29.6 g. (27%) of colorless prisms, m.p. 204–207°. An analytical sample melted at 205–207° (from chloroform).

Anal. Calcd. for $C_9H_{12}Cl_2O_2$: C, 48.45; H, 5.42. Found: C, 48.77; H, 5.69.

2-*exo*-Methyl-5,7-dichloronorbornane-2-*endo*-carboxamide (XXXIX).—The dichloro acid (22.0 g.) was refluxed for 3 hr. with 75 ml. of thionyl chloride and distilled through a short Vigreux column. The colorless acid chloride (21.6 g., 90%, b.p. 84–87° (0.03 mm.)) which crystallized as hard rosettes upon standing, was dissolved in 250 ml. of absolute ether. Gaseous ammonia was passed over the surface of the stirred solution for 1 hr. with ice-cooling. The suspension was allowed to stand overnight at room temperature, evaporated to dryness and the residue was washed well with water. The coarse crystalline product (19.5 g., 89%, m.p. 152–156°) was recrystallized twice from toluene to yield 15.8 g., m.p. 160–161°.

Anal. Calcd. for $C_9H_{13}Cl_2NO$: C, 48.67; H, 5.90; N, 6.31. Found: C, 48.46; H, 6.02; N, 6.26.

2-Bromobicyclo[2.2.2]octane-2-carboxamide (XXXVIII).—A solution of 16.0 g. of bicyclo[2.2.2]octane-2-carboxylic acid[28] and 50 g. of thionyl chloride in 500 ml. of chloroform was refluxed for 6 hr. and distilled under reduced pressure on a steam bath. The residue of bicyclo[2.2.2]octane-2-carbonyl chloride was dissolved in 100 ml. of thionyl chloride, heated to reflux and 17.0 g. of bromine was added dropwise in 4 hr. Refluxing was continued for 1 hr. longer and the solution was distilled. The 2-bromobicyclo[2.2.2]octane-2-carbonyl chloride (20.0 g., b.p. 132–134°(15 mm.)) was dissolved in ether, cooled in an ice bath, and saturated with gaseous ammonia. The ether was allowed to evaporate and the residue was washed well with water. Recrystallization from hexane gave 18.0 g. of colorless crystals, m.p. 107–108°.

Anal. Calcd. for $C_9H_{14}BrNO$: C, 46.57; H, 6.08; N, 6.04. Found: C, 46.82; H, 6.18; N, 5.85.

(28) C. A. Grob, H. Kny, and A. Gagnieux, *Helv. Chim. Acta*, **40**, 130 (1957).

The Synthesis and γ-Aminobutyric Acid Transaminase Inhibition of Aminoöxy Acids and Related Compounds

E. L. SCHUMANN, L. A. PAQUETTE, R. V. HEINZELMAN, D. P. WALLACH, J. P. DAVANZO, AND M. E. GREIG

The Upjohn Company Research Laboratories, Kalamazoo, Michigan

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A series of forty-six aminoöxy derivatives and thirty-six analogs of γ-aminobutyric acid was prepared and tested for inhibition of γ-aminobutyric acid transaminase and for protection against thiosemicarbazide-induced convulsions. Good *in vivo* activity in these tests was generally limited to certain α-aminoöxyacids or their easily hydrolyzed derivatives, such as esters. None of the compounds studied was superior to aminoöxyacetic acid.

The current interest in γ-aminobutyric acid (GABA) as a possible mediator of central nervous system transmission prompted the synthesis of the two series of compounds presently reported. One series consisting of various aminoöxy derivatives was initiated following the discovery in our laboratory that aminoöxyacetic acid was a potent inhibitor of γ-aminobutyric acid-α-ketoglutaric acid (GABA-AKG) transaminase both *in vitro* and *in vivo*.[1] A corollary series of GABA analogs and precursors was also prepared in the hope of obtaining competitive inhibitors of GABA-AKG-transaminase or compounds that would cross the blood-brain barrier and then be hydrolyzed or degraded to GABA. Recent work by Farquharson and Maclean[2] concerned similar attempts to obtain GABA-like activity.

Several approaches were followed in the design of analogs of aminooxyacetic acid. First, since the apparent oral absorption of aminooxyacetic acid was poor, derivatives such as esters, amides, N-alkylidene and N-acyl derivatives were prepared in the hope of obtaining compounds with a more favorable absorption rate which would be hydrolyzed to the active agent after absorption. This effort was only partially successful. Lower alkyl esters of aminoöxyacetic acid were highly potent although less so than the parent acid. The esters also showed erratic species differences. Amides and N-acyl derivatives were much less potent and N-alkylidene derivatives were uniformly inactive *in vivo*.

The preparation of β-aminoöxyacids was undertaken because of the apparently greater isosteric similarity to γ-aminobutyric acid. β-Aminoöxypropionic acid was, in fact, much more potent *in vitro* than aminoöxyacetic acid but was totally inactive *in vivo*. Derivatives of β-aminoöxyacetic acid designed to facilitate absorption and transport were equally inactive *in vivo*.

In a series in which the α-aminoöxyacid moiety was kept intact, various α-substituents were introduced. Single groups such as α-methyl, α-ethyl and α-phenyl reduced potency somewhat, but still resulted in very potent enzyme inhibitors *in vitro*. It is of interest that of the two optical isomers of α-aminoöxypropionic acid studied, both compounds were active in the *in vitro* system but only the levorotatory isomer was effective *in vivo*. The presence of two α-methyl groups eliminated all *in vivo* activity. Alkylating the aminoöxy nitrogen and the use of simple hydroxylamine derivatives resulted in a complete loss of *in vivo* activity.

Because of the possibility that a derivative formed from aminooxyacetic acid and pyridoxal was the effective inhibitor at the *in vivo* receptor site, pyridoxal carboxymethoxime and several related species

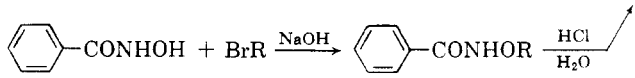
were prepared. These derivatives were uniformly inactive *in vivo*.

The series of thirty-six new and known GABA analogs tested was inactive in inhibiting GABA-AKG transaminase both *in vitro* and *in vivo* and no compound caused a rise in brain GABA levels.

In general, protective activity of compounds against thiosemicarbazide-induced convulsions paralleled the *in vivo* GABA transaminase inhibition.

Most of the compounds described were prepared by alkylation of a hydroxylamine derivative such as acetoxime or benzohydroxamic acid in the presence of base, followed by hydrolysis of the protecting group, *e.g.*

$$(CH_3)_2C{=}NOH + BrR \xrightarrow{NaOH} (CH_3)_2C{=}NOR \xrightarrow[H_2O]{HCl} H_2NOR{\cdot}HCl$$



Products having the aminoöxy group in the β-position were best prepared by Michael addition of acetoxime to a suitable α,β-unsaturated compound, and then acid hydrolysis.

New aminoöxy compounds and GABA analogs are listed in Table I.

Pharmacology Methodology

Inhibition of γ-Aminobutyric Acid–α-Ketoglutaric Acid Transaminase *in Vitro* **and** *in Vivo*.—Inhibition of this enzyme both *in vitro* and *in vivo* was studied using the methods described previously.[1] Results are given in Table II. All compounds not listed in Table II were inactive *in vivo*.

Thiosemicarbazide Studies.—For each compound to be tested, 8 rats (or mice)[3] were generally used. The animals were pretreated 1 hr. before receiving thiosemicarbazide (20 mg./kg. I.P.). Three doses (2 animals/dose) were employed, namely 10, 25 and 50% of the LD_{50}. Two animals served as controls and were given thiosemicarbazide alone. The animals were observed for 24 hr. at which time survival rates were determined. Virtually 100% of the controls died within 2-3 hr. after receiving thiosemicarbazide. Compounds found active in one species were always tested in at least one other species.

Summary of *in Vitro* **and** *in Vivo* **Screening.**[4]—In the aminoöxy series, these compounds were inactive in inhibiting GABA-AKG transaminase *in vitro* and *in vivo:* benzalaminoöxyacetic acid, benzamidoöxyacetic acid, dimethylaminoöxyacetic acid, 2-isopropylideneaminoöxyethanol, α-phenyl-α-benzamidoöxyacetic acid, α-benz-

(1) D. P. Wallach, *Biochem. Pharmacol.*, **5**, 323 (1960).
(2) M. E. Farquharson and J. A. R. Maclean, *J. Med. Pharm. Chem.*, **4**, 31 (1961).
(3) Male Sprague-Dawley rats and male Carworth Farm mice were used.
(4) This summary includes known compounds tested.

amidoöxybutyric acid, ethyl α-aminoöxyisobutyrate, methyl β-isopropylideneaminoöxypropionate, ethyl β-acetamidoöxypropionate, β-aminoöxypropionitrile, β-isopropylidineaminoöxypropionitrile, ethyl α-amino-β-aminoöxypropionate, azaserine, N,N-dimethylhydroxylamine, pyridoxal oxime, and pyridoxal carboxymethoxime.

The following compounds, not listed in Table I, were found active in *in vitro* screening but inactive *in vivo:* isopropylideneaminoöxyacetic acid, α-aminoöxybutyric acid, α-aminoöxyisobutyric acid, β-aminoöxypropionic acid, cycloserine, methoxamine.

Known compounds in the GABA analog series which were found inactive both *in vivo* and *in vitro* were: various derivatives of GABA such as N-methyl and dimethyl, N-acetyl and phthalimido derivatives, the corresponding esters, amides and nitriles, L-α,γ-diaminobutyric acid, β-hydroxy-γ-aminobutyric acid, γ,γ-dimethyl-γ-butyrolactam, 4-aminobutanol, 4-piperidinecarboxylic acid, (*trans*)-γ-amino-2-butenoic acid, β-guanidinopropionic acid, γ-guanidinobutyric acid, 5-aminolevulinic acid, oximes and semicarbazones of levulinic and β-benzoylpropionic acids, methyl 3-aminopropyl sulfone, γ-aminopropanesulfonic acid, 2-aminoethoxyacetic acid and 2-morpholinone.

Pharmacological data for all compounds active *in vivo* are summarized in Table II.

Experimental[5]

Ethylideneaminoöxyacetic Acid.—A solution of 10.9 g. (0.1 mole) of aminooxyacetic acid hemihydrochloride[6] and 4.3 g. (0.1 mole) of acetaldehyde in 35 ml. of water was rendered alkaline with 20% NaOH and warmed on the steam bath for 0.5 hr. The solution was cooled in an ice bath, extracted once with ether (discarded), then acidified with HCl and extracted several times with ether. The combined ether extracts were dried over anhydrous magnesium sulfate and distilled to give the desired product.

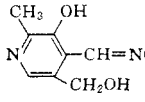
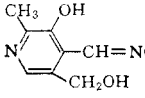
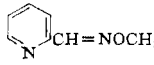
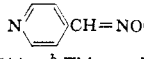
Benzalaminoöxyacetic Acid.—This compound was prepared, using benzaldehyde, in the same manner as described above except that during acidification of the reaction mixture the product precipitated as a solid and was purified by recrystallization.

Acetamidoöxyacetic Acid.—A solution of 10.9 g. (0.1 mole) of aminoöxyacetic acid hemihydrochloride in 50 ml. of glacial acetic acid and 15.3 g. (0.15 mole) of acetic anhydride was heated under reflux for 4 hr., then concentrated under reduced pressure. The residual oil was triturated with methylene chloride until solid, then recrystallized from ethyl acetate–Skellysolve B to yield 3 g. (29%) of pure product.

(5) All temperatures are uncorrected.

(6) "Organic Syntheses," Collective Volume III, John Wiley and Sons, Inc., N. Y., 1955 p. 172.

TABLE
NEW AMINOÖXY COMPOUNDS

Number	Compound	Formula
1	$CH_3CH{=}NOCH_2COOH$	$C_4H_7NO_3$
2	$C_6H_5CH{=}NOCH_2COOH$	$C_9H_9NO_3$
3	$CH_3CONHOCH_2COOH$	$C_4H_7NO_4$
4	$(CH_3)_2NOCH_2COOH\cdot HCl$[b]	$C_4H_9NO_3\cdot HCl$
5	$(CH_3)_2C{=}NOCH_2COSH$	$C_5H_9NO_2S$[d]
6	$H_2NOCH_2CONH_2\cdot HCl$	$C_2H_6N_2O_2\cdot HCl$
7	$(CH_3)_2C{=}NOCH_2CONH_2$	$C_5H_{10}N_2O_2$
8	$H_2NOCH_2CON(CH_3)_2\cdot HCl$	$C_4H_{10}N_2O_2\cdot HCl$
9	$H_2NOCH_2COOCH_3\cdot HCl$	$C_3H_7NO_3\cdot HCl$
10	$H_2NOCH_2COOC_2H_5\cdot HCl$	$C_4H_9NO_3\cdot HCl$
11	$H_2NOCH_2COOC_3H_7\cdot HCl$	$C_5H_{11}NO_3\cdot HCl$
12	$H_2NOCH_2COOC_4H_9\cdot HCl$	$C_6H_{13}NO_3\cdot HCl$
13	$H_2NOCH_2COOC_{12}H_{25}\cdot HCl$	$C_{14}H_{29}NO_3\cdot HCl$
14	$H_2NOCH_2CH_2Cl\cdot HCl$	$C_3H_6ClNO\cdot HCl$
15	$H_2NOCH_2CH_2OH\cdot HCl$	$C_2H_7NO_2\cdot HCl$
16	(+) $H_2NOCHCOOH\cdot HCl$[f] (CH₃)	$C_3H_7NO_3\cdot HCl$
17	(−) $H_2NOCHCOOH\cdot HCl$[h] (CH₃)	$C_3H_7NO_3\cdot HCl$
18	$H_2NOCH(C_6H_5)COOH\cdot HCl$	$C_8H_9NO_3\cdot HCl$
19	$C_6H_5CONHOCH(C_6H_5)COOH$	$C_{15}H_{13}NO_4$
20	$H_2NOC(CH_3)_2COOC_2H_5\cdot HCl$	$C_6H_{13}NO_3\cdot HCl$
21	$H_2NOCH_2CH_2COOC_2H_5\cdot HCl$	$C_5H_{11}NO_3\cdot HCl$
22	$(CH_3)_2C{=}NOCH_2CH_2COOCH_3$	$C_7H_{13}NO_3$
23	$CH_3CONHOCH_2CH_2COOC_2H_5$	$C_7H_{13}NO_4$
24	$H_2NOCH_2CH_2CN\cdot HCl$	$C_3H_6N_2O\cdot HCl$
25		$C_{10}H_{12}N_2O_5$
26		$C_{11}H_{14}N_2O_5$
27		$C_8H_8N_2O_3$
28		$C_8H_8N_2O_3$

[a] $n^{25}D$ 1.4514. [b] This product contained a small amount of the hydrobromide salt. [c] With decomposition. [d] $n^{26}D$ 1.4858. Calcd.: S, 21.78. Found: S, 21.59. [e] Hygroscopic. [f] $[\alpha]D$ in methanol was +113°. [g] The intermediate α-isopropylideneaminoöxypropionic acid was prepared and resolved as described by M. S. Newman and W. B. Lutz. [h] $[\alpha]D$ in methanol was −112°. [i] $n^{25}D$ 1.4229. [j] $n^{25}D$ 1.4470. [k] Obtained as the ethanol solvate before drying *in vacuo*. [l] Calcd.: Cl, 11.52. Found: Cl, 11.21.

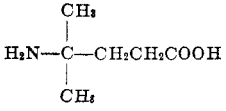
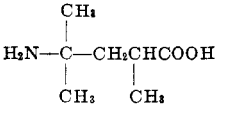
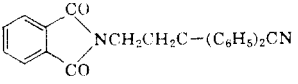
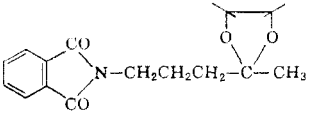
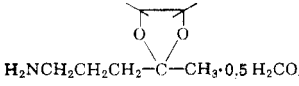
I

AND GABA ANALOGS

M.p. or b.p.		Yield,	Recrystallization	Calcd., %			Found, %		
°C.	mm.	%	solvents	C	H	N	C	H	N
73–75	0.13[a]	47		41.02	6.03	11.96	41.22	6.38	11.51
96–99		68	I	60.33	5.06	7.82	60.10	4.90	7.43
136–137		29	II	36.09	5.30	10.53	36.15	5.62	10.53
124–126[c]		62	III	30.88	6.47	9.00	28.91	6.10	8.41
84–85	13	75	..	40.80	6.16	9.52	41.23	6.11	9.57
124–125[c]		14	IV	18.98	5.58	22.14	19.19	5.51	22.33
90–91		33	II	46.14	7.74	21.53	46.07	8.09	21.37
155–156		20	V	31.07	7.17	18.12	30.68	7.20	18.41
100–102		50	III	25.45	5.69	9.90	25.53	5.83	10.03
115–117		61	III	30.88	6.48	9.00	31.07	6.52	8.78
73–75		46	VI	35.40	7.13	8.26	35.02	6.93	8.36
84–85		71	VI	39.24	7.68	7.63	39.26	7.62	7.89
114–116		42	III	56.83	10.22	4.74	57.04	10.45	4.86
180[c]		20	VI	18.20	5.35	10.61	18.78	5.71	10.79
62–65[e]		85	III	21.15	7.10	12.34	20.96	7.09	12.56
178–179[c,g]		68	V	25.45	5.70	9.90	25.69	5.72	9.71
184–185[c,g]		69	III	25.45	5.70	9.90	25.41	5.50	9.83
178–179[c]		70	III	47.18	4.95	6.88	46.93	5.31	7.05
187–188		22	VII	66.41	4.83	5.16	66.07	5.17	5.34
130–131		16	III	39.24	7.68	7.63	39.55	7.78	8.04
85–86		50	VIII	35.40	7.13	8.26	35.83	7.34	8.43
91–92	30[i]	65	..	52.81	8.23	8.80	53.02	8.61	8.53
110–115	0.05[j]	82	..	47.99	7.48	8.00	48.42	7.09	8.04
135–136[c]		7	V	29.40	5.76	22.86	29.22	5.48	22.92
209–210[c]		83	IX	50.00	5.04	11.67	49.79	5.08	11.81
204[c]		81	IX	51.96	5.55	11.02	..	..	11.00
181–183[c]		57	IX	53.33	4.48	15.55	53.47	4.93	15.43
222[c]		59	X	53.33	4.48	15.55	53.36	4.82	15.65

Recrystallization solvents: I, benzene; II, ethyl acetate–Skellysolve B; III, isopropyl alcohol–anhydrous ether; IV, chloroform–anhydrous ether; V, absolute ethanol–anhydrous ether; VI, ethyl acetate–anhydrous ether; VII, ethyl acetate; VIII, isopropyl alcohol; IX, 95% ethanol; X, water; XI, methyl ethyl ketone; XII, acetone; XIII, aqueous ethanol; XIV, methanol; XV, aqueous methanol.

TABLE I

Number	Compound	Formula
29	$H_2NCH_2CH_2CH_2COOCH_2CH_2Cl \cdot HCl$	$C_6H_{12}ClNO_2 \cdot HCl$
30	$H_2NCH_2CH_2CH_2COOC_4H_9 \cdot HCl$	$C_8H_{17}NO_2 \cdot HCl$
31	$H_2NCH_2CH_2CH_2COOC_{12}H_{25} \cdot HCl$	$C_{16}H_{33}NO_2 \cdot HCl$
32		$C_6H_{13}NO_2$
33		$C_7H_{15}NO_2$
34		$C_{24}H_{18}N_2O_2$
35		$C_{15}H_{17}NO_4$
36		$C_7H_{15}NO_2 \cdot 0.5H_2CO_3$

Dimethylaminoöxyacetic Acid Hydrochloride.—A mixture of 5.1 g. (0.036 mole) of bromoacetic acid and 10 g. of ice was cooled in an ice bath during the addition of 8 ml. (0.04 mole) of 20% sodium hydroxide. The cooled solution was then treated with 3.5 g. (0.036 mole) of N,N-dimethylhydroxylamine hydrochloride and an additional 16 ml. (0.08 mole) of 20% sodium hydroxide. The cold solution was then added, dropwise, to the top of a 75 cm. steam-jacketed straight condenser which was inclined at 20° to the horizontal. Hot solution issuing from the bottom of the condenser was collected in a water-cooled flask. The total addition time was 20 min. After acidification with 10 ml. of concd. hydrochloric acid, the solution was evaporated to dryness under reduced pressure. The solid residue was boiled with 25 ml. of isopropyl alcohol, filtered to remove insoluble material, diluted with anhydrous ether and refrigerated. The first crop of material obtained was inorganic. A second crop, obtained by further dilution with anhydrous ether, contained the desired crude product which decomposed at 127–129°. Since the crude product was found to be a mixture of the hydrochloride and hydrobromide salts, the solid was twice mixed with 50 ml. of concd. hydrochloric acid and evaporated to dryness under reduced pressure. The resulting solid was recrystallized twice from isopropyl alcohol–anhydrous ether to give 3.5 g. of product which still contained a small amount of the hydrobromide and decomposed at 124–126°.

Isopropylideneaminoöxythiolacetic Acid.—A mixture of 187 g. (1.42 moles) of isopropylideneaminoöxyacetic acid[6] and 600 g. (5 moles) of thionyl chloride was heated under reflux for 1 hr., then concentrated under reduced pressure. Distilla-

(*Continued*)

M.p. or b.p. °C.	mm.	Yield, %	Recrystallization solvents	Calcd., % C	H	N	Found, % C	H	N
93–95		78	XI	35.66	6.48	6.93	35.77	6.38	7.12
80–84		90	V	49.10	9.27	7.16	49.21	9.39	7.44
93–96		97	XII	62.41	11.13	(*l*)	62.05	11.09	(*l*)
268–269		73	XIII	54.94	9.99	10.68	55.14	9.98	10.77
185–186[c]		60	XIII	57.90	10.41	9.65	58.14	10.58	10.01
129–130		93	XIV	78.67	4.95	7.65	78.14	4.88	7.77
77–78		81	XV	65.44	6.22	5.09	65.82	6.15	5.18
66–69[c]		76	V	51.12	9.15	7.95	52.30	9.15	8.28

tion of the residual black oil gave 110 g. (52%) of isopropylideneaminoöxyacetyl chloride, b.p. 46–56° (8 mm.). *Caution:* when air was admitted to the hot still residue, a violent reaction ensued, spraying black tar over a considerable area. Suitable precautions are indicated when this distillation is carried out.

Pyridine (50 ml.), cooled in an ice bath, was saturated with hydrogen sulfide. A solution of 9 g. (0.06 mole) of isopropylideneaminoöxyacetyl chloride in 25 ml. of anhydrous benzene was added in several portions and the resulting solution was allowed to stand at room temperature for 0.5 hr., with occasional swirling. Ice (200 g.) was then added and the mixture was acidified with 6 *N* hydrochloric acid and extracted with three 150 ml. portions of chloroform. The combined chloroform extracts were dried over anhydrous magnesium sulfate, concentrated and distilled to give 6.6 g. of colorless product which boiled at 84–85° (13 mm.); $n^{26}D$ 1.4858.

Isopropylideneaminoöxyacetamide.—A mixture of 7.0 g. (0.054 mole) of isopropylidineaminoöxyacetic acid[6] and 64 g. (0.54 mole) of thionyl chloride was heated under reflux for 0.5 hr. and concentrated under reduced pressure. Anhydrous benzene, 100 ml., was added and the mixture was again concentrated under reduced pressure. The crude acid chloride was dissolved in 200 ml. of anhydrous benzene and treated with excess gaseous ammonia. After filtration to remove by-products, the solution was concentrated under reduced pressure. Two recrystallizations of the residual solid from ethyl acetate–Skellysolve B gave 2.3 g. (33%) of pure, golden yellow product, m.p. 90–92°.

Aminoöxyacetamide Hydrochloride.—A mixture of 11.2 g. (0.086 mole) of

TABLE II

PHARMACOLOGICAL DATA SUMMARY: INHIBITION OF GABA–α-KETOGLUTARIC ACID TRANSAMINASE

Compound	Approx. LD_{50}, mg./kg. I.P., mice	GABA-AKG inhibition *in vitro* at 6.6×10^{-2} *M*	Rat			Mouse			Protection against thiosemicarbazide			
			Dose, mg./kg.	Route	% Incr. in Brain GABA	Dose, mg./kg.	Route	% Incr. in Brain GABA	Dose, mg./kg. mice	Survival rate	Dose, mg./kg. rats	Survival rate
$H_2NOCH_2COOH \cdot 0.5\ HCl$[a]	65	100%	6.25	I.V.	0	10	S.C.	143	20	5/10	20	1/6
			12.5	I.V.	208	25	S.C.	262	30	9/10	30	2/6
			25	I.V.	272	50	S.C.	300	40	9/9	40	6/6
			50	I.V.	407				50	9/10	50	5/6
			25	P.O.	0							
			50	P.O.	145							
			100	P.O.	169							
			200	P.O.	169							
			6.25	S.C.	0							
			12.5	S.C.	162							
			25	S.C.	316							
			50	S.C.	438							
$CH_3CONHOCH_2COOH$	100	34%	12.5	I.V.	144	12.5	S.C.	0		Inactive	..	...
			25	I.V.	188	25	S.C.	123		at all		
			50	I.V.	248	50	S.C.	166		doses		
$H_2NOCH_2CONH_2 \cdot HCl$	650	88%	50	I.V.	0	12.5	S.C.	0		Inactive		
			50	S.C.	0	25	S.C.	180		at all	.	...
						50	S.C.	206		doses		
CH_3 \ C=$NOCH_2CONH_2$ / CH_3	>1000	20%	50	(inactive I.V. and S.C.)	50	50	S.C.	143		Inactive at all doses	..	..
$H_2NOCH_2COOCH_3 \cdot HCl$	42	96%	12.5	I.V.	132	12.5	S.C.	133	10	1/2	10	Inactive
			25	I.V.	195	25	S.C.	173	30	1/2	30	Inactive
			50	I.V.	447	50	S.C.	264	50	2/2	50	1/2
			12.5	S.C.	132							
			25	S.C.	137							
			50	S.C.	373							

Compound	Approx. LD_{50}, mg./kg. I.P., mice	GABA-AKG inhibition *in vitro* at 6.6×10^{-2} *M*	Rat			Mouse			Protection against thiosemicarbazide			
			Dose, mg./kg.	Route	% Incr. in Brain GABA	Dose, mg./kg.	Route	% Incr. in Brain GABA	Dose, mg./kg. mice	Survival rate	Dose, mg./kg. rats	Survival rate
$H_2NOCH_2COOC_2H_5 \cdot HCl$	10	75%	127	P.O.	0	12.5	S.C.	163	30	1/2	30	Inactive
			12.5	S.C.	118							
			25	S.C.	129	25	S.C.	248	60	2/2	60	2/3
			50	S.C.	300	50	S.C.	335	90	2/2	90	3/3
$H_2NOCH_2COOC_4H_7 \cdot HCl$	65	95%	12.5	I.V.	188	12.5	S.C.	247	10	Inactive	10	Inactive)
			25	I.V.	232	25	S.C.	237	30	2/2	30	Inactive
			50	I.V.	485	50	S.C.	350	50	Inactive	50	2/2
			12.5	S.C.	0							
			25	S.C.	175							
			50	S.C.	188							
$H_2NOCH_2COOC_4H_9 \cdot HCl$	69	100%	50	I.V.	fatal	50	S.C.	0	20	Inactive	20	Inactive
									40	1/2	40	Inactive
			50	S.C.	357				60	2/2	60	1/3
$H_2NOCH_2COOC_{12}H_{25} \cdot HCl$	83	65%	50	I.V.	140	50	S.C.	0		Inactive at all doses	..	...
			50	S.C.	0							
$(\pm)H_2NOCHCOOH \cdot HBr$[b] (CH$_3$)	100	37% (at 3.3×10^{-4})	12.5	I.V.	159	12.5	S.C.	251		Not run	..	...
			25	I.V.	185	25	S.C.	253				
			50	I.V.	323	50	S.C.	226				
			25	S.C.	0							
			50	S.C.	196							
$(-)H_2NOCHCOOH \cdot HCl$ (CH$_3$)	65	82%	12.5	I.V.	146	12.5	S.C.	253	30	3/3	30	Inactive
			25	I.V.	300	25	S.C.	262	40	3/3	50	1/2
			50	I.V.	296	50	S.C.	332	50	2/3		
			12.5	S.C.	0							
			25	S.C.	132							
			50	S.C.	214							

[a] For preparation *cf.* footnote 6. [b] D. McHale, J. Green, and P. Mamalis, *J. Chem. Soc.*, 225 (1960).

isopropylideneaminoöxyacetamide, 200 ml. of water and 7.1 ml. (0.086 mole) of concd. hydrochloric acid was warmed on the steam bath for 10 min., then evaporated to dryness under reduced pressure. Approximately halfway through the evaporation the solution was decanted from a brown, gummy precipitate, which was discarded. The semi-solid residue was triturated with anhydrous ether, then extracted with one 100-ml. portion of boiling ethanol and two 100-ml. portions of boiling methanol. The combined extracts were diluted with anhydrous ether to yield 4.4 g. of product which decomposed at 145–147°. This product, although mainly the desired compound, was contaminated with ammonium chloride. In order to obtain analytically pure material, a portion of the impure hydrochloride was treated with liquid ammonia and the solution was allowed to evaporate. The residual solid was extracted with several portions of boiling isopropyl alcohol and the combined extracts were treated with ethereal hydrogen chloride and diluted with anhydrous ether to precipitate the pure hydrochloride.

Methyl Aminoöxyacetate Hydrochloride.—A solution of 50 g. (0.46 mole) of aminoöxyacetic acid hemihydrochloride[6] in 300 ml. of absolute methanol was saturated at room temperature with hydrogen chloride, then allowed to stand for 24 hr. The solution was concentrated under reduced pressure, then cooled in an ice bath, dissolved in 100 ml. of water, then rendered alkaline with sodium carbonate. The free base was extracted with ether, dried over anhydrous potassium carbonate and distilled to give 24 g. of product which boiled at 60° (8 mm.); $n^{25}D$ 1.4292. The *hydrochloride* was prepared by treating a solution of the base in anhydrous ether with ethereal HCl. Other esters were prepared similarly.

α-Aminoöxy-N,N-dimethylacetamide Hydrochloride.—A solution of 20.3 g. (0.17 mole) of ethyl aminoöxyacetate in 100 ml. of absolute ethanol was cooled to 0° and saturated with dimethylamine. The solution was then sealed in a pressure vessel and allowed to stand at room temperature for 3 days. The vessel was cooled, opened, and the solution was filtered to remove a small amount of insoluble material. After concentration under reduced pressure, the mixture was dissolved in methanol and again concentrated to remove any residual dimethylamine. The residue was dissolved in methanol, treated with ethereal hydrogen chloride and diluted with anhydrous ether to precipitate the hydrochloride. Recrystallization from absolute ethanol-anhydrous ether yielded the pure product.

1-Aminoöxy-2-chloroethane Hydrochloride.—1-Isopropylideneaminoöxy-2-chloroethane was first prepared by the dropwise addition of 13 g. (0.11 mole) of thionyl chloride to a stirred solution of 12 g. (0.1 mole) of 2-isopropylideneaminooxyethanol[7] in 9 g. (0.11 mole) of pyridine, with ice bath cooling. (*Caution:* during the early part of the addition, the mixture suddenly turned dark, became quite warm and evolved a brown vapor. Larger runs should be made with appropriate precautions.) After the addition the mixture was stirred for 4 hr. at room temperature, then poured into 100 ml. of ice water and extracted with four 100 ml. portions of ether. The combined ether extracts were dried over anhydrous magnesium sulfate, then distilled to give 5.2 g. (38%) of somewhat impure product, b.p. 51–56° (16 mm.); $n^{26}D$ 1.4424.

Anal. Calcd. for $C_5H_{10}ClNO$: C, 45.28; H, 7.43; N, 10.33; Cl, 26.15. Found: C, 45.59; H, 7.98; N, 10.48; Cl, 22.36.

The isopropylidene derivative was mixed with 70 ml. of 6 *N* hydrochloric acid, steam-distilled for 0.5 hr., then evaporated to dryness under reduced pressure and recrystallized to give the pure, hygroscopic hydrochloride.

2-Aminoöxyethanol Hydrochloride.—2-Isopropylideneaminoöxyethanol[7] was

(7) G. B. Bachman and T. Hokama, *J. Am. Chem. Soc.*, **81**, 4223 (1959).

hydrolyzed in 6 *N* hydrochloric acid in the same manner as described in the preceding preparation.

(+)-α-Aminoöxypropionic Acid Hydrochloride.[8]—A mixture of 4.5 g. (0.031 mole) of (+)-α-isopropylideneaminoöxypropionic acid[9] and 70 ml. of 6 *N* hydrochloric acid was steam-distilled for 0.5 hr., then evaporated to dryness under reduced pressure. Two recrystallizations of the residual solid from absolute ethanol-anhydrous ether yielded 3 g. (68%) of pure hydrochloride. The levorotatory isomer was prepared similarly from (−)-α-isopropylideneaminoöxypropionic acid.[9] Decomposition points of both isomers and the (±) mixture varied considerably with the rate of heating.

α-Benzamidoöxyphenylacetic Acid.—A solution of 16 g. (0.4 mole) of sodium hydroxide in 500 ml. of 50% ethanol was mixed with 27.4 g. (0.2 mole) of benzohydroxamic acid and 43 g. (0.2 mole) of α-bromophenylacetic acid, heated under reflux for 5 hr., then evaporated to dryness under reduced pressure. The residual solid was dissolved in 500 ml. of ice-cold water, rendered acidic with cold, concd. hydrochloric acid and extracted with ethyl acetate. After drying over anhydrous magnesium sulfate, the ethyl acetate solution was concentrated under reduced pressure and the residue was recrystallized from ethyl acetate.

α-Aminoöxyphenylacetic Acid.—A mixture of 3 g. (0.01 mole) of α-benzamidooxyphenylacetic acid, 10 ml. of glacial acetic acid and 30 ml. of 5 *N* hydrochloric acid was heated under reflux for 2 hr., then refrigerated. The precipitated mixture of product hydrochloride and benzoic acid was separated by filtration and triturated thoroughly with anhydrous ether to remove the benzoic acid. Recrystallization of the ether-insoluble residue from isopropyl alcohol-anhydrous ether gave 1.5 g. of pure product.

Ethyl α-Aminoöxyisobutyrate Hydrochloride.—Ethyl α-bromobutyrate (59 g., 0.33 mole), was added to a solution of 22 g. (0.33 mole) of sodium ethoxide and 24 g. (0.33 mole) of acetoxime in 500 ml. of absolute ethanol. The solution was heated under reflux for 2 hr., cooled, filtered to remove sodium bromide, and concentrated under reduced pressure. Ether was added to the residue to precipitate additional sodium bromide. After filtration the solution was concentrated and distilled to yield 19 g. of impure ethyl α-aminoöxyisobutyrate, b.p. 66–86° (8 mm.); $n^{25}D$ 1.4261.

The impure ethyl ester was hydrolyzed to the free acid hydrochloride with 6 *N* hydrochloric acid in the manner described previously. Since several recrystallizations of the acid from absolute ethanol–anhydrous ether did not give a pure product, the acid was re-esterified with ethanol saturated with hydrogen chloride in the manner described previously. Distillation of the crude ethyl α-aminooxybutyrate gave 3 g. of product which boiled at 77° (13 mm.); $n^{26}D$ 1.4248. The base was dissolved in anhydrous ether and treated with ethereal hydrogen chloride to yield the pure hydrochloride.

Methyl β-Isopropylideneaminoöxypropionate.—A mixture of 73 g. (1 mole) of acetoxime, 240 ml. of anhydrous dioxane and 2.4 g. (0.045 mole) of sodium methoxide was stirred at room temperature until solution was complete. Methyl acrylate, 86 g. (1 mole) was then added, dropwise, to the stirred solution over a 1 hr. period. Stirring was continued for 24 hr. The mixture was then neutralized with 5% hydrochloric acid, filtered to remove insoluble material and distilled to yield 104 g. of product.

(8) The resolved intermediates were generously supplied by Professor M. S. Newman of The Ohio State University Chemistry Department.

(9) M. S. Newman and W. B. Lutz, *J. Am. Chem. Soc.*, **78**, 2469 (1956).

Pyridoxal Carboxymethoxime.—A solution of 5.1 g. (0.025 mole) of pyridoxal hydrochloride in 10 ml. of water was added to a solution containing 2.7 g. (0.025 mole) of aminoöxyacetic acid hemihydrochloride and 4.1 g. (0.05 mole) of sodium acetate. After refrigeration of the mixture, the precipitated product was separated by filtration, washed well with water and dried *in vacuo* to give a nearly quantitative yield of pure product which decomposed at 209–210°. Recrystallization of a sample from ethanol did not change the decomposition point.

Pyridoxal O-(2-Carboxyethyl)-oxime.—This product was prepared by the procedure used for the preceding compound using β-aminoöxypropionic acid. However, recrystallization of the crude product from 95% ethanol gave a light yellow ethanol solvate which decomposed at 204°.

Anal. Calcd. for $C_{11}H_{14}N_2O_5 \cdot C_2H_5OH$: C, 51.99; H, 6.71; N, 9.33. Found: C, 51.64; H, 6.42; N, 9.68. The compound was dried at 100° under high vacuum to produce the nonsolvated product after a weight loss of 15%. The calculated weight loss on removal of ethanol was 14.6%.

4-Amino-4-methylvaleric Acid.—A mixture of 71 g. (0.63 mole) of 5,5-dimethyl-2-pyrrolidone,[10] 428 g. (2.5 moles) of barium hydroxide and 1400 ml. of water was heated under reflux for 24 hr. The cooled solution was made slightly acidic with 6 *N* sulfuric acid and neutralized with solid barium carbonate. After removal of solids by filtration, the filtrate was evaporated under reduced pressure to yield 60 g. (73%) of crude product which melted at 270°. Three recrystallizations from aqueous ethanol gave an analytically pure product.

4-Amino-2,4-dimethylvaleric acid was prepared similarly from 3,5,5-trimethyl-2-pyrrolidone.[11]

2,2-Diphenyl-4-phthalimidobutyronitrile.—A mixture of 56.5 g. (0.188 mole) of 4-bromo-2,2-diphenylbutyronitrile,[12] 46.3 g. (0.25 mole) of potassium phthalimide and 300 ml. of dimethylformamide was stirred and heated at 100° for 8 hr. Solvent was removed under reduced pressure and the residue was mixed with 1 l. of water and cooled. The precipitated solid was recrystallized from methanol to give 64 g. (93%) of product which melted at 127–129°. An analytical sample recrystallized twice from methanol melted at 129–130°.

1-Phthalimido-4-pentanone Ethylene Ketal.—A mixture of 39.5 g. (0.33 mole) of 1-chloro-4-pentanone,[12] 43.5 g. (0.70 mole) of ethylene glycol, 600 ml. of anhydrous benzene and 2 drops of concd. sulfuric acid was heated under a Dean–Starke trap for 20 hr. The cooled benzene solution was washed with water and saturated sodium bicarbonate solution, dried over anhydrous magnesium sulfate and distilled to give 48 g. (89%) of colorless oil which boiled at 84–85° (9 mm.); $n^{29}D$ 1.4468. This ketal, 16.4 g. (0.1 mole), was mixed with 18.5 g. (0.1 mole) of potassium phthalimide and 125 ml. of dimethylformamide, then stirred and heated on a steam bath for 24 hr. Most of the solvent was removed under reduced pressure and the residue was poured into 750 ml. of ice water. The solid precipitate was separated by filtration and dried to give 27.1 g. (97%) of crude product. Three recrystallizations from methanol gave 22.6 g. (81%) of material, m.p. 77–78°.

2-Methyl-1,3-dioxolane-2-propylamine Carbonate.—A mixture of 10.8 g. (0.039 mole) of 1-phthalimido-4-pentanone ethylene ketal, 3.9 g. (0.078 mole) of hydrazine hydrate and 100 ml. of absolute ethanol was heated under reflux for

(10) R. B. Moffett, *Org. Syn.*, **32**, 59 (1952).
(11) E. H. Woodruff, U. S. Patent 2,655,511 (October 13, 1953).
(12) Purchased from The Aldrich Chemical Co., Inc.

2 hr., then concentrated under reduced pressure. The residual white solid was mixed with 200 ml. of ether and the slurry was shaken with 200 ml. of 30% potassium hydroxide solution. The ether layer was separated and the aqueous layer was extracted with three 200 ml. portions of ether. After drying over anhydrous magnesium sulfate, the combined ether solutions were distilled to give 4.3 g. (76%) of product which boiled at 91–92° at 14 mm.; $n^{25}D$ 1.4516. Exposure to CO_2 gas of a 3.2 g. (0.022 mole) sample of the base gave 3.8 g. (99%) of the carbonate which decomposed at 66–69°. Recrystallization from ethanol–ether (−70°) did not change the melting point.

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Anabolic Agents: Derivatives of 2-Halo-5α-androst-1-ene

R. E. COUNSELL AND P. D. KLIMSTRA

Division of Chemical Research of G. D. Searle & Co., Chicago 80, Illinois

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Various derivatives of 2-halo-5α-androst-1-ene were synthesized in the hope of obtaining compounds with high anabolic and minimal androgenic activity. Anabolic and androgenic activities are reported for a number of these compounds as well as for some of the synthetic intermediates.

In a previous publication,[1] the high anabolic activity exhibited by derivatives of 5α-androst-1-ene[2] was described. Since substitution of halogen for hydrogen at C-4 of testosterone produced a favorable effect on the anabolic/androgenic ratio,[3,4] the preparation of the 2-halo analogs of 17β-hydroxy-5α-androst-1-en-3-one (Ia) and its derivatives seemed inviting.

The preparation of these compounds involved the same sequence of reactions employed by other investigators to arrive at 4-halo-Δ^4-

(1) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).
(2) The 1957 IUPAC rules on steroid nomenclature as set forth in *J. Am. Chem. Soc.*, **82**, 5577 (1960), have been followed.
(3) B. Camerino, B. Patelli, and A. Vercellone, *ibid.*, **78**, 3540 (1956).
(4) G. Sala and G. Baldratti, *Proc. Soc. Exptl. Biol. Med.*, **95**, 22 (1957).