

Potential Hypotensive Compounds: Substituted 3-Aminopropionates and 3-Aminopropionohydroxamic Acids

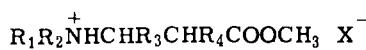
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Abstract □ As a continuation of a previous study, a series of substituted methyl 3-aminopropionates and 3-aminopropionohydroxamic acids were prepared and evaluated for their ability to lower blood pressure of rats. Many of the compounds possessed hypotensive properties but of very short duration. Some of the compounds were screened for their ability to protect mice against a lethal dose of diisopropyl fluorophosphate but none was active.

Keyphrases □ 3-Aminopropionates, methyl—synthesis, screened as potential hypotensive agents □ 3-Aminopropionohydroxamic acids—synthesis, screened as potential hypotensive agents □ Hypotensive agents, potential—synthesis, screening of methyl 3-aminopropionates and 3-aminopropionohydroxamic acids

Some studies were already reported (1, 2) on the synthesis of certain methyl 3-aminopropionates (I) and 3-aminopropionohydroxamic acids (II), in which the substituents R_1 and R_2 were alkyl groups, or an alkyl group and a hydrogen atom, or a ring system; R_3 was a hydrogen atom; and R_4 was a hydrogen atom or a methyl group. Some of these compounds possessed potent hypotensive properties in rats and cats, and it was shown that the nature of the groups R_1 , R_2 , and R_4 influenced significantly the magnitude and duration of the fall in blood pressure produced.

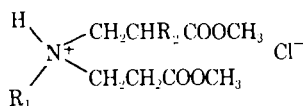
The results so far obtained prompted a continuation of this study to see if more potent hypotensive compounds could be produced. In particular, the result on hypotensive activity of varying the nature of groups R_1 and R_2 in I and II was sought. In all of the compounds



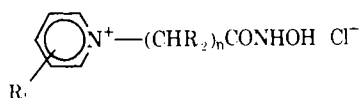
I



II



III



IV

synthesized, the substituents R_3 and R_4 were hydrogen atoms or one of these substituents was a methyl group. In addition, three diesters of general Structure III were synthesized, in which R_1 was a benzyl or phenethyl group and R_2 was a hydrogen atom or a methyl group.

Coe (3) synthesized a number of quaternary pyridinium hydroxamic acids of Structure IV and found them effective in the prophylaxis and treatment of organo-

phosphate poisoning. For this reason, all of the hydroxamic acids and some of the esters prepared in this study were evaluated for their ability to protect mice against lethal doses of diisopropyl fluorophosphate. None was active.

CHEMISTRY

The 3-aminopropionate esters (I) prepared in this study (Tables I and II) were obtained in two ways. Most were the products of the addition of an amine across the α,β -double bond present in methyl acrylate, methyl methacrylate, and methyl crotonate. When equimolecular quantities of the amine and the α,β -unsaturated ester were interacted, the major product was the desired one (I). Yields varied but were generally fair. The products were oils but, in some instances, small amounts of colorless solids precipitated during the reaction. They were presumed to be amides (2) but were not characterized.

When excess methyl acrylate was reacted with benzylamine or phenethylamine, two molecules of the ester reacted with one molecule of the amine, and monobasic diesters were the result.

A few amino-esters (I) were obtained by the interaction of an amine (2 moles) with a 3-bromopropionic ester (1 mole). The hydrogen bromide liberated during the reaction was trapped by the excess amine. Yields of amino-esters using this method of synthesis were generally good.

In most instances, the esters were readily purified by fractional distillation under reduced pressure. With some high boiling amino-esters (Table I, Compounds 41-45), purification was difficult because distillation resulted in the decomposition of the desired product. Dibenzylamine, for example, combined with methyl methacrylate, as evidenced by the gradual diminution of the N—H band in the IR spectrum of the reaction mixture. However, during distillation, dissociation of the molecule occurred, and the starting materials, dibenzylamine and methyl methacrylate, were regenerated. Similar difficulties were encountered when attempts were made to distill the diesters (Table II). McElvain and Stork (4) previously reported that condensations of amines with unsaturated esters are reversible, and Harper *et al.* (5) suggested a mechanism to explain the decomposition of tertiary amino-diester during distillation.

The synthesis of the 3-aminopropionohydroxamic acids (Table III) was accomplished by treating the appropriate amino-ester with hydroxylamine (base or hydrochloride) in methanol. Products were invariably contaminated with varying amounts of the hydrochloride salts of the amino-ester precursors, and lengthy purification procedures were required. In many instances, hydroxamic acids were oils and could not be purified. These acids were not investigated further.

PHARMACOLOGY

The effect of intravenous administration of the aminohydroxamic acid hydrochlorides (Table III) and many of the amino-ester salts (Tables I and II) on the blood pressure of anesthetized rats was investigated. Most were inactive (*i.e.*, a dose of 4 mg/kg. caused no fall in blood pressure), and those that showed activity (Table IV) had only an evanescent effect. Some of these were examined for muscarinic and ganglion-blocking activity.

Only one compound, *N,N*-di-(2-carbomethoxyethyl)phenethylamine hydrochloride (Compound 47), exhibited muscarinic properties. Activity was abolished by the administration of scopolamine. Most of the others were ganglion blockers (Table IV), as shown by their abilities to abolish the pressor response to nicotine but not noradrenaline. None of the compounds had any adrenergic neuron-blocking activity on the rabbit ileum preparation.

Table I—Methyl 3-Aminopropionates: $R_1R_2NCHR_3CHR_4COOCH_3$

Compound	R_1	R_2	R_3	R_4	Method ^a	Yield ^b , %	Boiling Point/mm.	Lit. Boiling Point/mm. (ref.)	Salt	Melting Point	Formula	Analysis, %	
												Calc.	Found
1	CH ₃	H	H	CH ₃	Ia	34	74-75/26	48.8-49.5/8 (7)	HCl	80°	C ₆ H ₁₃ NO ₂	54.94	55.29
2	CH ₃ CH ₂	H	H	CH ₃	Ia	47	76°/18.5	51°/0.5 (8)	HCl	104-105°	C ₇ H ₁₆ ClNO ₂	9.99	10.03
3	CH ₃ (CH ₂) ₂	H	H	CH ₃	Ia	54	88-89°/17.5	31°/0.15 (9)	HCl	127-128°	C ₈ H ₁₇ NO ₂	46.26	46.30
4	CH ₂ =CHCH ₂	H	H	H	Ia	32	93-95°/25.5	—	HCl	75-77°	C ₇ H ₁₄ ClNO ₂	8.88	8.57
5	CH ₂ =CHCH ₂	H	H	CH ₃	Ia	70	95°/24.5	72-74°/3 (10)	HCl	108° ^d	C ₈ H ₁₆ ClNO ₂	60.34	60.58
6	(CH ₃) ₂ CH	H	H	H	Ia	67	80-82°/24.5	—	HCl	107°	C ₇ H ₁₆ ClNO ₂	10.76	10.99
7	(CH ₃) ₂ CH	H	H	CH ₃	Ia	44	83-84°/22.5	41°/0.1 (9)	HCl	111-112°	C ₈ H ₁₈ ClNO ₂	46.80	46.85
8	CH ₃ (CH ₂) ₃	H	H	CH ₃	Ia	40	104°/19	58-60°/1 (11)	HCl	130-131°	C ₉ H ₁₉ NO ₂	7.85	7.75
9	CH ₃ CH ₂ CH(CH ₃)	H	H	H	Ia	38	95-97°/22.5	—	HCl	71-72°	C ₈ H ₁₈ ClNO ₂	49.60	49.69
10	CH ₃ CH ₂ CH(CH ₃)	H	H	CH ₃	Ia	39	99°/22.5	—	HCl	75-78°	C ₉ H ₂₀ ClNO ₂	8.33	8.38
11	(CH ₃) ₃ C	H	H	H	Ia	56	89-90°/24	—	HCl	142°	C ₈ H ₁₈ ClNO ₂	46.28	46.66
12	(CH ₃) ₃ C	H	H	CH ₃	Ia	25	55-57°/9	49-50°/1 (11)	HCl	98-99°	C ₉ H ₂₀ ClNO ₂	8.88	9.44
13	CH ₃ (CH ₂) ₄	H	H	H	Ia	51	115°/22	—	HCl	195°	C ₉ H ₁₉ NO ₂	49.10	48.89
14	CH ₃ (CH ₂) ₄	H	H	CH ₃	Ia	58	120°/22.5	—	HCl	140°	C ₁₀ H ₂₁ NO ₂	9.27	9.56
15	(CH ₃) ₂ CH(CH ₂) ₂	H	H	H	Ia	34	111-113°/21	—	HCl	208°	C ₉ H ₁₉ NO ₂	51.55	51.50
16	(CH ₃) ₂ CH(CH ₂) ₂	H	H	CH ₃	Ia	46	116-117°/22.5	—	HCl	119°	C ₁₀ H ₂₁ NO ₂	9.61	9.54
17	Cyclopentyl	H	H	H	Ia	38	119-121°/20	—	HCl	104-105°	C ₉ H ₁₈ ClNO ₂	49.10	49.31
18	Cyclopentyl	H	H	CH ₃	Ia	22	122-123°/18.5	—	HCl	85-86°	C ₁₀ H ₂₀ ClNO ₂	9.27	9.53
19	CH ₃ (CH ₂) ₆	H	H	CH ₃	Ia	37	133-134°/21	—	HCl	126-127°	C ₁₁ H ₂₃ NO ₂	51.55	51.76
20	Cyclohexyl	H	H	H	Ia	63	132-133°/17.5	125-128°/4.5 (12)	HCl	161-162°	C ₁₀ H ₂₀ ClNO ₂	9.61	9.87
21	Cyclohexyl	H	H	CH ₃	Ia	42	141°/22	110°/7 (13)	HCl	157° ^f	C ₁₁ H ₂₂ ClNO ₂	62.39	62.05
22	CH ₃ (CH ₂) ₆	H	H	H	Ia	45	86-89°/0.06	—	HCl	181-182°	C ₁₁ H ₂₃ NO ₂	11.06	10.80
23	CH ₃ (CH ₂) ₆	H	H	CH ₃	Ia	52	82-84°/0.03	—	HCl	115°	C ₁₂ H ₂₅ NO ₂	64.13	64.23
24	CH ₃ (CH ₂) ₇	H	H	H	Ia	56	110-112°/1	—	HCl	177°	C ₁₂ H ₂₅ NO ₂	11.30	11.23
25	CH ₃ (CH ₂) ₇	H	H	CH ₃	Ia	52	118-119°/0.6	—	HCl	111-112°	C ₁₃ H ₂₈ ClNO ₂	62.39	62.48
26	(CH ₃) ₃ CCH ₂ C(CH ₃) ₂	H	H	H	Ia	57	89-91°/0.1	—	HCl	135°	C ₁₂ H ₂₅ ClNO ₂	11.06	10.89
27	(CH ₃) ₃ CCH ₂ C(CH ₃) ₂	H	H	CH ₃	Ia	33	89°/0.07	75°/0.5 (11)	HCl	148-149°	C ₁₃ H ₂₈ ClNO ₂	64.13	64.31
												52.04	51.74
												8.73	9.06
												54.16	54.26
												9.09	9.20
												65.63	65.81
												11.52	11.54
												541.6	54.11
												9.12	9.12
												6.32	6.38
												56.04	56.12
												9.41	9.47
												65.63	65.27
												11.52	11.42
												66.93	66.93
												11.70	11.40
												66.93	66.68
												11.70	11.47
												58.74	58.85
												10.62	10.47
												57.24	57.50
												10.41	10.50
												58.74	58.62
												10.62	10.33

28	$C_8H_5CH_2$	H	H	H	H	Ia	77	123 ² /2	145-147°/7 (12)	HBr	135-137°	$C_{11}H_{16}BrNO_2$	C	48.19
29	$C_8H_5CH_2$	H	H	CH_3	CH_3	Ia	64	134-135°/5	96-98°/0.3 (14)	HBr	91-92°	$C_{12}H_{17}NO_2$	H	5.88
30	$C_8H_5CH_2$	H	CH_3	H	H	Ia	59	115-117°/1	—	HBr	136-137°	$C_{12}H_{17}NO_2$	N	5.23
31	$C_8H_5(CH_2)_2$	H	H	H	H	Ia	64	140-142°/3	—	HBr	140-141°	$C_{12}H_{17}NO_2$	C	69.77
32	$C_8H_5(CH_2)_2$	H	H	CH_3	CH_3	Ia	69	124°/1	136°/5 (13)	HBr	154-155°	$C_{13}H_{20}BrNO_2$	H	8.27
33	$C_8H_5(CH_2)_2$	H	CH_3	H	H	Ia	64	130-132°/2	—	HBr	146-147°	$C_{13}H_{19}NO_2$	N	6.75
34	$C_8H_5(CH_2)_2$	H	H	CH_3	CH_3	Ia	61	153-155°/3	126-128°/3 (13)	HCl	114-115°	$C_{14}H_{21}NO_2$	C	6.50
35	C_8H_5	H	H	H	H	Ib	28	144-146°/3	125-126°/3 (7)	HBr	105-106°	$C_{10}H_{13}NO_2$	H	8.27
36	C_8H_5	H	H	CH_3	CH_3	Ib	19	124-126°/2	152°/15 (15)	Oxalate	121° ^a	$C_{11}H_{15}NO_2$	N	6.40
37	C_8H_5	H	CH_3	H	H	Ib	28	125-127°/2	—	HBr	139-140°	$C_{11}H_{15}NO_2$	C	70.55
38	$C_8H_5CH_2$	CH_3	H	H	H	II	86	102-104°/1	—	HCl	99-100°	$C_{12}H_{17}NO_2$	H	8.65
39	$C_8H_5CH_2$	CH_3	H	CH_3	CH_3	II	53	100-102°/1	94°/0.3 (16))	HCl	149-150°	$C_{13}H_{19}NO_2$	N	7.82
40	$C_8H_5CH_2$	CH_3	CH_3	H	H	II	68	111-112°/1.5	—	Oxalate	114-115°	$C_{13}H_{19}NO_2$	C	71.45
41	$C_8H_5(CH_2)_2$	$C_6H_5(CH_2)_2$	H	H	H	II	95 ^a	—	—	HBr	122-123°	$C_{20}H_{26}BrNO_2$	H	8.99
42	$C_8H_5(CH_2)_2$	$C_6H_5CH_2$	H	H	H	II	94 ^a	—	—	HBr	97-99°	$C_{19}H_{24}BrNO_2$	N	5.95
43	$(C_8H_5)_2CH$	H	H	H	H	Ia	98 ^a	—	—	HBr	237-238°	$C_{17}H_{20}BrNO_2$	C	67.01
44	$C_8H_5CH(CH_2C_6H_5)$	H	H	H	H	Ia	96 ^a	—	—	HBr	170-171°	$C_{18}H_{22}BrNO_2$	H	7.31
45	$(C_8H_5)_2CH(CH_2)_2$	H	H	H	H	Ia	96 ^a	—	—	HBr	131-132°	$C_{19}H_{23}NO_2$	N	7.81

^a See Experimental section for details. ^b Yield after distillation except where noted. ^c Lit. (2) m.p. 130-132°. ^d Lit. (10) m.p. 103-104°. ^e Lit. (9) m.p. 114-114.5°. / Lit. (13) m.p. 157°. ^f Lit. (15) m.p. 120°. ^g Not distilled.



Table II—Dicarboxylic Esters

Compound	R ₁	R ₂	Reference	Method ^a	Yield, % ^b	Formula	Analysis, %		Hydrochloride Melting Point
							Calc.	Found	
46	C ₆ H ₅ CH ₂	H	17	III	97	C ₁₃ H ₂₁ NO ₄	C 64.49 H 7.57 N 5.01	64.79 7.40 4.95	84-85°
47	C ₆ H ₅ (CH ₂) ₂	H	18	III	97	C ₁₆ H ₂₃ NO ₄	C 65.49 H 7.90 N 4.77	65.39 8.20 4.83	99-100°
48	C ₆ H ₅ CH ₂	CH ₃	19	IV	91	C ₁₆ H ₂₃ NO ₄	C 65.49 H 7.90 N 4.77	65.35 7.99 4.74	76-77°

^a See Experimental section for details. ^b Not distilled.

Table III—3-Aminopropionhydroxamic Acid Hydrochlorides: R₁R₂NHCHR₃CHR₄CONHOH Cl⁻

Compound	R ₁	R ₂	R ₃	R ₄	Method ^a	Yield, %	Melting Point (dec.)	Formula, RC/N ₂ O ₂ R =	Analysis, %	
									Calc.	Found
49	CH ₃	H	H	CH ₃	I	44	137-138°	C ₅ H ₁₃	C 35.62 H 7.77	35.40 7.92
50	CH ₃ CH ₂	H	H	CH ₃	I	15	105-106°	C ₆ H ₁₅	C 39.46 H 8.28	16.95 39.33
51	CH ₃ (CH ₂) ₂	H	H	CH ₃	I	23	122-123 ^{ab}	C ₇ H ₁₇	C 42.75 H 8.71	15.30 43.04
52	CH ₂ =CHCH ₂	H	H	CH ₃	I	31	60°	C ₇ H ₁₅	C 43.19 H 7.77	14.31 43.36
53	CH ₃ (CH ₂) ₃	H	H	CH ₃	I	55	140°	C ₈ H ₁₉	C 45.60 H 9.09	14.49 45.61
54	(CH ₃) ₃ C	H	H	H	I	30	168-169°	C ₇ H ₁₇	C 42.75 H 8.71	13.38 42.90
55	(CH ₃) ₃ C	H	H	CH ₃	I	66	181°	C ₈ H ₁₉	C 45.60 H 9.09	13.99 45.53
56	CH ₃ (CH ₂) ₄	H	H	CH ₃	I	68	143-144°	C ₉ H ₂₁	C 48.10 H 9.42	13.49 48.18

57	Cyclopentyl	H	H	H	H	H	H	I	27	179-180°	C ₈ H ₁₇	C	46.04	45.83
58	Cyclopentyl	H	H	CH ₃	CH ₃	CH ₃	CH ₃	I	28	156-157°	C ₉ H ₁₉	N	8.21	8.31
59	CH ₃ (CH ₂) ₅	H	H	CH ₃	CH ₃	CH ₃	CH ₃	I	63	135-136°	C ₁₀ H ₂₂	C	13.42	13.63
60	Cyclohexyl	H	H	H	H	H	H	I	48	182-183°	C ₉ H ₁₉	N	48.54	48.34
61	Cyclohexyl	H	H	CH ₃	CH ₃	CH ₃	CH ₃	I	67	147-148°	C ₁₀ H ₂₁	C	8.60	8.68
62	CH ₃ (CH ₂) ₆	H	H	CH ₃	CH ₃	CH ₃	CH ₃	I	66	123°	C ₁₁ H ₂₃	N	12.58	12.84
63	CH ₃ (CH ₂) ₇	H	H	H	H	H	H	I	57	104°	C ₁₁ H ₂₃	C	50.30	50.42
64	(CH ₃) ₂ CCH ₂ C(CH ₃) ₂	H	H	H	H	H	H	I	68	153°	C ₁₁ H ₂₃	H	9.71	9.78
65	(CH ₃) ₂ CCH ₂ C(CH ₃) ₂	H	H	CH ₃	CH ₃	CH ₃	CH ₃	I	44	172°	C ₁₂ H ₂₇	N	11.73	11.74
66	C ₆ H ₅ CH ₂	H	H	H	H	H	H	II	68	158-159° ^c	C ₁₀ H ₁₈	C	48.16	48.16
67	C ₆ H ₅ CH ₂	H	H	CH ₃	CH ₃	CH ₃	CH ₃	II	41	166-167°	C ₁₁ H ₁₇	H	8.60	8.94
68	C ₆ H ₅ (CH ₂) ₂	H	H	H	H	H	H	II	35	149-150°	C ₁₁ H ₁₇	N	12.58	11.90
69	C ₆ H ₅ (CH ₂) ₂	H	H	CH ₃	CH ₃	CH ₃	CH ₃	II	83	135-136° ^d	C ₁₂ H ₁₉	C	50.74	50.74
70	C ₆ H ₅ (CH ₂) ₂	H	H	H	H	H	H	II	31	131-132°	C ₁₂ H ₁₉	H	8.94	9.39
71	C ₆ H ₅ (CH ₂) ₃	H	H	CH ₃	CH ₃	CH ₃	CH ₃	II	47	130-131°	C ₁₃ H ₂₁	N	11.83	11.94
72	C ₆ H ₅ CH ₂	CH ₃	CH ₃	H	H	H	H	I	17	168-169°	C ₁₂ H ₁₉	C	51.97	51.97
73	C ₆ H ₅ (CH ₂) ₂	C ₆ H ₅ (CH ₂) ₂	H	H	H	H	H	I	4	131-132° ^e	C ₁₃ H ₂₅	N	11.08	11.19
74	C ₆ H ₅ CH(CH ₂ C ₆ H ₅)	H	H	H	H	H	H	I	2	211-212° ^e	C ₁₇ H ₂₁	C	52.26	52.38

^a See *Experimental* section for details. ^b Lit. (2) m.p. 125-127°. ^c Lit. (1) m.p. 158-159°. ^d Lit. (2) m.p. 126-127°. ^e After numerous recrystallizations.

Table IV—Effect of 3-Aminopropionhydroxamic Acid Hydrochlorides and Methyl 3-Aminopropionate Salts on the Blood Pressure of the Anesthetized Rat

Compound ^a	Dose, mg./kg.	Average Fall in Blood Pressure, %	Average Duration, min.	Mechanism of Action
62	1	8	2	—
	2	14	3	
	4	26	3	
63	1	11	3	—
	2	17	4	
	4	24	5	
68	1	5	2	—
	2	10	3	
	4	13	7	
69	1	2	1	—
	2	4	1	
	4	8	1	
70	1	5	<1	—
	2	7	<1	
	4	10	<1	
71	1	4	1	—
	2	9	4	
	4	12	5	
72	1	4	2	—
	2	7	4	
	4	8	8	
73	1	9	1	—
	2	15	1	
	4	17	1	
5	1	8	1	—
	2	11	1	
	4	14	1	
14	1	15	3	—
	2	13	7	
	4	13	6	
16	1	9	1	—
	2	11	1	
	4	7	2	
22	1	7	2	—
	2	10	3	
	4	14	7	
25	1	13	6	Ganglionic blockade
	2	26	7	
	4	52	12	
31	1	13	<1	Ganglionic blockade
	2	19	1	
	4	24	3	
32	1	21	<1	Ganglionic blockade
	2	26	1	
	4	30	2	
33	0.5	14	<1	Ganglionic blockade
	1	15	<1	
	2	19	1	
34	1	28	<1	Ganglionic blockade
	2	30	<1	
	4	14	1	
41	1	9	<1	Ganglionic blockade
	2	37	1	
	4	63	<1	
44	1	4	<1	Partly ganglionic blockade
	2	15	<1	
	4	29	<1	
45	1	19	1	Partly ganglionic blockade
	2	26	2	
	4	31	2	
47	1	4	<1	Muscarinic
	2	15	<1	
	5	45	<1	
	25	50	1	

^a Each compound was tested at least twice in separate animals.

Esters—Esters were prepared in a variety of ways.

Method I. Secondary Amino-Esters (Table I)—(a) Methyl methacrylate, methyl acrylate, or methyl crotonate (0.25 mole) was added to a solution of the appropriate primary amine (0.25 mole) in anhydrous methanol (50–100 ml.). The reaction mixture was maintained at room temperature with stirring or was heated under reflux until the reaction was judged complete by the absence or near absence of a C=C stretching band near 1630 cm.⁻¹ in the IR spectrum of the crude reaction mixture. The solvent was removed, and the residual oil was fractionally distilled under reduced pressure. (b) The appropriate 3-bromo-ester (0.25 mole) was dissolved in anhydrous benzene (50–100 ml). To this solution, the appropriate primary amine (0.50 mole) was added dropwise with stirring. The reaction mixture was heated under reflux with stirring, and the accumulated precipitate of amine hydrobromide was removed from time to time. When precipitation was complete, the solvent was removed and the residual oil was fractionally distilled under reduced pressure.

Method II. Tertiary Amino-Esters (Table I)—These were prepared at reflux temperature using Method Ia, except that a 50–100% excess of methyl acrylate, methyl methacrylate, or methyl crotonate was added to a methanolic solution of the appropriate secondary amine and the reaction was judged complete when the IR spectrum of an aliquot from the reaction mixture exhibited no N—H stretching band near 3400 cm.⁻¹.

Method III. Amino-Diesters (Table II)—Excess methyl acrylate (0.60 mole) was added dropwise to a stirred solution of benzylamine or phenethylamine (0.25 mole) in anhydrous methanol (50–100 ml.), and the solution was cooled in an ice bath. The reaction mixture was allowed to come to room temperature and was then heated under reflux until the IR spectrum of an aliquot from the reaction no longer showed an N—H stretching band near 3400 cm.⁻¹. Solvent and excess methyl acrylate were removed under reduced pressure. Attempts to distill the products resulted in decomposition, so the products were purified and characterized as hydrochlorides.

Method IV. N-(2-Carbomethoxyethyl)-N-(2-carbomethoxypropyl)benzylamine (Table II, Compound 48)—Methyl acrylate (25 g.) was added dropwise to a stirred solution of methyl 2-methyl-3-benzylaminopropionate (26.5 g.) and the solution was chilled in an ice bath. The mixture was heated under reflux for 48 hr. and then treated as described in Method III.

Method V. Salts—The hydrochlorides and hydrobromides of the amino-esters were obtained by gassing a solution of the appropriate amino-ester in anhydrous diethyl ether with hydrogen chloride or hydrogen bromide dried by passage through concentrated sulfuric acid. The precipitated salts were recrystallized from anhydrous methanol-ether or acetone-ether.

In some cases, both the hydrochloride and hydrobromide salts were oils, so the acid oxalate was prepared for characterization purposes. The appropriate amino-ester was dissolved in methanol containing an exactly equivalent quantity of oxalic acid. The solution was chilled, and the resulting precipitate was collected and recrystallized from methanol.

All salts were characterized by their IR spectra and melting points, and they were colorless compounds.

Hydroxamic Acids—These were prepared by two methods.

Method I—A previously reported method (1) was used except that the reactants were allowed to stand at room temperature from 1 day to 5 weeks and the reaction was judged complete by the absence, or significant reduction in intensity, of the strong C=O stretching band near 1730 cm.⁻¹ (due to ester starting material) in the IR spectrum of the reaction mixture. Most aminohydroxamic acid hydrochlorides were recrystallized from anhydrous methanol-ether or acetone-ether.

If the IR spectrum of the crude hydroxamic acid hydrochloride showed appreciable contamination with ester hydrochloride, the crude product was dissolved in a few milliliters of distilled water, and dilute sodium hydroxide solution (10%) was added until the

¹ Melting and boiling points are uncorrected. The former were determined on a Thomas-Hoover capillary apparatus. IR spectra were recorded in mineral oil mulls or KBr disks on a Beckman IR-10 spectrophotometer, and NMR spectra were recorded on a Varian A-60D spectrometer. Microanalyses were performed by Mr. W. Dylke, Faculty of Pharmacy and Pharmaceutical Sciences, and in the Micro-analytical Laboratory, Department of Chemistry, University of Alberta.

solution was basic (pH approximately 8). The basified aqueous solution was extracted with ether to remove any amino-ester present: the aqueous layer was acidified with dilute hydrochloric acid and then concentrated under reduced pressure. Precipitated sodium chloride was removed by filtration, and the solution was concentrated further to yield a crude hydroxamic acid hydrochloride. This was recrystallized several times from anhydrous methanol to remove any sodium chloride still present and was finally recrystallized from anhydrous methanol-ether.

Method II—A previously reported method (2) was employed except that stirring was continued until the IR of an evaporated portion of the reaction mixture lacked ester carbonyl absorption (about 1730 cm^{-1}) and instead exhibited a lower frequency hydroxamate carbonyl absorption (about 1640–1680 cm^{-1}). Methanol was removed under reduced pressure, and the resulting oil was dried in a vacuum desiccator over anhydrous calcium sulfate². The resulting semisolid was purified as described in Method I of this section.

All hydroxamic acids were characterized by IR spectra which exhibited either one or two strong absorption bands due to carbonyl stretching within the range of 1630–1688 cm^{-1} . If present, the lower carbonyl absorption band was generally of equal or lower intensity than the higher carbonyl band.

The NMR spectra of all hydroxamic acid hydrochlorides were run in fully deuterated dimethyl sulfoxide. These showed a 3-proton or 4-proton broad band in the δ 8.05–11.10 range due to the one or two protons of the NH^+ or NH_2^+ groups, respectively, and the two protons of the NHOH group. These protons exchanged in deuterium oxide.

Pharmacological Methods—Anesthetized Rat Experiments—Rats weighing between 160 and 530 g. were anesthetized with urethan (1.9 g./kg. i.p.). The surgical procedures were similar to those given by D'Amour *et al.* (6). The blood pressure (carotid artery) was measured by means of a transducer³ coupled to an amplifier and pen recorder⁴. The drugs were dissolved in saline immediately before use and introduced *via* a cannula inserted into either a femoral or jugular vein.

Isolated Rabbit Intestine Experiments—A rabbit was killed and a length of ileum was taken with the mesenteric attachments intact. Portions 2–3 cm. in length were cut to which the mesenteric blood and nerve supply could be clearly seen. Threads were attached to this and to each end of the piece of intestine, and the tissue was suspended in a jacketed 300-ml. organ bath. The mesentery was then threaded through electrodes connected to a stimulator⁵. A modified Krebs solution was used of the following composition in grams per liter of distilled water: NaCl, 6.9; KCl, 0.35; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.36; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.29; NaHCO_3 , 2.1; KH_2PO_4 , 0.16; and glucose, 1.0. The solution was equilibrated with 95% oxygen and 5% carbon dioxide, and the temperature was maintained at 37°. The spontaneous contractions of the muscle were recorded by means of a strain gauge or an isotonic myograph transducer⁶ connected to an amplifier and pen recorder⁴.

Evaluation of Muscarinic Activity—The compound (4 mg./kg.) was administered intravenously to at least two scopolamine-treated rats to determine if the hypotensive action was muscarinic in nature. Acetylcholine (0.05, 0.1, and 0.2 mcg.) was given, followed by a suitable dose of the active compound. Scopolamine hydrobromide (1 mg.) was then given, and the procedure was repeated. Responses to acetylcholine were completely abolished, as were responses to some compounds, indicating that they cause hypotension by a muscarinic action.

Effect of Nicotine and Norepinephrine on Blood Pressure of Rats Treated with Active Nonmuscarinic Compounds—All nonmuscarinic active compounds were screened in the following manner. The anesthetized rat was given small intravenous doses (generally 20–40 mcg.) of nicotine, producing a rise in arterial blood pressure. Three doses of norepinephrine (0.05, 0.1, and 0.2 mcg.) were then administered. The rats were injected with the test compound (generally 5, 10, or 25 mg./kg.), and the administration of nicotine was repeated. An additional dose of test compound was given, and administration of norepinephrine was repeated.

It was found that in several cases the nicotine-induced rise in

arterial blood pressure was completely blocked (but returned after a period of time), while only a partial block was observed in other cases. The response to noradrenaline was undiminished in all cases. In a few cases, both nicotine and noradrenaline responses remained undiminished after administration of the test compound.

Determination of Effectiveness of Aminohydroxamic Acid against Diisopropyl Fluorophosphate Poisoning in Mice—Male Alas strain mice, weighing 20–25 g. each, were used. The LD_{50} of the diisopropyl fluorophosphate intraperitoneally in these mice was found to be 2.38 (2.09–2.71) mg./kg.⁷ The diisopropyl fluorophosphate was dissolved in distilled water and diluted such that 0.1 ml. contained 5 mg./kg. when administered intraperitoneally. Each aminohydroxamic acid was made up as a solution in distilled water so that 0.2 or 0.3 ml. contained the desired dose on subcutaneous injection. The general procedure was to inject groups of mice with the desired dose of the compound under investigation, followed either 20 or 30 min. later by an injection of diisopropyl fluorophosphate. In each set of experiments, one group of mice in which distilled water instead of an aminohydroxamic acid had been injected was used as a control.

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² Drierite.

³ E & M model P-100-A.

⁴ E & M physiograph desk model DMP-4A.

⁵ Harvard Apparatus Co. model 240.

⁶ E & M Instrument Co. Inc., MK II Ser 670.

⁷ The upper and lower confidence limits for 19/20 probability were determined by the method of Litchfield and Wilcoxon (20).