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.

Acknowledgment. We thank L. A. Dadah, R. Heideger, M. Hogan, L. J. Kofos, and L. S. Åberg for skillful technical assistance and M. R. Blair and G. H. Kronberg for many valuable discussions. We also thank J. B. Keenaghan for the mass spectral data.

References and Notes

- E. W. Byrnes, P. D. McMaster, E. R. Smith, M. R. Blair, R. N. Boyes, B. R. Duce, H. S. Feldman, G. H. Kronberg, B. H. Takman, and P. A. Tenthorey, J. Med. Chem., 22, first paper in a series of three in this issue.
- (2) (a) B. R. Duce, E. R. Smith, R. N. Boyes, and E. W. Byrnes, *Pharmacologist*, 15, 192 (1973); (b) E. W. Byrnes, M. R. Blair, H. S. Feldman, G. H. Kronberg, in preparation.
- (3) D. J. Coltart, T. B. Berndt, R. Kernoff, and D. C. Harrison, Am. J. Cardiol., 34, 35 (1974).

- (4) D. Lalka, M. B. Meyer, B. R. Duce, and A. T. Elvin, Clin. Pharmacol. Ther., 19, 757 (1976).
- (5) (a) D. G. McDevitt, A. S. Nies, G. R. Wilkinson, R. F. Smith, R. L. Woosley, and J. A. Oates, *Clin. Pharmacol. Ther.*, 19, 396 (1976); (b) R. A. Winkle, P. J. Meffin, J. W. Fitzgerald, and D. C. Harrison, *Circulation*, 54, 884 (1976); (c) P. J. Meffin, R. A. Winkle, T. F. Blaschke, J. Fitzgerald, D. C. Harrison, S. R. Harapat, and P. A. Bell, *Clin. Pharmacol. Ther.*, 22, 42 (1977); (d) R. L. Woosley, D. G. McDevitt, A. S. Nies, R. F. Smith, G. R. Wilkinson, and J. A. Oates, *Circulation*, 56, 980 (1977); (e) R. A. Winkle, P. J. Meffin, and D. C. Harrison, *Circulation*, 57, 1008 (1978).
- (6) P. D. McMaster, E. W. Byrnes, H. S. Feldman, B. H. Takman, and P. A. Tenthorey, J. Med. Chem., 22, second paper in a series of three in this issue.
- (7) N. Löfgren and B. Lundqvist, Svensk. Kem. Tidskr., 58, 206 (1946).
- (8) N. Löfgren, "Studies on Local Anesthetics", Ivar Haeggstroms, Stockholm, 1948, p 25.
- (9) U. H. Lindberg, B. Nylén, and B. Akerman, Acta Pharm. Suec., 5, 429 (1968).
- (10) J. C. Sheehan and B. A. Bolhofer, J. Am. Chem. Soc., 72, 2786 (1950).
- (11) J. W. Lawson, J. Pharmacol. Exp. Ther., 160, 22 (1968).
- (12) J. Berkson, J. Am. Stat. Assoc., 48, 565 (1953).
- (13) S. A. Harris, Circulation, 1, 1318 (1950).
- (14) D. T. Korkin, Astra Research Laboratories, unpublished.

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

Jehan F. Bagli,* T. Bogri, B. Palameta,

Department of Chemistry

R. Martel,

Department of Pharmacology

W. Robinson,

Department of Biochemistry

T. Pugsley, and W. Lippmann

Department of Biochemical Pharmacology, Ayerst Laboratories, Montreal, Quebec, Canada. Received February 23, 1979

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 discovery¹ of disodium cromoglycate (DSCG, 1) has



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 quinazolinones⁶ 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 found⁶ that quinazolinecarboxylic acid esters of the type 2 were contaminated with an intermediate, which was found to have structure 3. These oxamates were found

0022-2623/79/1822-1186\$01.00/0 © 1979 American Chemical Society

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



^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



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 reported⁸ an analysis of ¹³C NMR spectra of 2substituted tropones. It was demonstrated that by using the ¹³C 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 carbonatemethyl 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 confirmed⁹ the structures of regioisomers 6b,c, 8b,c, and



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

ц

Table II.C-3 Signals of 13C NMR of Substituted2-Oxamate Derivatives of Tropone

		R ² R ² R ² R ¹	2H5	
no.	R¹	R ²	C-3	solv
19	Н	Н	121.9	CDCl ₃
19	H	н	121.2	Me,SÕ
27	H	OPh	122.4	Me,SO
26	OPh	Н	a	Me,SO
29	H	CH ₃	122.3	CDC1,
28		Н	а	CDCl,
30	н	Br	122.1	Me,SŎ
32	H	OCH ₃	122.9	Me ₂ SO
36	н	Ph	122.2	CDCl,
37	н	CH ₂ COOCH ₃	122.2	CDCl,
31	н	OH	119.6	Me,SÔ
33	H	-N(CH ₃)SO ₂ PhCH ₃	121.4	Me ₂ SO

^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 ¹³C NMR analysis and shifted ¹³C signals of C-3 will be reported separately.

10b,c using ¹³C NMR spectroscopy. It is noted that the ¹³C 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 ¹³C 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



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 described¹¹ 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 arbitrarily³⁴ assigned structure 16 and the more polar one the regioisomer 17. The structure of isomer 17 was later confirmed by ¹³C NMR spectroscopy.⁹

A modification of the Sandmeyer reaction described earlier¹² 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 mercaptotropone¹³ 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

Ĩ2

с^ч

R2

la ^g		õ	6,			4	9			65	ი	0	9		3I 21	15		31		53	d by e is
r eden	od	0		0	0	7	4	0	0	 	0		0	0	ი ო	8	10			 	terized hexan f 35%
of paw			6	Ñ		ŝ	က်	-	-	õ	-			-	ú	4	5	2		õõ	charac etate/ tion o
hibn e	ip	10	87	•			40		ç	24		73	26	57		73		93	62	70	were o hyl ac Inhibi
% in		30^{e}	84	0	16	47	60	52	61	52	32	83	51	72	8	67	27	84	77	98	ounds sing et ie. g
		formula ^f (anal.)	CHNO. (C. H. N)	C, H, NO, (C, H, N)	C, H, NO, (C, H, N)	ĊĹ,H,NO ^c	C, H, NO c	C, H, BrNO, (C, H, N)	C,H,NO	C, H, NO (C, H, N)	C, H, N, O, S (C, H, N)	C,,,H,,N,O, (C, H, N)	C, H, N, O, (C, H, N)	C, H, NO, (C, H, N)	C, H, NO, (C, H, N)	C, H, NO c	C, H, CINO, (C, H, N)	C, H, N, O,	8 5 6		Solution c and c
	crystin ^b	solv	B/C	A	A	Η	Η	D/E	A	A	A/C	E/C^{d}	E/C^{d}	A	A/C^{d}	A	A	A			; H = cyo ne/hexau within ±
		yield, %	66.6	27	74	68	52	53	83.5	80	50	20.5	52	80	18.5	18.5	78	6.9			G = water ing aceto ults were
		mp, °C	114-115	121 - 122	145-146	81-82	125 - 126	161-163	158 - 159	164 - 167	148.5 - 150	178-181	oil	152 - 154	132 - 134	157-158	178-179	217-218			me; F = ether; (the first two us eses, and all res
		\mathbf{R}^{s}	H	Н	OC, H,	H Č	CH,	Br	НО	OCH,	N(CH ₃)SO ₂ -Ph-CH ₃	NHCH	N(CH ₃)COCOOC ₃ H ₅	Ph	Н	Н	Н	Η			D = methanol; E = aceto ied by chromatography, ments shown in parenth
		R⁴	H	Н	Н	Н	Н	Н	Η	Н	Н	Η	Η	Н	Η	OCH ₃	Η	Н			 hexane; rere purif or the ele o of oc
		R³	Н	Н	Н	Н	Η	Н	Н	Н	Н	Н	Н	Н	Н	Η	C	NHCOCOOC ₂ H ₅			:: B = chloroform; C = inds 34, 35, and 38 w unds were analyzed f
		R²	Н	Н	Н	Н	Н	Н	Н	Η	Н	Н	Н	Н	OCH ₃	Η	Н	Н			d Compoi
		R'	Н	OC, H,	H	CH ₃	Н	Н	Н	Н	Н	Н	Η	Н	Н	Н	Н	Н			b A = eth and MS. in mg/kg.
		compd	19	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	DSCG	W8011 ^a	^a See ref 33. IR, UV, NMR, (4:6). ^e Dose i



						% inhibn of paw edema ^f		lema ^f	
				ervstn		i	р	p	<u></u> с
no.	R	mp, $^{\circ}$ C	yield, %	solv ^e	formula ^b (anal.)	30^d	10^d	100^d	30^d
19	COOC ₂ H 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	$C_{1,0}H_{1,1}NO_{4}(C, H, N)$	57	61		12
44	COOH	193-194	63.1	D	$C_{a}H_{7}NO_{4}(C, H, N)$	77	89	62	38
45	COOK		33.6	G/E	C _o H ₆ NO ₄ K ^c	75	70	68	53
46	$COO^{-+}NH_3C(CH_2OH)_3$	148 - 152	90.3	D/E	$C_{13}H_{18}N_{2}O_{7}(C, H, N)$			73	32
47	COOCH ₂ COOC,H,	110 - 112	60.9	B/C	$C_{13}H_{13}NO_{6}(C, H, N)$		69	64	31
48	COOCH,COOK	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,	193	95	D	$C_{H_0}H_0N_0O_1(C,H,N)$	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 $\pm 0.4\%$ 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).

		тр		po				
compd	dose, mg/kg	edema vol, mL	% inhibn	dose, mg/kg	edema vol, mL	% 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		

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

^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 reported⁶ 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 $[N-(2-\infty -3,5,7-\text{cyclo-hexatrien-1-yl})$ 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 $(\times - \times)$, acid 44 $(\bigtriangleup - \blacktriangle)$, and 2-aminotropone $(\bigcirc -\bigcirc)$ 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 ¹⁴C in the ethyl group or in the carbonyl carbons of the oxalic moiety. The blood levels of ¹⁴C 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 [¹⁴C]ethanol. In contrast, the rate of disappearance of radioactivity in serum due to ¹⁴C 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 found¹⁷ 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
Phosphodie	esterase A	ctivity	

· · · · · · · · · · · · · · · · · · ·	IC ₅₀ , μM						
compd	beef heart PDE ^a	guinea pig lung PDE ^a					
19	1515 ^b	1940 ^b					
44	4330 ^c						
45	5100°	5000 ^c					
theophylline	515 ^b	1475 ^b					

^a Final cyclic AMP concentration: beef heart PDE, 0.117 μ M; guinea pig lung PDE, 10.0 μ M. ^b See ref 17.

0.117 μ M; guinea pig lung PDE, 10.0 μ M. ⁶ See ref 17. ^c Extrapolated values due to limited solubilities of these compounds.

Table	VII.	Effect on	the	Activity	of	Guinea	Pig
Lung A	Adeny	l Cyclase					

compd	concn, M	% stimulation of basal act. ^a
19	$\frac{1 \times 10^{-4}}{1 \times 10^{-5}}$	7b 0b
45	1×10^{-5} 1×10^{-5}	2
DSCG theophylline epinephrine	$\begin{array}{c} 2.5 \times 10^{-3} \\ 1 \times 10^{-3} \\ 1 \times 10^{-4} \end{array}$	0 ^b 5 ^b 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 previous¹⁹ 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 cells²⁰ 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*-toluenesulfonamidotropolone²⁷ 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 (reported²⁸ 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 (CDCl₃) δ 10.9 (1 H, br, NH), 7.8–9.0 (5 H, m, vinylic), 4.5 (2 H, q, -OCH₂-), 1.45 (3 H, t, CCH₃).

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, OCH₂-), 4.2 (3 H, s, OCH₃), 1.5 (3 H, t, CCH₃).

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 Me₂SO (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 Me₂SO (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 (CHCl₃) 3270, 1758, 1730, 1705 cm⁻¹; NMR (CDCl₃) δ 10.9 (1 H, br, NH), 9.0 (1 H, m, vinylic), 7.35 (4 H, m, vinylic), 4.4 (2 H, t, OCH₂), 1.85 (2 H, m, CCH₂C), 1.5 (4 H, m, CCH₂C), 0.92 (3 H, t, CCH₃).

2-[(1-Methylethoxy)-1,2-dioxoethyl]amino]-2,4,6-cycloheptatrien-1-one (41) and 2-[2-[(2-oxo-3,5,7-cycloheptatrien-1yl)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]-2,4,6cycloheptatrien-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 (CHCl₂) 3280, 1755, 1705, 1565 cm⁻¹; NMR (CDCl₃) 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-(CH₃)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 (D₂O) δ 7.3 (5 H, m, vinylic), 4.7 (2 H, s, OCH₂).

[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 heart³¹ and guinea pig lung²² were carried out essentially as described. The protein concentration was determined by the method of Lowry.³⁰ IC₅₀ 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 non-inhibited 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 [8-³H]AMP (specific activity 21 Ci/mmol, Schwarz-Mann), [α -³²P]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).

Acknowledgment. The authors express their appreciation to Dr. G. Schilling and his associates for microanalytical and spectral data. The skillful assistance of Miss M. Mihelic and Mr. J. Csakvary in chemical synthesis and of Ms. M. Wong and Ms. M. T. Silvestre in the biochemical studies is gratefully acknowledged. We also thank Dr. E. Greselin and Dr. D. Hicks for their cooperation in the bioavailability studies and in the synthesis of ¹⁴C-labeled esters, respectively.

References and Notes

- H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, J. Med. Chem., 15, 583 (1972).
- (2) J. S. G. Cox, Adv. Drug Res., 5, 115 (1970).
- (3) R. R. Martel and J. Klicius, Int. Arch. Allergy Appl. Immunol., 54, 205 (1977).
- (4) A. Nohara, H. Kuriki, T. Saijo, H. Sugihara, M. Kanno, and Y. Sanno, J. Med. Chem., 20, 141 (1977).
- (5) W. D. Jones, W. L. Albrecht, N. L. Munro, and K. T. Stewart, J. Med. Chem., 20, 594 (1977).
- (6) J. H. Stellstedt, C. J. Guinosso, A. J. Begany, S. C. Bell, and M. Rosenthale, J. Med. Chem., 18, 926 (1975).
- (7) B. Belleau and J. Burba, J. Med. Chem., 6, 755 (1963).
- (8) J. F. Bagli and M. St. Jaques, Can. J. Chem., 56, 578 (1978).
- (9) J. Bagli, M. St. Jaques, T. Bogri, and B. Palameta, Can. J.
- Chem., in press (1979).
 (10) T. Nozoe, S. Seto, and H. Takeda, Sci. Rep. Tohoku Univ., Ser. 1, 36, 126 (1952).
- (11) T. Yamatani, M. Yasunami, and K. Takase, *Tetrahedron Lett.*, 1725 (1970).
- (12) T. Nozoe, S. Seto, and M. Sato, Proc. Jpn. Acad., 27, 426 (1951).
- (13) T. Nozoe, M. Sato, and K. Matsui, Proc. Jpn. Acad., 29, 22 (1953).
- (14) R. Martel, J. Klicius, and J. Pinski, Can. J. Physiol. Pharmacol., 56, 1005 (1978).

- (15) N. Lavin, G. S. Rachelefsky, and S. A. Kaplan, J. Allergy Clin. Immunol., 57, 80 (1976).
- (16) J. C. Foreman and L. G. Garlan, Br. Med. J., 1, 820 (1976).
- (17) T. A. Pugsley and W. Lippmann, Experientia, 35, 88 (1979).
- (18) I. Weinryb and I. M. Michel, Experientia, 27, 1386 (1971).
- (19) H. Bergstrand, J. Kristoffersson, B. Lundquist, and A. Schurmann, Mol. Pharmacol., 13, 38 (1977).
- (20) F. Carpendo, R. M. Gaion, and G. Fassina, Biochem. Pharmacol., 24, 2069 (1975).
- (21) W. von E. Doering and L. H. Knox, J. Am. Chem. Soc., 73, 828 (1951).
- (22) T. Nozoe, M. Sato, and T. Matsuda, Sci. Rep. Tohoku Univ., Ser. 1, 37, 407 (1953).
- (23) N. Soma, J. Nakazawa, T. Watanabe, Y. Sato, and G. Sunagawa, Chem. Pharm. Bull., 13, 457 (1965).
- (24) T. Nozoe and S. Seto, Proc. Jpn. Acad., 27, 188 (1951); Chem. Abstr., 4522 (1952).
- (25) H. C. Steven, J. K. Reinhart, J. M. Lavanish, and G. M. Frenta, J. Org. Chem., 36, 2780 (1971).
- (26) K. Takase, Bull. Chem. Soc. Jpn., 37, 1288 (1964).
- (27) Y. Kitahara, Sci. Rep. Tohoku Univ., Ser. 1, 40, 83 (1956).
- (28) T. Sato, Nippon Kagaku Sasshi, 80, 1171 (1959); Chem. Abstr., 4389e (1961).
- (29) J. E. Tateson and D. G. Trist, Life Sci., 18, 153 (1976).
- (30) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
- (31) W. Lippmann, Experientia, 30, 237 (1974).
- (32) I. Weinryb, I. M. Michel, and S. Hess, Arch. Biochem. Biophys., 154, 240 (1973).
- (33) W 8011 [3-(hydroxymethyl)-8-methoxychromone, i] was used



to serve as an orally active, positive control. For reference, see "Immunopharmacology", M. E. Rosenthale and H. C. Mansmann, Jr., Eds., Spectrum Publications, New York, 1975, pp 112–113.

- (34) TLC polarity was used as arbitrary means of assignment, because of the relationship between isomer at C-3 and C-7 as described in Table I. The ¹³C NMR spectra of these compounds are under study, and the results will be reported in a separate publication.
- (35) J. B. Wright and H. G. Johnson, J. Med. Chem., 20, 166 (1977).

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

Robert J. Weinkam* and Huey-Shin Lin

Department of Pharmaceutical Chemistry, School of Pharmacy, and the Brain Tumor Research Center, Department of Neurological Surgery, School of Medicine, University of California, San Francisco, California 94143. Received November 10, 1978

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-