_3$
19	H	H	121.2	Me_2SO
27	H	OPh	122.4	Me_2SO
26	OPh	H	*a*	Me_2SO
29	H	CH_3	122.3	$CDCl_3$
28		H	*a*	$CDCl_3$
30	H	Br	122.1	Me_2SO
32	H	OCH_3	122.9	Me_2SO
36	H	Ph	122.2	$CDCl_3$
37	H	CH_2COOCH_3	122.2	$CDCl_3$
31	H	OH	119.6	Me_2SO
33	H	$-N(CH_3)SO_2PhCH_3$	121.4	Me_2SO

[a] These regioisomers were assigned C-7 regiostructure based on the absence of the C-3 signals in the upfield region of the spectra. Their detailed ^{13}C NMR analysis and shifted ^{13}C signals of C-3 will be reported separately.

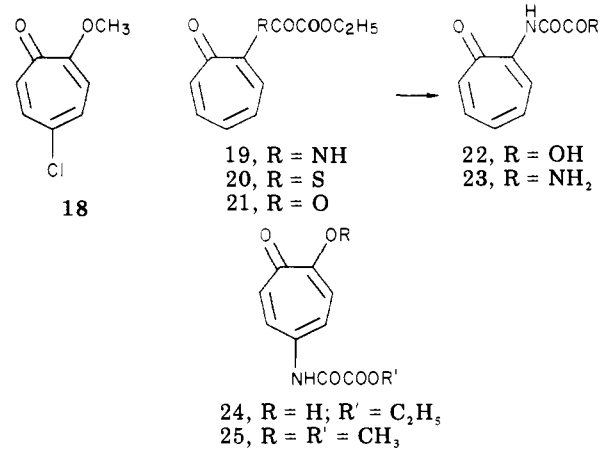
10b,c using ^{13}C NMR spectroscopy. It is noted that the ^{13}C signal due to protonated carbon-3 in this series of compounds appears most upfield of all the ring carbons and is therefore easily identifiable in the majority of cases. Carbon-3 signals of ^{13}C NMR spectra of the oxamates are recorded in Table II.

Two consistent observations that emerge from the data in Table I are related to the yield and TLC polarity of the two regioisomers. With the exception of **10a**, all the other substituted tropolones yielded the C-3 isomer as the minor component in the methylation reaction. Also, the C-3 isomer is consistently less polar on TLC relative to the C-7

Scheme II



Scheme III



isomer. In fact, the structures **7b,c**, **9b,c**, and **11b,c** were assigned based on these two observations and later confirmed by ^{13}C NMR spectra of their more advanced derivatives.

Methylation of **7a** yielded in addition to **7b** and **7c** a minor product which was found to be a monomethyl derivative and was assigned structure **12** based on its spectral and elemental analyses. The above assignment was confirmed as follows: Compounds **7c** and **12** were treated with sulfuric acid to generate the corresponding amino derivatives **13** and **14** (Scheme II). Compound **14** was found to be identical in all respects with that obtained from **8c** by treatment with ammonia.

4-Methoxytropolone (**15**) was prepared as described[11] and separated from its C-3 regioisomer by chromatography. Methylation of **15** led to the formation of isomeric methyl ethers **16** and **17**. The gross structures of these isomers were elucidated by spectroscopy. The less polar of the two isomers was arbitrarily[34] assigned structure **16** and the more polar one the regioisomer **17**. The structure of isomer **17** was later confirmed by ^{13}C NMR spectroscopy.[9]

A modification of the Sandmeyer reaction described earlier[12] was used to prepare 5-chlorotropolone, which was transformed to its methyl ether **18** in the usual manner (Scheme III).

The methoxytropones described above were generally reacted with ammonia or methylamine in a pressure bottle to yield the corresponding amino derivatives. It is interesting to note that, except in the case of 3-Me-**11b** and 3-phenoxy-2-methoxytropone (**9b**), no other 3-substituted derivatives yielded the corresponding amines under the conditions employed.

The oxamates of the amino derivatives above were prepared using ethyloxalyl chloride in the presence of (a) pyridine or (b) methylene chloride–triethylamine as solvent. To investigate the structural requirements for antiallergy activity, various modified oxamate side chains were synthesized. The thia and oxa derivatives **20** and **21** of the biologically interesting derivative **19** were synthesized starting with mercaptotropone[13] and tropolone. The latter was quite unstable and was isolated only when the sodium salt of tropolone was reacted with ethyloxalyl chloride in dry benzene and the reaction worked up without contact with water.

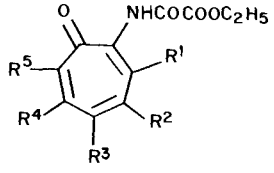
The hydrolysis of oxamate **19** under alkaline conditions led mainly to the cleavage of the -NHCO- bond attached to the ring, yielding 2-aminotropone. However, the hydrolysis proceeded smoothly in boiling water containing 1 equiv of potassium acetate to yield the acid **22**. Diamide **23** was obtained by reaction of acid **22** with carbonyldiimidazole, followed by treatment with ammonia.

Pharmacology. The compounds reported in this paper were screened for their antiallergy activity.

Passive anaphylaxis (reaginic antibody mediated) was induced in the rat hind paw (PPA) instead of in the skin (PCA) using a recently described method.[3] In the paw, inhibition of edema rather than inhibition of dye leakage was used to determine antiallergic activity. Rat serum containing reaginic antibody was injected into the left hind paw. After 24 h, antigen was injected intravenously. Paw volume was measured by mercury displacement. The inhibition of this reaction produced by disodium cromoglycate is comparable to that of cutaneous anaphylaxis.[3] However, in our hands the procedure is more accurate and can be done rapidly in normal unrestrained rats. The compounds were first screened at dose levels of 30 and 10 mg/kg intraperitoneally (ip), followed by oral (po) administration at 100 and 30 mg/kg.

Structure–Activity Relationship. The results of [*N*-(2-oxo-3,5,7-cycloheptatrien-1-yl)amino]oxoacetic acid ethyl ester **19** and the effect of ring substitution on the antiallergy activity are listed in Table III. Although the limitations of the synthetic aspect of tropone chemistry allowed mainly the preparation of C-7 substituted compounds, in a few cases the C-3, C-4, and C-5 substituted derivatives prepared did not show any encouraging biological profile. In general, the substitution at C-7 significantly reduced the biological activity. It is worth noting that, although the C-7 substituents, e.g., phenyl (**36**), methyl (**29**), methoxy (**32**), and methylamino (**34**), retained a significant degree of activity when given ip, they were only slightly active or essentially inactive when administered orally. Substituents at C-3, e.g., phenoxy (**26**) and methyl (**28**), as well as at C-4, e.g., methoxy (**37**), also lowered significantly the potency relative to the unsubstituted parent compound **19**. The only two exceptions were the dioxamic acid ester **40** and the 6-methoxy derivative **38**. These compounds retained to a large degree the oral activity of the parent oxamate. In contrast, replacing the C-2 oxamate grouping of **40** by an hydroxy, as

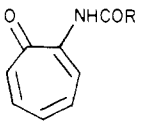
Table III. Antianaphylactic Activity of Substituted 2-Oxamic Acid Ethyl Esters of Tropone



compd	R¹	R²	R³	R⁴	R⁵	mp, °C	yield, %	crystn[b] solv	formula[f] (anal.)	% inhibn of paw edema[g] ip 30[e]	ip 10	po 100	po 30
19	H	H	H	H	H	114-115	66.6	B/C	$C_{11}H_{11}NO_4$ (C, H, N)	84	87	91	79
26	OC_6H_5	H	H	H	H	121-122	27	A	$C_{17}H_{15}NO_5$ (C, H, N)	0		20	
27	H	H	H	H	OC_6H_5	145-146	74	A	$C_{17}H_{15}NO_5$ (C, H, N)	16		9	
28	CH_3	H	H	H	H	81-82	68	H	$C_{12}H_{13}NO_4$[c]	47		37	4
29	H	H	H	H	CH_3	125-126	52	H	$C_{12}H_{13}NO_4$[c]	60	40	34	6
30	H	H	H	H	Br	161-163	53	D/E	$C_{11}H_{10}BrNO_4$ (C, H, N)	52		0	
31	H	H	H	H	OH	158-159	83.5	A	$C_{11}H_{11}NO_5$	61	5	0	
32	H	H	H	H	OCH_3	164-167	80	A	$C_{12}H_{13}NO_5$ (C, H, N)	52	24	56	59
33	H	H	H	H	$N(CH_3)SO_2$-Ph-CH_3	148.5-150	50	A/C	$C_{19}H_{20}N_2O_6S$ (C, H, N)	32		0	3
34	H	H	H	H	$NHCH_3$	178-181	20.5	E/C[d]	$C_{12}H_{14}N_2O_4$ (C, H, N)	83	73		0
35	H	H	H	H	$N(CH_3)COCOOC_2H_5$	oil	52	E/C[d]	$C_{16}H_{18}N_2O_7$ (C, H, N)	51	26	0	6
36	H	H	H	H	Ph	152-154	80	A	$C_{17}H_{15}NO_4$ (C, H, N)	72	57	0	
37	H	OCH_3	H	H	H	132-134	18.5	A/C[d]	$C_{12}H_{13}NO_5$ (C, H, N)	8		53	31
38	H	H	H	OCH_3	H	157-158	18.5	A	$C_{12}H_{13}NO_5$[c]	67	73	48	45
39	H	H	Cl	H	H	178-179	78	A	$C_{11}H_{10}ClNO_4$ (C, H, N)	27		25	
40	H	H	$NHCOCOOC_2H_5$	H	H	217-218	6.9	A	$C_{15}H_{16}N_2O_7$[c]	84	93	76	61
DSCG										77	62		
W8011[a]										98	70	83	53

[a] See ref 33. [b] A = ethyl acetate; B = chloroform; C = hexane; D = methanol; E = acetone; F = ether; G = water; H = cyclohexane. [c] These compounds were characterized by IR, UV, NMR, and MS. [d] Compounds 34, 35, and 38 were purified by chromatography, the first two using acetone/hexane (3:7) and the last one using ethyl acetate/hexane (4:6). [e] Dose in mg/kg. [f] Compounds were analyzed for the elements shown in parentheses, and all results were within ±0.4% of the calculated value. [g] Inhibition of 35% is considered statistically significant by Student's t test ($p < 0.05$).

Table IV. Comparative Intraperitoneal and Oral Activity of Oxamic Acid Esters and Salts



no.	R	mp, °C	yield, %	crystn solv[e]	formula[b] (anal.)	% inhibn of paw edema[f] ip 30[d]	ip 10[d]	po 100[d]	po 30[d]
19	$COOC_2H_5$					84	87	91	79
41	$COOCH(CH_3)_2$	91-93	52.6	F/C	$C_{12}H_{13}NO_4$ (C, H, N)	96	76	72	63
42	$COOC(CH_3)_3$	76-78	60	A/C	$C_{13}H_{15}NO_4$ (C, H, N)	79		82	47
43	$COO(CH_2)_4CH_3$	87-89	74.6	F/C	$C_{19}H_{17}NO_4$ (C, H, N)	57	61		12
44	COOH	193-194	63.1	D	$C_9H_7NO_4$ (C, H, N)	77	89	62	38
45	COOK		33.6	G/E	$C_9H_6NO_4K$[c]	75	70	68	53
46	$COO^-{}^+NH_3C(CH_2OH)_3$	148-152	90.3	D/E	$C_{13}H_{18}N_2O_7$ (C, H, N)			73	32
47	$COOCH_2COOC_2H_5$	110-112	60.9	B/C	$C_{13}H_{13}NO_6$ (C, H, N)		69	64	31
48	$COOCH_2COOK$	257-259	91.7	F/D	$C_{11}H_8NO_4K$[c]	93	66	50	45
49	COOPh	131-132	30	D	$C_{15}H_{11}NO_4$ (C, H, N)	73	68	71	31
50	$CONHNH_2$	193	95	D	$C_9H_9N_3O_3$ (C, H, N)	78	64	78	41
W8011[a]						98	70	83	53

[a] See ref 33. [b] Compounds were analyzed for the elements shown in parentheses, and all results were within ±0.4% of the calculated value. [c] These salts were prepared from purified acids and analyzed spectroscopically (IR, UV, and NMR). [d] Dose in mg/kg. [e] A = ethyl acetate; B = chloroform; C = hexane; D = methanol; E = acetone; F = ether; G = water. [f] Inhibition of 35% or more is considered statistically significant by Student's t test ($p < 0.05$).

Table V. Comparative Effect of Ester 19 and Its Free Acid and Salts in the PPA Test

compd	ip dose, mg/kg	ip edema vol, mL	ip % inhibn	po dose, mg/kg	po edema vol, mL	po % inhibn
control		1.1 ± 0.08			0.95 ± 0.06	
19	10	0.22 ± 0.09[a]	80	30	0.17 ± 0.03[a]	81
	3	0.41 ± 0.05[a]	62	10	0.24 ± 0.04[a]	75
	1	0.95 ± 0.11	16	3	0.47 ± 0.07[a]	50
control		1.16 ± 0.11			1.43 ± 0.09	
44	10	0.12 ± 0.03[a]	89	30	0.88 ± 0.10[a]	38
	3	0.75 ± 0.09[b]	35	10	1.11 ± 0.07	22
control		1.28 ± 0.13			0.95 ± 0.06	
45	10	0.36 ± 0.04[a]	80	30	0.44 ± 0.05[a]	53
	5	0.69 ± 0.17[b]	46	10	0.66 ± 0.12[b]	31
	2	0.69 ± 0.09[a]	46	3	0.61 ± 0.12	35
control					0.95 ± 0.06	
46				30	0.64 ± 0.09[b]	32
				10	0.88 ± 0.15	7
				3	0.83 ± 0.08	13
control		1.10 ± 0.09			1.34 ± 0.05	
W8011[33]	10	0.32 ± 0.09[a]	70	30	0.63 ± 0.06[a]	53
	3	0.68 ± 0.09[a]	39	10	0.74 ± 0.12[a]	44
	1	1.00 ± 0.09	14	3	1.13 ± 0.04[a]	15

[a] $p < 0.01$ (Student's t test). [b] $p < 0.05$ (Student's t test).

in **24**, or by a methoxy group, as in **25**, markedly lowered the potency (30 mg/kg ip showed 10% inhibition for **24** and 36% for **25**).

The loss of activity observed in the above compounds in vivo can be attributed either to the fact (1) that the compound is intrinsically inactive, (2) that the product is indiscriminately bound to the tissue and is not absorbed, or (3) that it cannot cross the transport barriers to reach the site of action. It is observed that many of the compounds in Table III retain a high degree of intraperitoneal activity, suggesting a possible lack of absorption when administered orally.

The effect on the biological activity of changing the ester function is recorded in Table IV. Substituting the ethyl group of ester **19** with more bulky functions, such as isopropyl as in **41** or *tert*-butyl as in **42**, led to a slight drop in the oral activity. A similar change in activity was observed when the ethyl group was replaced by an aromatic ring, as in **49**, or by the $-CH_2COOC_2H_5$ group, as in **47**. In contrast, a marked drop in the oral activity was observed when the ester was prepared from *n*-pentanol as in **43**. The hydrazide **50**, on the other hand, retained a significant degree of both oral and intraperitoneal activity. Replacing the nitrogen atom of **19** by sulfur or oxygen, as in **20** and **21**, led to totally inactive compounds.

It has been reported[6] earlier in another series of oxamic acid esters that the oral activity of an ester was lost when the esters were hydrolyzed to the acid. It was therefore of interest to compare in our series the activity of the ester, acid, and the salt. These results are reported in Table V.

In contrast to the earlier report,[6] the oxamic acids of the tropone series retain a significant degree of oral activity. It is interesting to observe that both the K salt and the THAM salt of acid **44** are also active; the former is slightly more potent than the acid itself.

It was reported earlier that [*N*-(2-oxo-3,5,7-cyclohexatrien-1-yl)amino]oxoacetic acid ethyl ester **19** inhibited the liberation of mediators from the mast cell (intraperitoneal anaphylaxis) when administered ip. It also antagonized the histamine release when administered po.[14]

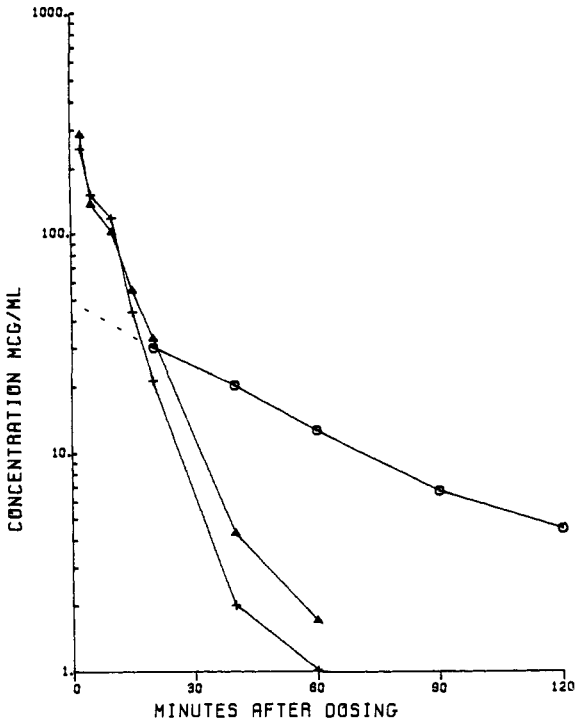


Figure 1. The change in serum concentration with time of ester **19** (×–×), acid **44** (▲–▲), and 2-aminotropone (○–○) in rats given an iv bolus of 0.23 mmol/kg of each compound.

These and other experiments demonstrated that the oxamate **19** is similar in its mode of action to DSCG.

In order to investigate the compound(s) appearing in the serum of rats treated with ester **19**, a method was developed based on the quantification of 2-aminotropone in the UV. Following an iv injection of equimolar doses of the acid **44**, its ester **19**, and 2-aminotropone, the rate of disappearance of drug-derived material from the serum after **44** and **19** was virtually identical and considerably faster than after 2-aminotropone (Figure 1). The finding suggested that the ester **19** is rapidly hydrolyzed to the acid **44** but not to 2-aminotropone when administered iv. Supporting evidence was obtained in rats injected iv with the ester **19** labeled with ^{14}C in the ethyl group or in the carbonyl carbons of the oxalic moiety. The blood levels of ^{14}C after the injection of ester **19** labeled at the ethyl group were similar to those found after an iv injection of an equimolar dose of [^{14}C]ethanol. In contrast, the rate of disappearance of radioactivity in serum due to ^{14}C after the injection of ester **19** labeled at the oxalyl carbonyl was comparable to the values obtained with the chemical method. A similar observation was made when the above experiments were repeated after one oral administration of either ester **19** or acid **44**. In this case, however, some biodegradation to 2-aminotropone was noted.

The antianaphylactic drug DSCG has been suggested to exert its pharmacological effect by inhibition of lymphocytis cyclic nucleotide phosphodiesterase (PDE).[15] The resulting rise in the intracellular cyclic AMP is postulated to lead, via a lowering of the transport of calcium ions across the mast cell membrane,[15] to the inhibition of the release of histamine. In view of the ability of oxamate ethyl ester **19** to inhibit the release of histamine, it was of interest to examine the PDE inhibitory activity of this compound.

It was found[17] that the compound **19** was as equally potent as the known PDE inhibitor theophylline in inhibiting guinea pig lung PDE and was three times less active than theophylline in inhibiting beef heart PDE.

Table VI. Effect on Cyclic AMP Phosphodiesterase Activity

compd	IC_{50}, μM: beef heart PDE[a]	IC_{50}, μM: guinea pig lung PDE[a]
19	1515[b]	1940[b]
44	4330[c]	
45	5100[c]	5000[c]
theophylline	515[b]	1475[b]

[a] Final cyclic AMP concentration: beef heart PDE, 0.117 μM; guinea pig lung PDE, 10.0 μM. [b] See ref 17. [c] Extrapolated values due to limited solubilities of these compounds.

Table VII. Effect on the Activity of Guinea Pig Lung Adenyl Cyclase

compd	concn, M	% stimulation of basal act.[a]
19	1×10^{-4}	7[b]
	1×10^{-5}	0[b]
45	1×10^{-5}	2
	1×10^{-5}	0
DSCG	2.5×10^{-3}	0[b]
theophylline	1×10^{-3}	5[b]
epinephrine	1×10^{-4}	84[b]

[a] The figures represent percent stimulation of basal activity, which is 60.88 ± 2.78 pmol (100 μg of protein)$^{-1}$ per 15-min incubation. [b] See ref 17.

However, it is important to point out that higher levels of cyclic AMP resulting from the inhibition of PDE are perhaps only a minor pathway to the mode of action of ester **19**.

Since the blood-level studies had shown that the ester is readily hydrolyzed to the corresponding acid, it was of interest to determine the PDE inhibitory activity of the free acid **44** and the potassium salt **45**. Furthermore, since the level of cyclic AMP appears to be of importance with regard to the inhibition of the mediator release and since the formation of intracellular cyclic AMP is in turn a function of the enzyme adenyl cyclase, the effect of these compounds on the activity of adenyl cyclase was also determined. The PDE inhibitory activities of the free acid **44** and the potassium salt **45** were much less than that of the ester **19**, both on the beef heart and on the guinea pig lung preparations (Table VI).

The ester **19** is more active when administered orally in the PPA test (Table V) than the acid **44**. Furthermore, it is more active as a PDE inhibitor than the acid **44**. These two observations tend to suggest that the ester function, although readily biodegraded, may play, due to its lipophilicity, a vital role in transport of the compound to the site of action when administered orally.

The guinea pig lung adenyl cyclase assay system can be employed to measure the effects of a compound on this enzyme, since negligible cyclic nucleotide PDE activity is associated with this preparation.[18] It can be seen from the previous[19] and present results (Table VII) that neither compound **19** nor **45** had any stimulating effect on the enzyme, thus suggesting a lack of any direct effect on the formation of cyclic AMP.

Finally, a point of relevance is the following. It has been shown,[19] particularly with DSCG, that this compound preferentially inhibits cyclic GMP PDE in comparison with cyclic AMP PDE, thus affecting the ratio of cyclic AMP to cyclic GMP. As pointed out,[17] it would be of interest to examine the ability of the ester **19** to inhibit the cyclic GMP PDE to further delineate its mode of action. Modulation of adenyl cyclase and phosphodiesterase

systems by Ca^{2+} ion transport across the mast cells[20] may also be relevent in the mode of action of ester **19**.

Experimental Section

The infrared and ultraviolet spectra were recorded on Perkin-Elmer diffraction grating and Zeiss DMR-21 spectrophotometers, respectively. The melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The NMR spectra were performed on a Varian A-60A and CFT-20 spectrometer. Organic extracts were dried over anhydrous magnesium sulfate, and solvents were always removed under vacuum. Merck silica gel 60 (70–230 mesh) was used for column chromatography.

Tropolone,[25] 2-aminotropone,[21] 2,5-diaminotropone,[22] 2-(*N*-methylamino)tropone,[23] 5-aminotropone,[24] 5-chlorotropolone,[12] 2-phenoxytropolone,[26] and *p*-toluenesulfonamidotropolone[27] were synthesized using slight modifications of the described procedure.

Methylation of Substituted Tropolone. Two procedures were used and a typical case of each one is as follows.

(a) Dimethyl Sulfate Procedure. 5-Chlorotropolone (40 g, 1 equiv) was suspended in methyl ethyl ketone (548 mL) and heated to 70 °C. Potassium carbonate (39.2 g, 1.1 equiv) was added and stirred for 10 min. Dimethyl sulfate (55.6 mL, 1.7 equiv) was added and the mixture was refluxed for 1.5 h. It was cooled to room temperature and filtered. The filtrate was evaporated, and the residue was taken up in ether and filtered to yield the product (39.2 g): mp 120–122 °C (reported[28] mp 123 °C).

(b) Diazomethane. The methylation with diazomethane was done in the usual manner, using methanol or ether–methanol as solvent.

Preparation of Substituted 2-Aminotropone. These compounds were generally prepared from the methoxy derivatives. A solution of the 2-methoxy derivative in methanol was saturated with ammonia at low temperatures (0 to −10 °C) and heated in a pressure bottle to 80 °C for 4 to 8 h. The reaction mixture was cooled, the solvent was evaporated, and the product was isolated by crystallization. In most cases, the crude product was characterized spectroscopically and utilized for the next step, i.e., acylation with ethyl oxalyl chloride, without further purification.

Preparation of Ethyl Oxamates from Aminotropones. Method A. 2-Aminotropone (0.24 g, 1 equiv) was dissolved in pyridine (0.5 mL) and to it was added a solution of ethyloxalyl chloride (0.3 g, 1.1 equiv) in pyridine (1.5 mL). The mixture was stirred at room temperature for 45 min. It was then diluted with water, and the product **19** (0.29 g) was filtered. Crystallization from chloroform–hexane gave an analytical sample: NMR ($CDCl_3$) δ 10.9 (1 H, br, NH), 7.8–9.0 (5 H, m, vinylic), 4.5 (2 H, q, $-OCH_2-$), 1.45 (3 H, t, CCH_3).

Method B. To a solution of 2-amino-6-methoxytropone (1.05 g, 1 equiv) and triethylamine (0.84 g, 1.2 equiv) in methylene chloride (20 mL) was added dropwise ethyloxalyl chloride (0.96 g, 1.05 equiv) in methylene chloride (10 mL) at room temperature. The reaction mixture was stirred for 3 h, washed with water, and dried, and the solvent was removed. The crude product was purified by chromatography to yield a pure sample of [*N*-(4-methoxy-2-oxo-3,5,7-cycloheptatrien-1-yl)amino]oxoacetic acid ethyl ester **38** (0.52 g): mp 157–158 °C; IR (Nujol) 3270, 1732, 1715 cm^{-1}; NMR (TFA) δ 9.5 (1 H, d, vinylic), 7.95 (3 H, m, vinylic), 4.58 (2 H, q, OCH_2-), 4.2 (3 H, s, OCH_3), 1.5 (3 H, t, CCH_3).

2-[(Carboxycarbonyl)amino]-2,4,6-cycloheptatrien-1-one (44). To a suspension of ethyl ester **19** (2.21 g, 1 equiv) in water (15 mL) was added potassium acetate (0.98 g, 1 equiv) in water (5 mL). The mixture was heated to reflux for 5 h. The solution was cooled, diluted with water, treated with charcoal, and filtered. The filtrate was acidified with hydrochloric acid (10%) to yield the acid (1.2 g, 63%), mp 193–194 °C. The spectroscopic data (IR, UV, and NMR) were in consonance with the expected structure **44**.

2-[[1-(Pentyloxy)-1,2-dioxoethyl]amino]-2,4,6-cycloheptatrien-1-one (43). To a solution of acid **44** (1.54 g, 1 equiv) in Me_2SO (8 mL) were added potassium carbonate (0.82 g, 1.5 equiv) and then a solution of bromopentane (1.52 mL, 1.5 equiv) in Me_2SO (8 mL). The mixture was heated to 80 °C for 40 min, cooled to room temperature, poured over ice (ca. 100 g), stirred for 10 min, and filtered. The residue was dissolved in ether, treated with charcoal, and crystallized by adding hexane to yield pure product (1.56 g, 74.6%): mp 87–89 °C; IR ($CHCl_3$) 3270, 1758, 1730, 1705 cm^{-1}; NMR ($CDCl_3$) δ 10.9 (1 H, br, NH), 9.0 (1 H, m, vinylic), 7.35 (4 H, m, vinylic), 4.4 (2 H, t, OCH_2), 1.85 (2 H, m, CCH_2C), 1.5 (4 H, m, CCH_2C), 0.92 (3 H, t, CCH_3).

2-[(1-Methylethoxy)-1,2-dioxoethyl]amino]-2,4,6-cycloheptatrien-1-one (**41**) and 2-[2-[(2-oxo-3,5,7-cycloheptatrien-1-yl)amino]-1,2-dioxoethoxy]acetic acid ethyl ester (**47**) were prepared in an analogous manner as described above.

2-[[(1,1-Dimethylethoxy)-1,2-dioxoethyl]amino]-2,4,6-cycloheptatrien-1-one (42). To a solution of acid **44** (1.90 g, 1 equiv) in dry tetrahydrofuran (30 mL) was added carbonyldiimidazole (1.6 g, 1 equiv). The mixture was stirred at room temperature for 2 h. *tert*-Butyl alcohol (8 mL, 5 equiv) was then added and the mixture refluxed for 1 h. It was cooled to room temperature and filtered. The residue was washed with tetrahydrofuran and the total filtrate evaporated to dryness. The product was isolated by chromatography to yield a crystalline solid (1.4 g, 60%): mp 76–78 °C; IR ($CHCl_3$) 3280, 1755, 1705, 1565 cm^{-1}; NMR ($CDCl_3$) δ 10.6 (1 H, br, NH), 8.8 (1 H, m, vinylic), 7.15 (4 H, m, vinylic), 1.58 [9 H, s, C-$(CH_3)_3$].

[*N*-(2-Oxo-3,5,7-cycloheptatrien-1-yl)amino]oxoacetic Acid Potassium Salt (45). To a solution of potassium hexanoate (0.92 g, 3 equiv) in methanol–water (7 mL, 1:1) was added a hot solution of acid **44** (0.386 g, 1 equiv) in methanol (35 mL). The mixture was stirred at room temperature for 0.5 h and cooled. The resulting solid was filtered and crystallized to yield the potassium salt (0.37 g), mp 245–265 °C. The IR, UV, and NMR spectra were in accord with the desired structure.

2-[[[(2-Oxo-3,5,7-cycloheptatrien-1-yl)amino]oxoacetyl]oxy]acetic Acid Potassium Salt (48). Acid **44** was allowed to react with bromo-*tert*-butyl acetate as described above to form the corresponding *tert*-butyl ester. This ester (8.4 g) was suspended in concentrated hydrochloric acid (20 mL) and stirred at −20 °C for 2 h. The mixture was then diluted with ice and filtered to yield the corresponding acid (7.1 g). This acid was dissolved in dry tetrahydrofuran (100 mL), and a solution of potassium hexanoate (12.7 g) in dry tetrahydrofuran (30 mL) was added to it. The mixture was heated to 80 °C for 2 h, cooled, and filtered to give a crude product (7.23 g), mp 228–230 °C. A sample was crystallized from water–methanol to yield the pure potassium salt: mp 255–257 °C; IR (Nujol) 3220, 1630, 1542, 1450, 1345, 1345 cm^{-1}; NMR (D_2O) δ 7.3 (5 H, m, vinylic), 4.7 (2 H, s, OCH_2).

[*N*-(2-Oxo-3,5,7-cycloheptatrien-1-yl)amino]oxoacetic Acid Hydrazide (50). To a solution of compound **19** (1.2 g, 1 equiv) in ethanol was added anhydrous hydrazine (0.18 g, 1 equiv), and the mixture was stirred at room temperature for 5 h. The precipitate which formed was filtered, dried, and crystallized from methanol to give a pure product (1.12 g): mp 193 °C; IR, UV, and NMR were in agreement with the expected structure.

Biochemical Studies. Male Sprague–Dawley albino CD (BioBreeding Laboratories) rats with body weights of 170–190 g were used. Sonified suspensions of ester **19** made up with 0.2% aqueous Tween-80 were used for iv injection. At specified times after dosing, the rats were killed by decapitation, in groups of five, and the blood was collected and allowed to clot. Serum was separated by centrifugation and stored frozen until analyzed. Radioactivity was measured in 0.2 mL of serum digested with 1.0 mL of Soluene-350 (Packard Instrument Co., Inc.) after the addition of 15 mL of Omnifluor solution (New England Nuclear; 4 g/L of toluene).

2-Aminotropone Determination. To 1.0 mL of serum, 3.0 mL of distilled water, 1.0 mL of 1 N NaOH, and 12 mL of 10% *n*-heptane in ethyl acetate were added and shaken; the supernatant was added to 5 mL of 0.1 M HCl and shaken, and the aqueous phase was used for the measurement of 2-aminotropone by UV spectroscopy.

Biochemical Pharmacology Studies. The measurement of PDE activity of beef heart[31] and guinea pig lung[22] were carried out essentially as described. The protein concentration was determined by the method of Lowry.[30] IC_{50} was determined by plotting percent inhibition vs. the logarithm of the inhibitor concentration and is the inhibitor concentration producing 50% reduction in enzyme reaction velocity compared with the noninhibited controls.

The measurement of adenyl cyclase activity of guinea pig lung was carried out as previously described.[32] The materials used were beef heart PDE (Boehringer Mannheim, 15153, EPAY, control no. 7205306), Russel's viper venom (Sigma Chemical Co.), cyclic [8-^{3}H]AMP (specific activity 21 Ci/mmol, Schwarz-Mann), [α-^{32}P]ATP [adenosine 5'-triphosphate, tetrakis(triethylammonium salt), specific activity 33.54 Ci/mmol, New England Nuclear], Dowex 1-X-2 (~400 mesh, chloride form), Dowex 50 W-X-8 (100–200 mesh, hydrogen form, Bio-Rad Laboratories), and theophyllinemonoethylamine (K and K laboratories).

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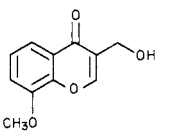

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Reactions of 1,3-Bis(2-chloroethyl)-1-nitrosourea and 1-(2-Chloroethyl)-3-cyclohexyl-1-nitrosourea in Aqueous Solution

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Products formed from the reaction of two chloroethylnitrosoureas in neutral aqueous solution have been identified and quantified. Mixture components recovered after a 1-h incubation period accounted for 75–85% of the starting nitrosourea. Approximately 65–85% of the reaction products were formed by an initial cleavage of the nitrosourea to the proposed intermediates 2-chloroethyl azohydroxide and an isocyanate and by subsequent hydrolytic reactions. A minor pathway, 5–10% of products, involves denitrosation of the nitrosourea with oxazoline formation. Stable isotope labeling and mass spectrometry have been used to determine the reaction sequence and product origins. Reaction product identification has been made using high-performance LC isolation and comparison with synthetic material.

1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) and other chloroethylnitrosoureas are used in the treatment of brain tumors,[1,2] lymphomas,[3] and other malignant diseases. These agents are not biologically active in their parent form but are converted to active alkylating intermediates by chemical reactions in aqueous media.[4] The active alkylating agent is thought to be 2-chloroethyl azohydroxide or diazonium ion.[5,6] BCNU and related chlo-

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