Pharmacologically Active Acetylene Compounds. I.[1,2] Structural Modifications of Oxotremorine

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A number of structural modifications of oxotremorine were synthesized and their pharmacological properties examined. All of the derivatives tested were less potent than oxotremorine itself, although several compounds showed pharmacological properties similar to oxotremorine. These studies indicate specific structural requirements for oxotremorine activity.

In our continuing study of structures containing carbon–carbon triple bonds, we have focused our attention to an investigation of the structure and steric requirements of the potent muscarinic agent oxotremorine, N-(4-pyrrolidino-2-butynyl)-2-pyrrolidone. Tremorine (1,4-dipyrrolidino-2-butyne) induces both central effects and peripheral cholinergic effects in experimental animals. Everett, *et al.*,[3] have suggested tremorine as a tool in screening compounds for antiparkinsonism activity. The central effects of this drug resemble some of the symptoms seen in Parkinson's disease, *e.g.*, ataxia, tremor, and rigidity, and can be blocked by many drugs which are clinically useful in this disorder.

Baker, *et al.*,[4] noted a latent period following tremorine administration before effects occurred. They suggested that this latency could result if tremorine were being converted to an active metabolite. Kocsis and Welch[5] found that tremorine was metabolized by mouse, hamster, and rat liver *in vitro* to a substance many times more active than tremorine itself, which elicited effects immediately following administration. This *in vitro* conversion was blocked by known inhibitors of liver microsomal activity. They also observed that premedication of animals with β-diethylaminoethyldiphenylpropyl acetate blocked the effects of tremorine but not of its active metabolite, which indicated that a similar metabolic conversion occurred also *in vivo*. The active metabolite of tremorine was isolated, identified, and subsequently synthesized by Cho, *et al.*[6] Leslie and Maxwell[7] suggested that the pharmacological actions of tremorine may be due entirely to metabolically formed oxotremorine, and, hence, that clinically effective antiparkinsonism drugs may inhibit tremorine activity by antagonizing the action of the metabolically formed oxo derivative. They pointed out that oxotremorine is a more desirable tool in screening compounds for antiparkinsonism activity than is tremorine.

Recently Cho and Jenden[8] reported the preparation of a series of acetylenic diamines related to tremorine in which the pyrrolidine ring was substituted by a number of cyclic and alicyclic secondary amino moieties. Their structure modifications failed to produce any compounds possessing any of the effects of tremorine or oxotremorine and these authors suggested that their tremorine analogs failed to be metabolized to the active oxotremorine, or that the structural requirements for oxotremorine-like activity are so specific that the corresponding metabolites are inactive. Our studies reported herein and those recently reported by Bebbington, *et al.*,[9] show that the structural requirements of oxotremorine are also specific. Oxotremorine was still the most potent agent of the numerous structural modifications prepared by the two groups.

Chemistry.—The following general methods were used to obtain the oxotremorine analogs described in Table I.

Method A involved a Mannich condensation[10] of the acetylene derivatives and an appropriate secondary amine with formaldehyde in dioxane to yield structures related to oxotremorine.

The saturated oxotremorine analogs **2** and **9** were prepared by method B. Heating N-(4-aminobutyl)-pyrrolidine[11] or N-(6-aminohexyl)pyrrolidine[12] with γ-butyrolactone in a sealed tube at 250° gave **2** and **9** in satisfactory yields. A similar condensation of γ-butyrolactone with 4-pyrrolidino-2-butynylamine failed to yield any of the desired oxotremorine under a variety of conditions.

Another alternative method for the synthesis of oxotremorine and its derivatives which proved unsatisfactory in the ultimate step involved the condensation of N-chloromethyl-2-pyrrolidone[13] with the sodium salt of N-propargylpyrrolidine. Pure oxotremorine could not be isolated by this method.

The preparation of N-(4-pyrrolidino-2-butynyl)-

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(2) This work was performed under Contract DA18-108-AMC-103(A) with the U. S. Army Edgewood Arsenal, Chemical Research Laboratory, Edgewood Arsenal, Md.

(3) G. M. Everett, L. E. Blockus, and I. M. Shepperd, *Science*, **124**, 79 (1956).

(4) W. W. Baker, M. J. Hosko, W. J. Rutt, and J. R. McGrath, *Proc. Soc. Exptl. Biol. Med.*, **104**, 214 (1960).

(5) J. J. Kocsis and R. M. Welch, *ibid.*, **107**, 731 (1961).

(6) A. K. Cho, W. L. Haslett, and D. J. Jenden, *Biochem. Biophys. Res. Commun.*, **5**, 276 (1961).

(7) G. B. Leslie and D. R. Maxwell, *Nature*, **202**, 97 (1964).

(8) A. K. Cho and D. J. Jenden, *Intern. J. Neuropharmacol.*, **3**, 27 (1964).

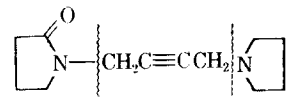
(9) A. Bebbington, R. W. Brimblecombe, and D. Shakeshaft, *Brit. J. Pharmacol.*, **26**, 56 (1966).

(10) C. Mannich and F. T. Chang, *Ber.*, **66**, 418 (1933); J. L. Neumeyer, J. G. Cannon, and J. P. Buckley, *J. Med. Pharm. Chem.*, **5**, 784 (1962).

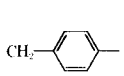
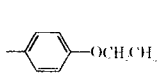
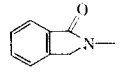
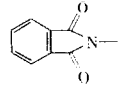
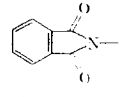
(11) A. Lespagnol, E. Cuingnet, and M. Diebaret, *Bull. Soc. Chim. France*, 1162 (1960); *Chem. Abstr.*, **55**, 7283 (1961).

(12) A. Weichmann (to Badische Anilin und Soda Fabrik), German Patent 803,903; *Chem. Abstr.*, **45**, 8046 (1951).

TABLE I: STRUCTURAL MODIFICATIONS OF OXOTREMORINE



X Y Z

Compd	X	Y	Z	Method	Bp, °C (mm)	$n^{25}D$	Mp, °C	Formula	%C Calcd	%C Found	%H Calcd	%H Found	%N Calcd	%N Found
1		$(CH_2)_3C\equiv CCH_2$		A	118–112 (0.005)	1.5105		$C_{14}H_{12}N_2O$	71.75	71.51	9.46	9.39	11.96	12
2		$(CH_2)_4$		B	125–130 (0.01)[a]	1.4908[a]		$C_{12}H_{22}N_2O$	68.53	68.46	10.54	10.74		
3		$CH_2C\equiv CCH_2$		A	103 (0.05)	1.5321		$C_{16}H_{22}N_2O$	74.38	74.20	8.58	8.68	10.84	10.85
4		$CH_2C\equiv CCH_2$		A	130–131 (0.01)	1.5252		$C_{17}H_{20}N_2O$	74.96	74.66	8.88	8.97	10.29	10.34
5				D			120–121	$C_{15}H_{20}N_2O$	73.73	73.70	8.25	8.32	11.47	11.34
6				D			110–115[b]	$C_{15}H_{20}N_2O$	73.73	73.74	8.25	8.19	11.47	11.43
7				D			96.5–97[c]	$C_{16}H_{22}N_2O_2$	70.04	70.67	8.08	8.08	10.21	10.18
8			$-OOCCH_3$	D			114–116[d]	$C_{12}H_{13}NO_3$	65.74	65.19	5.98	5.89	6.39	6.54
9		$(CH_2)_5$		B	146 (3)	1.4908		$C_{17}H_{26}N_2O$					11.75	11.50
10		$CH_2C\equiv CCH_2$		A			152–156[e]	$C_{20}H_{22}N_2O_3$[e]	64.85	65.02	5.99	5.95	7.56	7.39
11		$CH_2C\equiv CCH_2$		C			107–109[f] 198–200[g]	$C_{16}H_{16}N_2O_2$ $C_{16}H_{17}ClN_2O_2$[g]	71.62 63.00	70.37 63.06	6.01 5.63	6.22 5.72	10.44 9.20	10.19 9.14
12		$(CH_2)_3C\equiv CCH_2$		C			164–165[g]	$C_{18}H_{21}ClN_2O_2$[g]	64.80	64.79	6.35	6.30	8.26	8.22
13	$OHCONH-$	$CH_2C\equiv CCH_2$		C	[h] 97–105 (0.02)	1.5082		$C_{10}H_{16}N_2O$	66.63	66.62	8.95	8.98	15.54	15.59
14	$CH_3(CH_2)_6CONH-$	$CH_2C\equiv CCH_2$		C			80–83	$C_{26}H_{42}N_2O$	77.17	77.16	11.96	11.86	6.92	7.00

15		$CH_2C{\equiv}CCH_2$		C		65–67[a]	$C_{12}H_{18}N_2O$	69.87	69.95	8.80	8.92	13.58	13.61
16	H_2N—	$CH_2C{\equiv}CCH_2$		C	75–76 (15)	1.5005	$C_8H_{14}N_2$	69.52	69.38	10.21	10.12	20.27	20.25
17	H_2N—	$CH_2C{\equiv}C(CH_2)_3$		C	61–62 (0.5)	1.4938	$C_{10}H_{18}N_2$	72.24	72.35	10.91	11.05	16.85	17.07
18		$CH_2C{\equiv}CCH_2$		C		200–205	$C_{15}H_{18}ClN_3O$	61.74	61.73	6.22	6.21		
19	$ClCO_2$—	$CH_2C{\equiv}CCH_2$		E	108–110 (3)[j]	1.5747	$C_{10}H_{15}NO_2$	66.27	66.02	8.34	8.25	7.73	7.81
20	CH_3NHCO_2—	$CH_2C{\equiv}CCH_2$		E	123.5 (0.2)	1.4970	$C_{10}H_{16}N_2O_2$	61.20	61.46	8.22	8.13	14.28	14.10
21	NH_2CO_2—	$CH_2C{\equiv}CCH_2$		E		105–107	$C_9H_{14}N_2O_2$	59.32	59.49	7.74	7.71	15.37	15.54
22		$CH_2C{\equiv}CCH_2$		E		88.5–89.5[j]	$C_{15}H_{17}ClN_2O_2$	61.53	61.75	5.85	6.09	9.57	9.31
23	$(CH_3)_2NCO_2$—	$CH_2C{\equiv}CCH_2$		E	80 (0.05)[k]	1.4887[k]	$C_9H_{16}N_2O_2$	62.83	62.81	8.63	8.69	13.32	13.32
24	CH_3CO_2	$CH_2C{\equiv}CCH_2$		E	60–62 (0.04)	1.4858	$C_{10}H_{14}NO_2$	67.02	67.14	7.31	7.41	7.82	7.93

[a] Lit.[9] bp 100° (0.02 mm), $n^{25}D$ 1.4932. [b] Recrystallized from hexane. Thin layer chromatography in ethyl acetate gave one spot at R_f 0.58. [c] Recrystallized from water. [d] Lit.[14] mp 117°. [e] Melting point and analysis is for the hydrogen fumarate. [f] Lit.[9] mp 112–114°. [g] Melting point, analysis, and pharmacological activity are on the hydrochloride. [h] Recrystallized from ethanol. [i] Lit.[9] bp 68° (0.1 mm). [j] Recrystallized from hexane–benzene. [k] Lit.[9] bp 80–84° (0.05 mm), $n^{25}D$ 1.4885.

phthalimide (**11**) in 87% yield from **25a** by a Mannich condensation (method C) was more desirable than the

condensation of N-(4-chloro-2-butynyl)phthalimide[16] with pyrrolidine. Hydrazinolysis[17] of **11** and **12** yielded the acetylenic amines **16** and **17**. Subsequent acylation produced the amides **13**, **14**, and **15**, and the urea derivative **18**.

Compounds **5** and **6**, in which the butynyl moiety in oxotremorine was replaced by a phenyl ring, were synthesized by the following sequence shown as method D from **26a** and **26b**. Compound **7** was similarly prepared by the condensation of *p*-aminophenol with a slight excess of γ-butyrolactone. The resulting 1-(*p*-hydroxyphenyl)-2-pyrrolidone was subsequently alkylated with N-(2-chloroethyl)pyrrolidine to yield N-[4-2-pyrrolidinoethoxy)phenyl]-2-pyrrolidone (**7**) or with acetic anhydride to yield the ester (**8** in Table I).

The intermediate amino alcohol, 4-pyrrolidino-2-butynol, was prepared from 4-chloro-2-butynol[18] and pyrrolidine. The carbamates **20** and **22** were prepared directly from this alcohol with the appropriate isocyanate.

Method E which consists of treating the acetylenic alcohols with phenyl chloroformate[19] and then without purification with an appropriate amine yielded the acetylenic carbamates **21** and **23**.

(13) Considerable difficulty experienced in the synthesis of N-chloromethyl-2-pyrrolidone was traced to an erroneous assignment of structure to its precursor N-hydroxymethyl-2-pyrrolidone by Reppe.[14] By the action of 2-pyrrolidone on 30% aqueous formaldehyde solution in an acid medium Reppe obtained a product to which he ascribed the structure N-hydroxymethyl-2-pyrrolidone. Later J. W. Breitenbach and E. Wolf [*Monatsch.*, **87**, 367 (1956)] showed that the compound isolated by Reppe was 1,1′-methylenedi-2-pyrrolidone. The conversion to the chloro derivative proceeded without difficulty with authentic N-hydroxymethyl-2-pyrrolidone, prepared from 2-pyrrolidone and 30% aqueous formaldehyde in an alkaline medium by the method of Shostakovskii, *et al.*[15]

(14) W. Reppe and "Mitarbeiter," *Ann.*, **596**, 80 (1955); *Chem. Abstr.*, **50**, 16786 (1956).

(15) F. P. Sidel'kovskaya, M. G. Zelenskaya, and M. F. Shostakovskii, *Izv. Akad. Nauk SSSR, Otd. Khim. Nauk*, 901 (1959); *Chem. Abstr.*, **54**, 1286 (1960).

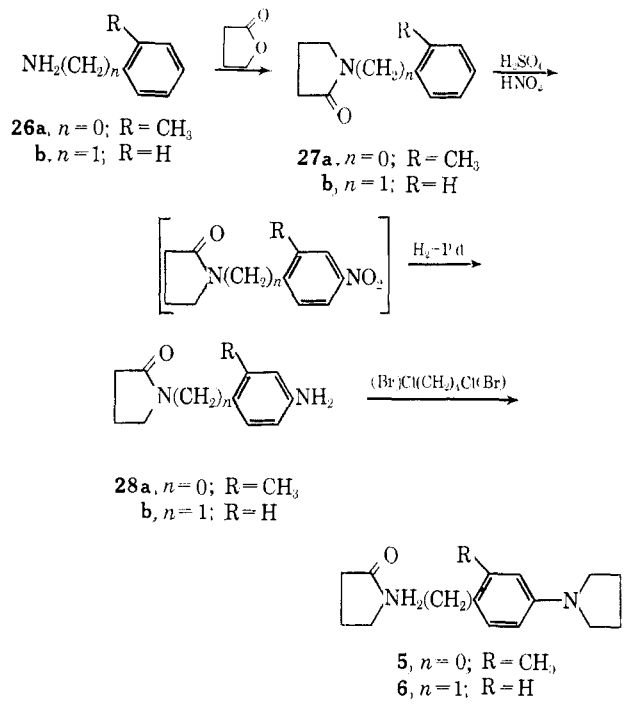
(16) J. L. Neumeyer and W. Firshein, *J. Pharm. Sci.*, **55**, 441 (1966).

(17) J. C. Sheehan and W. A. Golhafer, *J. Am. Chem. Soc.*, **72**, 2788 (1950).

(18) W. Bailey and E. Fujiwara, *ibid.*, **77**, 165 (1955).

(19) W. M. McLamore, S. Y. P'an, and A. Bavley, *J. Org. Chem.*, **20**, 1379 (1955).

method D



Results and Discussion[20]

The LD_{50} of oxotremorine in male albino mice was 1.6 mg/kg. A variety of pharmacological effects were noted at the highest nonlethal dose administered. Most prominent were a depression of spontaneous activity, tremors, salivation, lacrimation, respiratory depression, a decreased response to external stimuli, some reflex depression, and an impairment of motor performance. At progressively lower doses, the effects elicited by oxotremorine became fewer, less severe, and of shorter duration. It is of interest that, in this lessening dose progression, tremors were not the most persistent symptom. Thus, at oxotremorine's lowest effective dose, only one animal exhibited tremors, along with a depression of spontaneous activity; the other three animals exhibited only a depression of spontaneous activity. The ED_{50} for any observable effect was 0.018 mg/kg. The ED_{50} for tremors *per se* was 0.042 mg/kg. Our values agree well with those reported by Bebbington, *et al.*[9] (LD_{50} = 1.4 mg/kg; minimal effective dose for tremors, 0.05 mg/kg).

All of the oxotremorine derivatives tested were less potent than oxotremorine itself (Table II). The differences were so large as to be significant ($p \leqslant 0.05$; fiducial limits are shown in the table) even with the small number of animals used, and more definitive pharmacology did not seem warranted. Their LD_{50} values were at least six times as great as that for oxotremorine (range 6 to >60 times). Their ED_{50} values were at least 100 times as great as that for oxotremorine (range 100–3000 times). In effect, the various structural analogs of oxotremorine were not only less potent, but also had narrower dose ranges of activity. Indeed, five of the compounds had no effects at all below their lethal doses. The pharmacological activity of the other compounds was similar to that observed with oxotremorine. At their lowest effective doses, their predominant effects were the depression of spontaneous activity, and tremors were not seen. This was similar to the effects of oxotremorine at its lowest effective dose. Only six of the compounds had more than minimal effects below their lethal doses, and with these tremors were observed (Table II).

TABLE II

PHARMACOLOGICAL ACTIVITY OF ANALOGS OF OXOTREMORINE IN MICE[a]

Compd[b]	Group range of LD_{50}, mg/kg	Group range of ED_{50}, mg/kg	Compd of group exhibiting tremors	ED_{50} for tremors, mg/kg
Oxotremorine	1.6 (1.3–2.0)[c]	0.018 (0.011–0.030)[c]	Oxotremorine	0.042 (0.024–0.075)[c]
A. Alterations of Central Chain				
1, 2, 5–7, 9	18–>100 (5.6)[d]	10–56 (3.2)[d]	7	32 (10)[d]
B. Alterations of Pyrrolidine End Group. Ring Fusion				
3, 4	56 (18)	1.8–32 (0.6)	4	18 (5.6)
C. Alterations of Pyrrolidinone End Group. Ring Fusion and Further Oxidation				
10, 11	18 (5.6)	5.6–10 (1.8)	None	
D. Alterations of Pyrrolidinone End Group. Ring Breakage; Amide or Amine Link to Central Chain				
14–16, 18	>10–56 (5.6)	10–56 (3.2)	18	32 (10)
E. Alterations of Pyrrolidinone End Group. Ring Breakage; Ester Link to Central Chain				
19–23	10–56 (3.2)	5.6–18 (1.8)	19, 22, 23	18–32 (5.6)
F. Multiple Alterations. Alterations of Both End Groups or of Central Chain and an End Group				
8, 12, 24	18–56 (5.6)	5.6–>32 (1.8)	None	

[a] Two animals per dose (except oxotremorine, 4/dose in ED_{50} dose range); at least four doses per compound. [b] Compounds 13 and 17 were not tested. [c] The range in parentheses is the 95% confidence limit. [d] The figure in parentheses is the lower 95% confidence limit of the most potent compound in the group. This value may be compared with the upper fiducial limit for oxotremorine, for the evaluation of significant potency differences.

Our results did not show any particular portion of the oxotremorine structure to have a primary role in its activity. Any alteration of any part of the molecule markedly reduced activity. From these results we concluded that the structural requirements for oxotremorine activity are very specific.

Experimental Section[21]

N-(2-Propynyl)-2-pyrrolidone, bp 57–58° (0.01 mm), n^{25}D 1.4932 [lit.[22] 77° (0.05 mm), n^{25}D 1.4970], was prepared as previously described.[22]

[20] In conducting the research reported herein, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

[21] All melting points were recorded on a Thomas–Hoover melting point apparatus and were corrected. The microanalyses were performed by Dr. S. M. Nagy of the Massachusetts Institute of Technology. The reported yields were based frequently on one run and do not necessarily reflect the optimum ones attainable. The preparation of previously unreported intermediates, as well as of representative compounds for each of the methods outlined in the previous section and summarized in Table I, will be described in detail.

[22] A. Bebbington and D. Shakeshaft, *J. Med. Chem.*, **8**, 274 (1965).

Similarly prepared from 45 g (0.53 mole) of pyrrolidone and 51.8 g (0.5 mole) of 5-chloro-1-pentyne was **N-(4-pentynyl)-2-pyrrolidone**, a colorless oil, bp 123° (3.5 mm), $n^{25}D$ 1.4934. Thin layer chromatography in methanol on Adsorbosil showed one spot, R_f 0.73.

Anal. Calcd for $C_9H_{13}NO$: C, 71.49; H, 8.67. Found: C, 71.54; H, 8.72.

N-(2-Propynyl)phthalimidine.—In a dry 500-ml flask fitted with a stirrer, addition funnel, and condenser, 4.5 g (0.1 mole) of 53% NaH (in mineral oil) was dispersed in 50 ml of dry toluene, and 10 g (0.075 mole) of phthalimidine[23] was added in a slurry of 100 ml of toluene. The mixture was refluxed for 3 hr and cooled. 3-Bromopropyne (12 g, 0.1 mole) in 50 ml of toluene was added dropwise, the mixture was allowed to reflux for another 1 hr and then was stirred at room temperature overnight. The solids were filtered off, the toluene was evaporated, and the resulting oil was washed with aqueous Na_2CO_3 and extracted ($CHCl_3$). After evaporation of the $CHCl_3$ an oil remained which crystallized on standing. The solid was filtered and recrystallized from ethyl acetate–petroleum ether (bp 30–60°) to give 1.6 g of yellow crystals, mp 84–85°.

Anal. Calcd for $C_{11}H_9NO$: C, 77.2; H, 5.30; N, 8.25. Found: C, 77.40; H, 5.47; N, 8.15.

N-(2-Propynyl)phthalimide (25a).—Potassium phthalimide (74 g, 0.4 mole) was partially dissolved in 700 ml of dimethylformamide (DMF) by heating to reflux, and a solution of 47.6 g (0.4 mole) of propargyl bromide in 100 ml of DMF was added dropwise to the hot solution with stirring. The heating was continued at 100° for 3 hr. The mixture was then stirred at room temperature overnight and finally it was poured into ice water. The resulting precipitate was washed with H_2O and was recrystallized from ethanol. The product was obtained as colorless needles, mp 149–150° (lit.[24] 150–157°) in 80% yield.

Similarly prepared from potassium phthalimide and 5-chloro-1-pentyne in 93% yield was **N-(4-pentynyl)phthalimide (25b)**, mp 81–84°. An analytical sample, recrystallized from ethanol–H_2O, melted at 85–86°.

Anal. Calcd for $C_{13}H_{11}NO_2$: C, 73.22; H, 5.20. Found: C, 73.23; H, 5.45.

Mannich Condensation of a Monosubstituted Acetylene. Method A. 2-[4-(2-Pyrrolidino)-2-butynyl]-*cis*-hexahydro-4,7-methanoisoindoline (Table I, 4).—A mixture of 5.3 g (0.044 mole) of N-(2-propynyl)-2-pyrrolidone, 6.0 g (0.044 mole) of hexahydromethanoisoindoline, 1.45 g (0.048 mole) of paraformaldehyde, and 10 ml of dioxane was refluxed for 6 hr. The dioxane was evaporated and the oil remaining was dissolved in dilute HCl. The solution was washed with ether, made basic (aqueous NaOH) and extracted ($CHCl_3$). The $CHCl_3$ extract was dried (K_2CO_3), filtered, and evaporated. The residual oil, bp 169° (0.1 mm), was redistilled on a spinning-band column to give 2.7 g (23%) of product.

***cis*-Hexahydro-4,7-methanoisoindoline.**—The thimble of a Soxhlet extractor was charged with 79 g (0.47 mole) of norbornanedicarboximide[25] and the solid was extracted into a suspension of 36.8 g (0.89 mole) of $LiAlH_4$ in 1 l. of ether for 36 hr. The excess hydride was decomposed by dropwise addition of 10% aqueous Na_2SO_4 solution until no more hydrogen was evolved. The solids were removed by filtration and washed with ether. The ether solutions were dried (Na_2CO_3) and evaporated under reduced pressure. The residue, 33.7 g (52%) of a white waxy solid, was recrystallized from acetonitrile, but remained waxy and its melting point could not be determined. The compound sublimed at 40°.

Anal. Calcd for $C_9H_{15}N$: N, 10.21. Found: N, 10.09.

Method B. N-(4-Pyrrolidinobutyl)-2-pyrrolidone (Table I, 2).—In a 25-ml flask, a mixture of 6 g (0.0423 mole) of N-(4-aminobutyl)pyrrolidine and 3.54 g (0.0423 mole) of γ-butyrolactone was heated at 110–130° for 3 hr with stirring. The heating was continued at 200° for 3 hr and at 225° for 3 hr. Distillation at reduced pressure afforded 5.75 g (65%) of N-(4-pyrrolidinobutyl)-2-pyrrolidone as a colorless oil; infrared absorption peaks, 1650–1675 (amide C=O), 1775 cm^{-1} (weak, γ-lactone).

4-Pyrrolidinobutyronitrile.—Freshly distilled 4-bromobutyronitrile (Matheson Coleman and Bell) (23.2 g, 0.157 mole) was added below 10° to a solution of 22.0 g (0.32 mole) of pyrrolidine in 150 ml of ether. The resulting solution was refluxed for 3 hr and then was stirred overnight at room temperature. The ether layer was decanted and the remaining oil was extracted with 1 l. of ether in five portions. The ether extract was distilled at reduced pressure, to give 18.5 g (85%) of product, bp 112–113 (20 mm), $n^{25}D$ 1.4588 [lit.[11] bp 115° (18 mm)].

N-(4-Aminobutyl)pyrrolidine.—In a 1-l. flask fitted with a stirrer, condenser, and addition funnel, 10.2 g (0.262 mole) of $LiAlH_4$ was dissolved in 500 ml of ether, and 18.05 g (0.131 mole) of 4-pyrrolidinobutyronitrile in 20 ml of ether was added dropwise with stirring and cooling. The reaction mixture was refluxed for 20 hr. The excess hydride was decomposed with 10% Na_2SO_4 solution. The inorganic solids were removed by filtration and washed with 500 ml of ether. The ether filtrates were dried (K_2CO_3) and evaporated. Distillation yielded 13.71 g (74%) of N-(4-aminobutyl)pyrrolidine as a clear oil, bp (0.2 mm), $n^{25}D$ 1.4680 (lit.[11] bp 205°).

N-(5-Cyanopentyl)-2-pyrrolidone.—A solution of 32.6 g (0.29 mole) of ω-aminocapronitrile[26] and 25 g (0.29 mole) of γ-butyrolactone was heated at 110–130° for 3 hr and then at 250° for 2.5 hr, while H_2O was distilled off. The mixture was fractionated at reduced pressure and 42.5 g (80%) of the desired cyanopentylpyrrolidone was collected at 165–173° (2 mm), $n^{25}D$ 1.4780. The infrared spectrum showed peaks at 1680 (amide C=O), 1775 (γ-lactone), and 2259 cm^{-1} (nitrile). The product was 92% pure as determined by gas chromatography.

N-(6-Aminohexyl)pyrrolidine was prepared by $LiAlH_4$ reduction of N-(5-cyanopentyl)-2-pyrrolidone as described above for the preparation of N-(4-aminobutyl)pyrrolidine, except that the reducing agent was used in 3 *M* excess. The product, a colorless oil, was collected in 31% yield at 108–115° (6 mm), $n^{25}D$ 1.4682 [lit.[12] bp 126–127° (14 mm)]. The infrared spectrum showed neither carbonyl absorption peaks at 1680 and 1775 cm^{-1} nor nitrile absorption at 2250 cm^{-1}.

N-Hydroxymethyl-2-pyrrolidone was prepared as described.[15] The product, mp 82–83° (lit. 76–78°,[15] 83–84°[27]), was obtained in 40% yield.

N-Chloromethyl-2-pyrrolidone[13] was prepared in 80% yield as described.[15] The product was distilled at 107° (5 mm), $n^{25}D$ 1.5008 [lit.[15] bp 104–105° (5 mm), $n^{20}D$ 1.5022].

N-(4-Pyrrolidino-2-butynyl)phthalimide (Table I, 11).—To 3.1 g (0.044 mole) of pyrrolidine in 10 ml of DMF was slowly added 4.6 g (0.02 mole) of N-(4-chloro-2-butynyl)phthalimide[16] (**28**) dissolved in 30 ml of DMF. The mixture was stirred at 75° for 3 hr and for 12 hr at room temperature. The resulting orange solution was poured into 150 ml of ice water. The crystals which formed were filtered off and discarded. The DMF–H_2O filtrate was extracted with 400 ml of $CHCl_3$. The $CHCl_3$ extract was evaporated, and the remaining DMF was distilled under vacuum. The residual oil crystallized on standing to yield 0.5 g of N-(4-pyrrolidino-2-butynyl)phthalimide as a brown powder. This compound was also prepared by method A in 87% yield from N-(2-propynyl)phthalimide, pyrrolidine, and paraformaldehyde.

Method C. 4-Pyrrolidino-2-butynylamine (Table I, 16).—N-(4-Pyrrolidino-2-butynyl)phthalimide (10 g, 0.038 mole) (method A, Table I, 11), 2 g (0.038 mole) of 100% hydrazine hydrate, and 80 ml of ethanol were mixed and heated at reflux for 4 hr. The mixture was cooled, the precipitate was filtered off, and the ethanol solution was evaporated. The remaining brown oil was distilled and 2.8 g (53%) of amine was collected as a clear oil.

Similarly prepared in 78% yield from 10 g (0.03 mole) of N-(6-pyrrolidino-2-hexynyl)phthalimide hydrochloride and 3.1 g (0.061 mole) of 99% hydrazine in 100 ml of ethanol was **6-pyrrolidino-4-hexynylamine** (Table I, 17).

N-(4-Pyrrolidino-2-butynyl)stearamide (Table I, 14).—A solution of 6.6 g (0.022 mole) of stearoyl chloride in 25 ml of $CHCl_3$ was added dropwise with stirring to a solution of 3 g (0.022 mole) of 1-(4-amino-2-butynyl)pyrrolidine in 75 ml of $CHCl_3$ at 0°. The solution was stirred at room temperature for an additional 8 hr. The $CHCl_3$ was evaporated, the residue was dissolved in H_2O, and the solution was neutralized (Na_2CO_3), which precipi

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(24) K. Sato, *Nippon Kagaku Zasshi*, **76**, 1404 (1955); *Chem. Abstr.*, **51**, 17760 (1957).

(25) M. Morgan, R. S. Tipson, A. Lowy, and W. E. Baldwin, *J. Am. Chem. Soc.*, **66**, 404 (1944).

(26) ω-Aminocapronitrile ($n^{25}D$ 1.4505; Columbia Organic Chemicals Co., Inc.) was purified by fractionation, bp 116–118° (14 mm), $n^{25}D$ 1.4477. The purity of distilled material was 95% by gas chromatography (F and M Model 500; detector, flame ionization; carrier gas, helium; column, 6-ft Carbowax 2017 on Haloport F; run at 150°), with one major impurity of about 4%.

(27) H. Bohme, G. Driesen, and D. Schunemann, *Arch. Pharm.*, **294**, 344 (1961); *Chem. Abstr.*, **55**, 22335 (1961).

tated a white solid. The mixture was heated on the steam bath to drive off the last traces of $CHCl_3$, then was cooled and filtered. The dried solid amounted to 8.7 g (98%) of crude amide, mp 70–85°. The solid was recrystallized four times from ethanol with use of decolorizing carbon to give colorless platelets: mp 80–83°; infrared spectrum, 1640 (amide C=O) and 3300 cm^{-1} (NH).

1-(3-Chlorophenyl)-3-(4-pyrrolidino-2-butynyl)urea (Table I, 18).—A solution of 1 g (0.007 mole) of 1-(4-amino-2-butynyl)-pyrrolidine and 1.12 g (0.007 mole) of m-chlorophenyl isocyanate in 100 ml of ether was refluxed for several hours, cooled, and allowed to stand. Presently 1.8 g of the urea crystallized as a white solid, mp 200–205° dec. It was recrystallized twice from methanol–H_2O, but the melting point was not changed.

Method D. 1-*o*-Tolyl-2-pyrrolidone (Table I, 13).—A mixture of 88 g (0.8 mole) of *o*-tolnidine and 104 g (1.2 moles) of γ-butyrolactone was heated in an autoclave at 300° for 10 hr. Distillation afforded 118 g (88%) of 1-*o*-tolyl-2-pyrrolidone, bp 125° (0.2 mm) [lit.[14] bp 130–132° (1 mm)], mp 43–46° (lit.[14] mp 47°).

Similarly prepared from *p*-aminophenol and γ-butyrolactone was **1-(*p*-hydroxyphenyl)-2-pyrrolidone,** mp 161–163° (dioxane–H_2O) (lit.[14] mp 162°).

1-(4-Amino-2-methylphenyl)-2-pyrrolidone (28a).—A solution of 17.5 g (0.1 mole) of 1-(*o*-tolyl)-2-pyrrolidone in 84 ml of concentrated H_2SO_4 was cooled to 0°. To this was added dropwise a solution of 6.5 ml of concentrated HNO_3 in 18.5 ml of concentrated H_2SO_4, while the temperature was held at 0–5°. The mixture was then stirred at room temperature for an additional 2 hr and poured onto 200 g of ice. The resulting aqueous solution was neutralized (Na_2CO_3) and extracted ($CHCl_3$). The extract was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 20.8 g of an orange oil, presumed to be the nitrated product. This oil was reduced during 2 hr in methanol over 1 g of 5% Pd–C at 3.29 kg/cm^2. The filtered solution was evaporated under reduced pressure and the residue was recrystallized from H_2O to give 8.0 g (42%) of **28a** as tan crystals, mp 142–144° (lit.[27] mp 143°).

1-(2-Methyl-4-pyrrolidinophenyl)-2-pyrrolidone (Table I, 5).—A mixture of 3.8 g (0.02 mole) of 1-(4-amino-2-methylphenyl)-2-pyrrolidone, 5 g (0.023 mole) of 1,4-dibromobutane (Eastman), 4 g of K_2CO_3, and 25 ml of absolute ethanol was refluxed under N_2 with stirring for 3.5 days. The mixture was evaporated under reduced pressure and the residue was redissolved in CH_2Cl_2 and filtered to separate excess KBr. After removal of solvent the residue was crystallized from a benzene–petroleum ether mixture to give 3.5 g (72%) of the desired product as off-white crystals, mp 118–120°. Repeated recrystallizations from petroleum ether raised the melting point to 120–121°.

N-[4-(2-Pyrrolidinoethoxy)phenyl]-2-pyrrolidone (Table I, 7).—A mixture of 3.4 g of N-(2-chloroethyl)pyrrolidine hydrochloride (0.02 mole), 3.6 g of 1-(*p*-hydroxyphenyl)-2-pyrrolidone (0.02 mole), 7 g of anhydrous K_2CO_3 (0.1 mole), and 100 ml of acetone was refluxed for 3 days, then cooled, and filtered. The solvent was removed under reduced pressure and the solid residue was recrystallized from water to give 3 g (56%) of the pyrrolidone **7**. The infrared spectrum showed peaks at 1690 (NC=O stretching) and 1260 cm^{-1} (C–O stretching).

Method E. 4-Pyrrolidino-2-butynol.—4-Chloro-2-butynol (50.4 g, 0.5 mole)[18] was added dropwise with stirring to a solution of 70 g (1.0 mole) of pyrrolidine in 300 ml of ether, while the temperature was kept below 10°. The solution was stirred at this temperature an additional 30 min, heated at reflux for 3 hr, and then stirred overnight at room temperature. The ether layer was decanted, and the residual oily layer containing pyrrolidine hydrochloride was extracted three times with ether. The combined ether solutions were dried (K_2CO_3), the solvent was removed under reduced pressure, and the residue was distilled (28.4 g, 41%), 95–98° (0.05 mm), $n^{25}D$ 1.5028, infrared spectrum 3600–3100 cm^{-1} (OH).

4-Pyrrolidino-2-butynyl N-(3-Chlorophenyl)carbamate (Table I, 18).—A mixture of 7.0 g (0.05 mole) of 3-chlorophenyl isocyanate (Aldrich Chemical Co.), 5 drops of pyridine, and 100 ml of benzene was refluxed for 3 hr. The solution was then cooled to room temperature and poured into 150 ml of hexane. Soon 14.2 g (98%) of the carbamate precipitated as an off-white powder, mp 88.5–89.5°. It was recrystallized from hexane–benzene, but the melting point was unchanged.

4-Pyrrolidino-2-butynyl Carbamate (Table I, 21).—The method of McLamore[19] for the preparation of carbamates of acetylenic carbinols was used. A solution of 10 g (0.072 mole) of 4-pyrrolidino-2-butynol and 8.1 g (0.08 mole) of triethylamine was added dropwise to a solution of 11.3 g (0.072 mole) of phenyl chloroformate (Eastman) in 50 ml of ether at 0° and stirred at room temperature for 12 hr. Water was added and the ether layer was separated. The H_2O layer was extracted again with ether and the combined ether solutions were dried ($MgSO_4$) and concentrated to 200 ml. The chloroformate solution was then added dropwise to an equal volume of liquid NH_3 and the solution was allowed to react for 8 hr (Dry Ice–acetone condenser). The condenser was removed and the NH_3 was allowed to evaporate. Water was added, and the mixture was extracted several times with ether. The combined ether extracts were washed with 4% aqueous NaOH, then with saturated NaCl solution. The ether was evaporated, the residual oil was dissolved in dilute HCl, and the solution was washed with ether. The remaining H_2O extracts were made basic with concentrated NaOH solution and extracted with ether. The ether solution was evaporated to an oil which was crystallized from ethanol–ether, to give 0.7 g of **21** as colorless needles: infrared spectrum, 1725 (C=O), 3300 (amide, NH) cm^{-1}.

Pharmacological Test Methods.—Male albino mice (22 ± 2 g) were used. Compounds were administered intravenously to two animals per dose at each of four or more doses, at 0.5 log intervals; except oxotremorine, which was administered to four animals in its ED_{50} dose range and at 0.1 log intervals in its LD_{50} dose range. Compounds were administered in aqueous solution except **14** and **18**, which required 25% w/v polyethylene glycol for solubilization. Animals were observed at 3, 15, 30, and 60 min following medication and at hourly intervals thereafter through 24 hr, or until symptoms disappeared, whichever was longer, for the kind, time of onset, degree, and duration of effects. Observations were made on approximately 50 discrete components of behavior and appearance, response to external stimuli, reflex functioning, and neuromuscular integrity and performance, under standardized environmental conditions and using standardized manipulative procedures.

These observations included: (1) behavior and appearance, *e.g.*, general locomotor activity—differences in degree or kind from normal, carriage of tail, respiration—occurrence of exophthalmos, lacrimation, salivation, piloerection, catalepsy; (2) response to external stimuli, *e.g.*, touch, sound, mild pain, change of environment; (3) reflex functioning, *e.g.*, righting reflex, pupil size and pupillary light reflex, corneal reflex, pinnal reflex; (4) neuromuscular integrity and performance, *e.g.*, muscle tone, occurrence of ataxia, tremors, ptosis; seven different tests of motor coordination performance, *e.g.*, climb up vertical screen, walk down vertical pole, walk on rotating rod.

The observations thus described a compound's general pharmacological activity in the mouse. This test is similar to that described by Irwin[28] for the screening of new compounds. LD_{50}'s and ED_{50}'s (the minimum dose eliciting any observable effect in 50% of the animals) were calculated using the moving average method of Thompson[29] and the tables were constructed by the method of Weil.[30] A span of three doses was used for the moving average. Confidence limits (95%) were calculated as described by Weil.[30] Because of the small number of animals used, the LD_{50} and ED_{50} values were only approximate, *i.e.*, their 95% confidence limits were relatively large. The values did serve, however, to indicate the dose range over which a given compound exhibited activity and to indicate the existence of large potency differences between compounds.

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