mg/kg of **17**. However, further investigation using the Hardy, *et al.*,[29] procedure to detect analgesia in squirrel monkeys failed to yield any significant analgetic activity. Because a degree of docility was observed in squirrel monkeys following administration of **17**, this compound was examined for potential tranquilizing activity. The antifighting procedure of Tedeschi, *et al.*, indicated a 55% decreae in the incidence of fighting following the administration of 120 mg/kg, sc, of the test compound. A statistical ED_{50} of 135 mg/kg was calculated by the Litchfield and Wilcoxon technique.[30]

(29) J. D. Hardy, H. G. Wolff, and H. Goodell, *J. Clin. Invest.*, **19**, 694 (1940).

(30) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

Acetylene Compounds of Potential Pharmacological Value. XIV. *N*-(*t*-Aminoalkynyl)-Substituted Succinimides and Maleimides. A Class of Central Anticholinergic Agents[1]

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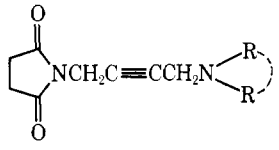
A series of 34 *N*-(*t*-aminoalkynyl)-substituted succinimides and maleimides has been prepared through the Mannich reaction from an *N*-alkynylimide, formaldehyde, and a secondary amine or by ring closure of an *N*-(*t*-aminoalkynyl)-substituted succinamic acid. These compounds have been investigated for antagonistic activity toward acetylcholine on isolated guinea pig ileal preparations and for mydriatic activity and blockade of oxotremorine in intact mice. Some of the compounds exceed atropine in tremorolytic activity, and are relatively selective in their central anticholinergic effects. The latter property tends to be associated with compounds which show evidence of partial agonism *in vitro*.

In two recent publications[2,3] we reported on the synthesis and pharmacological properties of a series of *N*-(4-*t*-amino-2-butynyl)-substituted succinimides.



Some compounds of this class were found to be quite potent in blocking the motor effects of oxotremorine, 1-(2-oxopyrrolidino)-4-pyrrolidino-2-butyne, while the effect on peripheral cholinergic symptoms, such as acetylcholine-induced spasms of guinea pig ileal strips, is of lower magnitude. Consequently, these compounds can be regarded as specific central anticholinergic agents. This discovery has led to the synthesis of a number of analogs with the aim of defining the limits of activity in the series and of enhancing the activity found in the parent compounds. In most of the compounds described in this paper, the chain connecting the imide and the amino nitrogens has been branched or lengthened, though structural modifications have been made also in the imido and amino groups. The most potent of the new compounds are about 100 times as active as the most potent compound in the parent series when tested for oxotremorine antagonistic activity.

Chemistry.—Two methods of synthesizing the *N*-(*t*-aminoalkynyl)-substituted cyclic imides listed in Tables I and II were utilized: Mannich reaction between an *N*-alkynylimide, formaldehyde, and a secondary amine in dioxane in the presence of small amounts of CuCl (method A) and ring closure of an *N*-(*t*-aminoalkynyl)-substituted succinamic acid (method B). The *N*-alkynylimides used as starting materials in method A were generally obtained by treating an alkynylamine with a succinic or maleic anhydride and subsequent ring closure of the *N*-alkynylsuccinamic or *N*-alkynylmaleamic acid formed. The *N*-(*t*-aminoalkynyl)-substituted succinamic acids used as starting materials in method B were prepared by treating succinic anhydride with a *t*-aminoalkynylamine, obtained either by aminoalkylation of an alkynylamine in the presence of $NaNH_2$ or by the reaction of the Grignard reagent of the alkynylamine with a ternary iminium salt. The reaction sequences are shown in Scheme I.

Pharmacological Results and Discussion.—Table III summarizes the pharmacological data. All but four of the compounds antagonized the tremorogenic effect of oxotremorine, and the dose required was in every case less than that which produced mydriasis, with the exception of the weakly active compound **8**. This is in marked contrast to atropine, which is less effective in blocking oxotremorine than in producing mydriasis. Nevertheless, as in previous series of compounds related to oxotremorine, there was a highly

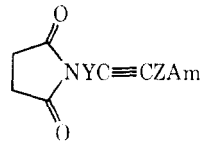
(1) Previous paper in this series: P. Moses and R. Dahlbom, *Acta Pharm. Suecica*, **6**, 359 (1969).

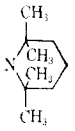
(2) R. Dahlbom, B. Karlén, R. George, and D. J. Jenden, *Life Sciences*, **5**, 1625 (1966).

(3) R. Dahlbom, B. Karlén, R. George, and D. J. Jenden, *J. Med. Chem.*, **9**, 843 (1966).

TABLE I

N-(*t*-AMINOALKYNYL)SUCCINIMIDES



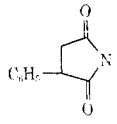
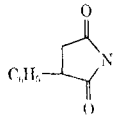
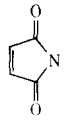
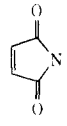
Compd	Y	Z	Am	Method of prepn	Yield, %	Derivative	Recrystn solvent[a]	Mp, °C	Formula[b]
1	CH_2	CH_2	$N(CH_3)_2$	A	23	HCl	Et–E	163.5–165	$C_{10}H_{15}ClN_2O_2$
2	CH_2	CH_2	$NCH_3C_2H_5$	A	63	HCl	Et–E	149–150	$C_{11}H_{17}ClN_2O_2$
3	CH_2	CH_2	$NCH_3C_3H_7$-*n*	A	77	HCl	Et–E	154–156	$C_{12}H_{19}ClN_2O_2$
4	CH_2	CH_2	$NCH_3C_4H_9$-*n*	A	60	Oxalate	Et–E	128–129	$C_{15}H_{22}N_2O_6$
5	CH_2	CH_2	$NCH_3(CH_2)_4OH$	A	72	HCl	Et–E	129.5–131	$C_{13}H_{21}ClN_2O_3$
6	CH_2	CH_2	$N(CH_2CH{=}CH_2)_2$	A	70	HCl	Et–E	136–137	$C_{14}H_{19}ClN_2O_2$
7	CH_2	CH_2		A	44	HCl	Et–E	184–185	$C_{14}H_{21}ClN_2O_2$
8	CH_2	CH_2		A	9	Base	P	80–81	$C_{17}H_{26}N_2O_2$
9	$CHCH_3$	CH_2	$N(C_2H_5)_2$	A	79	HCl	Et–E	155.5–157	$C_{13}H_{21}ClN_2O_2$
10	$CHCH_3$	CH_2		A	58	Base	P	56–58	$C_{13}H_{18}N_2O_2$
11	$CHCH_3$	CH_2		A	80	HCl	Ac	168–170	$C_{14}H_{21}ClN_2O_2$
12	$C(CH_3)_2$	CH_2	$N(CH_3)_2$	A	60	HCl	Et–E	147–148	$C_{12}H_{19}ClN_2O_2$
13	$C(CH_3)_2$	CH_2	$N(C_2H_5)_2$	A	39	HCl	Et–E	120–121	$C_{14}H_{23}ClN_2O_2$
14	$C(CH_3)_2$	CH_2		A	87	HCl	Et–E	153–154	$C_{14}H_{21}ClN_2O_2$
15	$C(CH_3)_2$	CH_2		A	74	HCl	Ac–E	155–156	$C_{16}H_{25}ClN_2O_2$
16	$C(CH_3)_2$	CH_2		A	78	HCl	Ac–E	144–145	$C_{15}H_{23}ClN_2O_2$
17	$(CH_2)_2$	CH_2	$N(CH_3)_2$	A	80	HCl	Ac–Et	141–143	$C_{11}H_{17}ClN_2O_2$
18	$(CH_2)_2$	CH_2	$NCH_3C_3H_7$-*n*	A	79	HCl	Et–E	136–137	$C_{13}H_{21}ClN_2O_2$
19	$(CH_2)_2$	CH_2	$N(C_2H_5)_2$	A	82	HCl	Ac–Et	138–139	$C_{13}H_{21}ClN_2O_2$
20	$(CH_2)_2$	CH_2		A	72	Citrate	Ac	97–98 dec	$C_{19}H_{26}N_2O_9$
21	$(CH_2)_2$	CH_2		A	70	HCl	Ac–Et	191–192	$C_{14}H_{21}ClN_2O_2$
22	$(CH_2)_2$	CH_2		A	71	Base	Et–Aq	89–90	$C_{16}H_{24}N_2O_2$
23	$(CH_2)_3$	CH_2	$N(CH_3)_2$	A	72	Maleate	Ac	96–97	$C_{16}H_{22}N_2O_6$
24	$(CH_2)_3$	CH_2	$N(C_2H_5)_2$	A	69	Citrate	Ac–Et	101–102 dec	$C_{20}H_{30}N_2O_9$
25	$(CH_2)_3$	CH_2		A	75	Maleate	Ac	109–110	$C_{18}H_{24}N_2O_6$
26	$(CH_2)_3$	CH_2		A	88	HCl	Ac–Et	207–209	$C_{15}H_{23}ClN_2O_2$
27	CH_2	$(CH_2)_2$	$N(C_2H_5)_2$	B	37	Citrate	Ac–EA	95–100 dec	$C_{19}H_{28}N_2O_9$
28	CH_2	$(CH_2)_2$		B	24	Base	P	75–76	$C_{14}H_{20}N_2O_2$
29	$CHCH_3$	$C(CH_3)_2$		B	74	Base	P	66–67	$C_{15}H_{22}N_2O_2$
30	$C(CH_3)_2$	$C(CH_3)_2$		B	80	Base	P	53–54	$C_{16}H_{24}N_2O_2$

[a] Ac, Me_2CO; Aq, H_2O; B, C_6H_6; E, Et_2O; EA, EtOAc; Et, EtOH; M, MeOH; P, petroleum ether. [b] All compounds were analyzed for C, H, N. The analytical results obtained for those elements were within ±0.4% of the theoretical value.

TABLE II

N-(*t*-AMINOALKYNYL)-SUBSTITUTED α-PHENYLSUCCINIMIDES AND MALEIMIDES

RYC≡CCH$_2$Am

Compd	R	Y	Am	Method of prepn	Yield, %	Derivative	Recrystn solvent[a]	Mp or bp (mm), °C	Formula[b]
31		$C(CH_3)_2$	$N(C_2H_5)_2$	A	66	Citrate	Ac–E	87–90 dec	$C_{26}H_{34}N_2O_9$
32		$(CH_2)_3$		A	66	Base		215 (0.3)	$C_{20}H_{24}N_2O_2$
33		CH_2	$N(C_2H_5)_2$	A	32	Oxalate	Et	147–148 dec	$C_{14}H_{18}N_2O_6$
34		$(CH_2)_2$	$N(C_2H_5)_2$	A	26	Citrate	Ac–Et	75–80 dec	$C_{19}H_{26}N_2O_9$

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I.

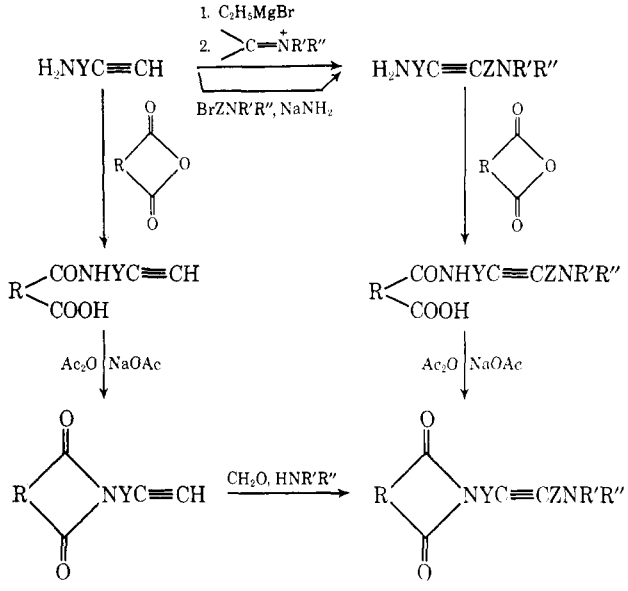
TABLE III

PHARMACOLOGICAL RESULTS

	In vivo dose (μmoles/kg) in mice required to produce		Effect on isolated guinea pig ileum	
Compd	Oxotremorine blockade	Mydriasis	Type of effect[a]	*Concentration* at which effect is seen (μmoles/l.)
1	A	74	A	~0.1
2	22	88	B	~10
3	180	740	B	~10
4	8.9	28	B	~10
5	14	>3000	G	>250
6	120	180	G	>250
7	47	130	G	>250
8	>500	410	G	>250
9	17	63	E	1.3
10	1.2	7.3	C	0.41
11	0.97	4.9	E	0.77
12	32	190	B	~10
13	30	31	E	1.0
14	3.8	7.7	E	0.84
15	4.1	4.2	E	0.65
16	4.7	11	E	0.28
17	41	310	D	~10
18	6.0	120	B	~100
19	94	370	B	~10
20	4.5	13	C	2.6
21	53	250	G	>250
22	25	270	G	>250
23	26	500	C	81
24	89	210	E	22
25	32	230	C	80
26	18	>670	C	180
27	43	130	B	~0.1
28	150	>810	G	>250
29	48	510	D	~1
30	A	360	B	~10
31	39	120	F	35
32	56	>140	G	>250
33	12	>400	E	1.5
34	>1000	1100	G	>250
Atropine	2.1	0.64	E	0.00026

[a] A, agonist; B, partial agonist; C, potentiates in low concentrations; competitive antagonist at higher concentrations; D, stimulates rhythmic contractions; E, competitive antagonist; F, noncompetitive antagonist; G, inactive at 2.5×10^{-4} *M*.

SCHEME I



significant correlation ($r = 0.682$, $P < 0.001$) between the two types of activity (analyzed as the logarithms of dose required), suggesting that a similar pattern of pharmacological specificity underlies both. Compound **1** had actions *in vivo* like oxotremorine, producing tremor, rigidity, analgesia, and marked parasympathomimetic effects. It also behaved as an agonist on the isolated guinea pig ileum and in each case its activity was about one-forth of that shown by oxotremorine. Compound **30** failed to block oxotremorine at the highest dose tested (400 μmoles/kg) but also produced weak agonistic effects *in vivo* at this dose.

Several types of activity were displayed on the isolated guinea pig ileum, ranging from pure agonist to competitive or noncompetitive antagonist. In addition, several compounds appeared to be devoid of activity at the highest concentration tested ($2.5 \times$

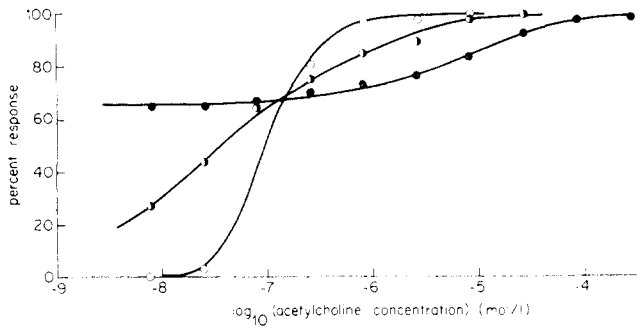


Figure 1.—Dose–response curves of acetylcholine on isolated guinea pig ileum alone (○) and in the presence of **4** at 2.5×10^{-5} M (◑) and 2.5×10^{-4} M (●).

10^{-4} M). Those which are listed as partial agonists displayed agonistic properties in low concentrations, although not producing a maximum contraction (relative to acetylcholine) at any concentration. At low concentrations the effect summed with that of acetylcholine, while at higher levels the action of acetylcholine was antagonized (Figure 1). Other compounds exhibited no agonistic properties, but showed a clear-cut potentiation (2–10 times) of the effect of acetylcholine at low concentrations, and behaved as competitive antagonists when the concentration was raised. Similar behavior was exhibited towards carbachol and oxotremorine, and it is therefore not attributable to cholinesterase inhibition. A detailed study of this phenomenon is in progress and will be reported separately. Two compounds in the series (**17** and **29**) induced rhythmic contractions which precluded obtaining dose–response curves to acetylcholine. In both cases this effect was seen at relatively low concentrations below which the action of acetylcholine was unaltered; it became more marked as the concentration was increased.

For the 13 compounds which behaved as competitive antagonists on the isolated ileum, their potency in this test was significantly correlated with both tremorolytic and mydriatic activity; these activities were also highly correlated (Table IV). Of the partial

TABLE IV

CORRELATION OF PHARMACOLOGICAL ACTIVITIES[a]

	Mydriasis		Oxotremorine blockade	
	r[b]	P	r[b]	P
Mydriasis			0.788	<0.01
			0.566	>0.05
Acetylcholine antagonism	0.823	<0.001	0.666	<0.02
	0.650	<0.05	0.049	>0.2

[a] Only compounds in categories C and E are included in this correlation analysis. Data are analyzed as the logarithms of concentrations. [b] Upper value of "r" is the total correlation coefficient; lower value is the partial correlation coefficient.

correlation coefficients, only that between mydriatic activity and acetylcholine antagonism was significant. A similar pattern was observed in a previous series of compounds[3] and the present data further support the conclusion that *in vitro* antagonism of acetylcholine on the isolated ileum is an excellent predictor of *in vivo* parasympatholytic activity but is unreliable in predicting ability to antagonize the central actions of oxotremorine, in which distributional factors are clearly important. The relative inactivity of **27** as a tremorolytic agent but its high activity *in vitro* may reasonably be explained by its stronger basicity owing to the greater distance between the amine N and the acetylenic bond, since base strength is known to be inversely correlated with ability to penetrate into the central nervous system. The only other compound with two CH_2 groups between the acetylenic bond and the amine N was inactive *in vitro*, and its weak central antagonism of oxotremorine may have been nonspecific.

It is difficult to find generalizations relating structure to activity which appear valid for the entire series of compounds. The most active members are those having pyrrolidine or piperidine as the amine components, with the parent 2-butyne chain branched with one or two Me groups or lengthened with an additional CH_2 group between the acetylenic bond and the imide N. Activity is generally less in compounds containing more bulky amine groups including diethylamine. Compound **21** is an exception to this generalization, and no explanation can be offered for its anomalously weak activity.

It has previously been observed that among analogs of oxotremorine, a dimethylamine or pyrrolidine group tends to confer agonistic properties on the molecule.[4] A similar conclusion may be drawn from the present series. If potentiation of ACh on the isolated ileum at low concentrations, or stimulation of rhythmic activity, are regarded as a manifestation of weak partial agonism, then 13 of the 14 active compounds with an *N*-methyl or pyrrolidine group show evidence of agonistic properties, the sole exception being **14**, which was a potent competitive antagonist. Of the remaining three compounds showing agonistic effects (**19**, **26**, **27**), a common feature was extension of the butyne chain by one or two CH_2 groups. These data suggest that a sterically unhindered basic N atom and conformational flexibility in the intermediate chain both favor agonistic activity; all of the 5 active compounds with both these features possessed this property (**17**, **18**, **20**, **23**, **25**).

The specificity of these compounds for anticholinergic activity on the CNS as opposed to the periphery can be assessed by the ratio of mydriatic to tremorolytic doses. As stated previously, this ratio is greater than unity for all active compounds in this series, in contrast to atropine. Certain members of the series have a remarkably high ratio, of which the highest (**5**) is over 200. Since oxotremorine-induced tremor can be suppressed nonspecifically, *e.g.*, by barbiturates, these very high ratios cannot be accepted alone as evidence of a selective central anticholinergic action, but if high anticholinergic activity is also observed *in vitro*, this would favor an anticholinergic mechanism for the tremorolytic effect. On this basis **10**, **11**, **20**, and **33** appear to be of particular interest as central anticholinergics and merit further investigation.

There is some indication that a high ratio of tremorolytic to mydriatic activity tends to be associated with agonistic properties on the isolated guinea pig ileum. If those compounds showing evidence of agonistic properties (groups B, C, and D in Table III)

(4) R. Dahlbom, B. Karlén, Å. Lindquist, R. George, and D. J. Jenden, *Acta Pharm. Suecica*, **4**, 247 (1967).

TABLE V

N-ALKYNYL-SUBSTITUTED SUCCINAMIC AND MALEAMIC ACIDS

HOCORCONHYC≡CH

R	Y	Yield, %	Recrystn solvent[a]	Mp, °C	Formula[b]
CH_2CH_2	$CHCH_3$	85	B	111–113	$C_8H_{11}NO_3$
CH_2CH_2	$C(CH_3)_2$	89	B	119–121	$C_9H_{13}NO_3$
CH_2CH_2	CH_2CH_2	88	Ac	105–106	$C_8H_{11}NO_3$
CH_2CH_2	$(CH_2)_3$	89	Ac	68–69	$C_9H_{13}NO_3$
$CH(C_6H_5)CH_2$[c]	$C(CH_3)_2$	79	Ac	133–134	$C_{15}H_{17}NO_3$
$CH(C_6H_5)CH_2$[c]	$(CH_2)_3$	100[d]			$C_{15}H_{17}NO_3$
CH=CH	CH_2	69	M–E	142–143	$C_7H_7NO_3$
CH=CH	$C(CH_3)_2$	90	B–Ac	150–151	$C_9H_{11}NO_3$
CH=CH	CH_2CH_2	59	B–Ac	93–96	$C_8H_9NO_3$[e]

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] Two isomers may be formed on the treatment of an amine with α-phenylsuccinic anhydride. It has been shown [R. Anschütz, *Justus Liebigs Ann. Chem.*, **354**, 117 (1907)] that reaction of ammonia with α-phenylsuccinic anhydride yielded the β-amide α-acid, the weaker carboxylic group being attached to N. We therefore propose the structure $HOCOCH(C_6H_5)CH_2CONHYC{\equiv}CH$ for the above compounds. On ring closure the two possible isomers give the same *N*-alkynylimide. [d] Not crystalline; the crude product which was not analyzed, was cyclized directly to the corresponding succinimide. [e] C: calcd, 57.48; found, 56.94. N: calcd, 8.38; found, 7.81.

are compared to pure antagonists (groups E and F), the (log) ratios of the former are significantly greater than the latter ($t_{22} = 2.125$, $P < 0.05$). The mean ratio is also higher for compounds containing an *N*-Me or a linearly extended butyne chain than for compounds containing neither, as might be expected from the structural correlations already pointed out; only in the first group was the difference statistically significant ($t_{22} = 2.150$, $P < 0.05$; $t_{22} = 1.872$, $P > 0.05$, respectively). It may be inferred that the central:peripheral activity ratio is more probably related to the agonistic properties of the compound *per se* than to the structural features which *inter alia* determine them. We suggest that in the case of compounds showing partial agonistic properties, differences in efficacy may contribute significantly to a selective action on central muscarinic receptors, in addition to differences in affinity and distribution.

Experimental Section

Melting points were taken in open capillary tubes in an electrically heated metal block using calibrated Anschütz thermometers. Ir spectra were run on a Perkin-Elmer 237 spectrophotometer equipped with a grating monochromator, using KBr discs. Microanalyses were carried out in the laboratories of Dr. A. Bernhardt, Mülheim, West Germany, or by AB Analytica, Sollentuna, Sweden.

Starting Materials.—3-Amino-1-butyne,[5] 3-amino-3-methyl-1-butyne,[6] 4-amino-1-butyne,[7] 5-amino-1-pentyne,[7] *N*-propargylsuccinimide,[3] and α-phenylsuccinic anhydride[8] were prepared according to methods described in the literature.

***N*-Alkynyl-Substituted Succinamic and Maleamic Acids (Table V).**—A solution of the appropriate acetylenic amine (0.1 mol) in Me_2CO (25 ml) was added dropwise to a refluxing solution of succinic or maleic anhydride (0.1 mol) in Me_2CO (50 ml). The stirred mixture was refluxed for 1 hr and the solvent was then removed. The crystalline residue was purified by recrystallization.

***N*-Alkynyl-Substituted Succinimides and Maleimides (Table VI).**—A mixture of the appropriate *N*-alkynyl-substituted succinamic or maleamic acid (0.1 mol), Ac_2O (50 ml), and anhyd NaOAc (5 g) was stirred on a boiling water bath for 1 hr and then cooled. Ice–water (150 ml) was added, and the mixture was stirred for 2 hr. The mixture was neutralized with solid K_2CO_3 under vigorous stirring and then extracted with six 50-ml portions of Et_2O. The extract was dried (K_2CO_3), the solvent was removed and the residue was purified by recrystallization or distillation *in vacuo*. The ir spectra of the compounds in Table VI showed absorption bands at 3280–3300 (≡CH), 2100–2130 (C≡C), 1770–1790, and 1700–1730 (C=O) cm^{-1}.

***t*-Aminoalkynylamines (Table VII).**—The preparation of these compounds is exemplified by the following typical procedures.

Method C. 5-Diethylamino-2-pentynylamine.—To a solution of $NaNH_2$ in liquid NH_3, prepared by standard methods from Na (9.66 g, 0.42 mol) and liquid NH_3 (1500 ml), propargylamine·HCl (18.3 g, 0.2 mol) was added portionwise during 10 min under stirring. After stirring for 0.5 hr, β-diethylaminoethyl bromide·HBr (26.1 g, 0.1 mol) was added in portions during 1 hr. The reaction mixture was stirred for 3 hr during which time the NH_3 gradually evaporated. When the volume had decreased to about 400 ml, anhyd Et_2O (400 ml) was added and the mixture was stirred at room temp for 1 hr. After addition of NH_4Cl (1.1 g) the mixture was left overnight. The salts were filtered off and the ppt was washed thoroughly with Et_2O. The filtrate and the Et_2O washings were combined and the solvent was removed at normal pressure. The excess of propargylamine was recovered by distillation and the residue was distd *in vacuo*.

Method D. 4-Pyrrolidino-1,1,4,4-tetramethyl-2-butynylamine.—To a solution of EtMgBr, prepared from Mg (2.9 g, 0.12 mol) and EtBr (12.8 g, 0.117 mol), in Et_2O (100 ml) was added dropwise 3-amino-3-methyl-1-butyne (7.2 g, 0.087 mol). The reaction mixture, protected from moisture, was stirred overnight at room temp, and *N*-isopropylidenepyrrolidinium perchlorate[9] (18.4 g, 0.087 mol) was added in portions. The mixture was then refluxed for 2 hr and satd NH_4Cl solution (10 ml) was added in order to destroy excess of Grignard reagent. The aq phase was separated and the Mg was pptd by the addition of a satd NaF solution. MgF_2 was filtered off, the filtrate was made alkaline with 5 *M* NaOH and the mixture extracted thoroughly with Et_2O. After drying over K_2CO_3 the solvent was removed and the residue distd *in vacuo*.

***N*-(*t*-Aminoalkynyl)-Substituted Succinamic Acids (Table VIII).**—These compounds were prepared from the *t*-aminoalkynylamines listed in Table VII and succinic anhydride in the same way as described for the compounds in Table V.

For data on the ***N*-(*t*-aminoalkynyl)succinimides and maleimides** see Tables I and II.

Method A. Mannich Condensation of an *N*-Alkynylimide.—A mixture of the *N*-alkynyl-substituted succinimide or maleimide (0.03 mol), the appropriate secondary amine (0.036 mol), paraformaldehyde (0.036 mol), glacial HOAc (3.5 ml), and CuCl (0.06 g) in peroxide-free dioxane (10 ml) was stirred at room temp for 5 min. The temp was then raised to 90° and kept there for 1 hr. After cooling, H_2O (100 ml) was added, the reaction mixture was acidified to pH 1 with 5 *M* HCl and extracted with two 50-ml portions of Et_2O. The aq phase was made alk with 1 *M* Na_2CO_3 and extracted with six 50-ml portions of $CHCl_3$. The $CHCl_3$ extract was dried (K_2CO_3) and the solvent removed *in vacuo*. The residue was dissolved in a small

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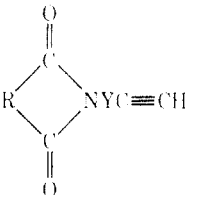
(6) G. F. Hennion and C. V. DiGiovanna, *J. Org. Chem.*, **30**, 2645 (1965).
(7) J. L. Dumont, J. Tohier, and P. Cadiot, *C. R. Acad. Sci.*, **256**, 3146 (1963).
(8) C. A. Miller and L. M. Long, *J. Amer. Chem. Soc.*, **73**, 4895 (1951).
(9) N. J. Leonard and J. V. Paukstelis, *J. Org. Chem.*, **28**, 3021 (1963).

TABLE VI

N-ALKYNYL-SUBSTITUTED SUCCINIMIDES AND MALEIMIDES



R	Y	Yield, %	Recrystn solvent[a]	Mp, °C	Bp (mm), °C	Formula
CH_2CH_2	$CHCH_3$	90	P–B	77–79		$C_8H_9NO_2$
CH_2CH_2	$C(CH_3)_2$	82	P–B	45–46		$C_9H_{11}NO_2$
CH_2CH_2	CH_2CH_2	87		40[c]	112 (1.5)	$C_8H_9NO_2$
CH_2CH_2	$(CH_2)_3$	87		35–36[c]	100 (0.4)	$C_9H_{11}NO_2$
$CH_2CH(C_6H_5)$	$C(CH_3)_2$	84	Et–Aq	75–76		$C_{15}H_{15}NO_2$
$CH_2CH(C_6H_5)$	$(CH_2)_3$	75	Ac–E	55–56	169 (0.7)	$C_{15}H_{15}NO_2$
CH=CH	CH_2	61		52–53[c]	88 (2)	$C_7H_5NO_2$
CH=CH	$C(CH_3)_2$	58			45 (0.2)	$C_9H_9NO_2$
CH=CH	CH_2CH_2	63			77 (0.1)	$C_8H_7NO_2$

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] Melting point of solidified oil.

TABLE VII

t-AMINOALKYNYLAMINES

$H_2NYC{\equiv}CZAm$

Y	Z	Am	Method of prepn	Yield, %	Bp (mm), °C	Formula[a]
CH_2	CH_2CH_2	$N(C_2H_5)_2$	C	39	55 (0.3)	$C_9H_{18}N_2$
CH_2	CH_2CH_2		C	32	94 (0.6)	$C_{10}H_{18}N_2$[b]
$CHCH_3$	$C(CH_3)_2$		D	52	58 (0.3)	$C_{11}H_{20}N_2$[c]
$C(CH_3)_2$	$C(CH_3)_2$		D	36	56 (0.3)	$C_{12}H_{22}N_2$

[a] See footnote *b*, Table I. [b] This compound was not analytically pure, but it could be satisfactorily used in the next step. [c] The dihydrochloride had mp 263–265° dec (from EtOH–Et_2O). *Anal.* ($C_{11}H_{22}Cl_2N_2$) C, H, N.

TABLE VIII

N-(*t*-AMINOALKYNYL)SUCCINAMIC ACIDS

$HOCOCH_2CH_2CONHYC{\equiv}CZAm$

Y	Z	Am	Yield, %	Recrystn solvent[a]	Mp, °C	Formula[b]
CH_2	CH_2CH_2	$N(C_2H_5)_2$	100[c]			$C_{13}H_{22}N_2O_3$
CH_2	CH_2CH_2		100[c]			$C_{14}H_{22}N_2O_3$
$CHCH_3$	$C(CH_3)_2$		85	Et–E	138–140	$C_{15}H_{24}N_2O_3$
$C(CH_3)_2$	$C(CH_3)_2$		86	Et–Ac	173–174	$C_{16}H_{26}N_2O_3$

[a] See footnote *a*, Table I. [b] See footnote *b*, Table I. [c] Viscous oil; the crude product, which was not analyzed, was cyclized directly to the corresponding succinimide.

volume of Et_2O, passed through an Al_2O_3 column, and the column was eluted with the same solvent. The Et_2O was evaporated and the compound isolated either as the free base or in the form of a suitable salt.

Method B. Cyclization of an *N*-(*t*-Aminoalkynyl)-Substituted Succinamic Acid.—The cyclization was carried out in the same manner as described for the *N*-alkynyl-substituted imides of Table VI. The crude products were purified as described above. The ir spectra of the compounds in Table I and II all showed two absorption bands in the C=O stretching region, one sharp of medium strength at 1760–1790 cm^{-1} and one strong and broad at 1690–1720 cm^{-1}.

Pharmacology. Methods.—Antagonism of tremor induced by oxotremorine was estimated using an electronic device to achieve an objective measurement of the tremor intensity.[10] Individual mice are placed in a light plastic bowl resting in the cone of a Quam 16A6PA moving coil loudspeaker. An amplifier with a sharp bandpass from 20.0–24.6 Hz is used to amplify selectively the signals generated by tremor induced by oxotremorine, while signals generated by random movement are rejected. The amplified signal is rectified and integrated over periods of 1 min with an integrator of essentially infinite time constant which is reset automatically at the end of 1 min. Integrator output was recorded on a potentiometric recorder. The total response during a 3-min period following the intravenous injection of oxotremorine was found to be a satisfactory measure of the drug response. This was recorded as positive or negative depending on whether it exceeded a predetermined threshold which was identical for all the experiments and corresponded to the mean response to 180 μg/kg of oxotremorine. The use of an all-or-none measurement was found to result in little or no

(10) R. W. Silverman and D. J. Jenden, *J. Appl. Physiol.*, in press.

loss of information and greatly simplified the subsequent calculations.

Each compound was screened initially to determine the dose range in which it was effective. Four linearly spaced doses including zero were then chosen, and estimates were made of the median effective doses of oxotremorine when administered intravenously 15 min after intraperitoneal injection of the test compound. The "up-and-down" method for small samples described by Dixon[11] was employed to estimate the median effective doses, using a logarithmic series of doses of oxotremorine with a spacing of 0.1 in the $\log_{10}$ dose scale and a nominal sample size of 5. When the median effective dose of oxotremorine was plotted against the dose of the test compound used for premedication, most compounds gave results characteristic of competitive antagonism (Figure 2). The intercept on the abscissa provides an estimate of the dose of antagonist which doubles the median effective dose of oxotremorine, and was determined by a weighted regression analysis to allow for the fact that the standard error of the estimate is constant on the log-dose scale.[11] Some compounds showed no significant linear regression, and are recorded as inactive at the doses tested.

Mydriatic activity was estimated by measuring the pupillary diameter of mice in groups of 5, both before and 15–20 min after the i.p. injection of the test compound. The measurements were made under standard lighting conditions with a binocular microscope fitted with calibrated eyepiece. The mydriatic dose was estimated graphically as that required to double the pupil size relative to the control.

Acetylcholine antagonism was measured in isolated guinea pig ileal strips suspended in oxygenated Krebs solution at 38°. Contractions were recorded isotonically at 1-g tension, using a Collins displacement transducer and potentiometric recorder. A series of cumulative dose–response curves was obtained using acetylcholine only; these were then repeated in the presence of a test compound at concentrations increasing in the ratio 1:3:10:30.... The preparation was allowed to equilibrate with each new concentration for 30 min before the ACh dose–response curve was obtained.

(11) W. J. Dixon, *J. Amer. Statist. Ass.*, **60**, 967 (1965).

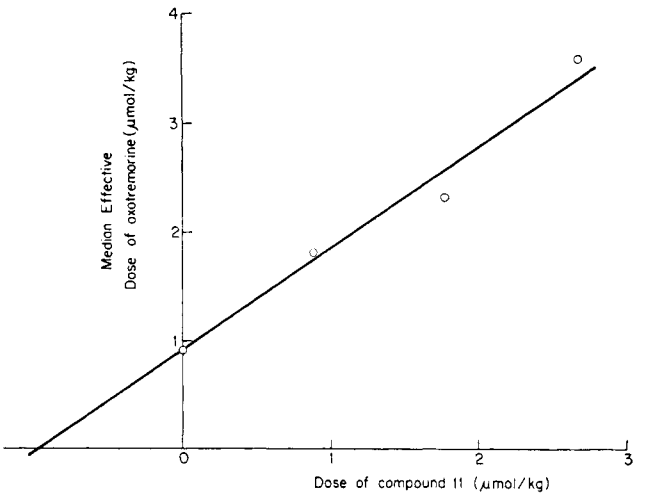


Figure 2.—Median effective dose of oxotremorine, estimated by the "up and down" method for small samples, plotted against dose of **11** used for premedication, showing typical competitive antagonism isobole.

In the case of competitive antagonists, the Ach concentration giving a 50% response was estimated by interpolation at each concentration of antagonist, and the antagonist concentration producing a twofold block of acetylcholine was estimated graphically. For noncompetitive antagonism, the effective concentration is recorded as that which reduces the maximum response to ACh by 50%.

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Modified Cardenolides. V.[1a] Replacement of the C-17 Lactone Substituent by Alkylating Groups[1b]

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The synthesis of a variety of potential alkylating substances derived from cardiac aglycones is described. For example, ozonolysis of digitoxigenin acetate gives 3β,14,21-trihydroxy-5β,14β-pregnan-20-one 3-acetate which is converted into the 21-chloroacetate and 21-iodoacetate. Biological evaluation in the usual cat toxicity test shows that some of these compounds have appreciable cardiotoxicity, but tests for cardiotonic activity are negative.

The discovery of the cardioactivity of 3β,5β,14β,19,-21-pentahydroxypregnan-20-one 3,19-diacetate 21-iodoacetate (**13**) in cats[1a] prompted us to prepare a number of related structures to investigate this structural lead systematically. The basis for the design of these compounds was the hypothesis that alkylation of an essential nucleophilic group on the receptor is required for drug action, and that the α,β-unsaturated lactone group in cardenolides, and the iodoacetate function in **13**, respectively, perform this function.

(1) (a) For a preliminary communication containing a small part of this work, see paper IV in this series: M. E. Wolff, W. Ho, and H. H. Chang, *J. Pharm. Sci.*, **57**, 1450 (1968); (b) This research was supported in part by Public Health Service Grant (HE-09578) from the National Heart Institute, U. S. Public Health Service.

It is known that 17α-lactones derived from cardenolides are inactive in the usual tests for cardioactivity, and it was desired to determine whether this loss of activity would also be seen in the cardiotoxic C-21 substituted alkylating agents.

The characteristic C/D *cis* fusion of cardiac aglycones is considered to be important for biological activity. To obviate the necessity for the synthesis of C/D *cis* steroids from ordinary C/D *trans* steroids, the naturally occurring cardiac glycosides strophanthin and digitoxin were used as starting materials in planning the syntheses. Without disturbing the steroid nucleus, the butenolide ring was replaced with functional groups which were capable of reacting with SH groups.