Bicyclic Homologs of Piperazine. Synthesis of Pharmacologically Active 8-Methyl-3,8-diazabicyclo[3.2.1]octanes. III.[1,2]

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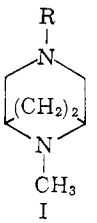
A number of 3-alkyl, 3-aralkyl, and 3-acyl substituted 8-methyl-3,8-diazabicyclo[3.2.1]octanes were synthesized for pharmacological screening. 3-Methyltropoyl-8-methyl-3,8-diazabicyclo[3.2.1]octane was found highly active in antagonizing the effect of acetylcholine. This activity was shown to reside largely in the (−) enantiomorph. Preliminary pharmacological results show substantial analogy of 8-methyl-3,8-diazabicyclo-[3.2.1]octane derivatives with the corresponding tropane derivatives. By contrast, the analogy with N-methylpiperazine seems rather limited.

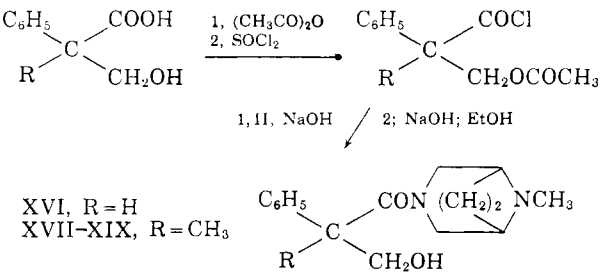
As part of a systematic investigation on substituted 3,8-diazabicyclo[3.2.1]octanes,[1,2] we wish to report the synthesis and preliminary pharmacological examination of a series of 3-substituted 8-methyl-3,8-diazabicyclo-[3.2.1]octanes of the general formula I. Some of the reported compounds were prepared because of the analogy of the 3,8-diazabicyclo[3.2.1]octane nucleus with piperazine. The pharmacological activity of piperazine derivatives is well documented; therefore it seemed of interest to compare the compounds of both groups in order to study the influence of the endoethylenic bridge in this structure. A further reason of investigation was given by the structural analogy of 8-methyl-3,8-diazabicyclo[3.2.1]octane [II, (I, R = H)] with tropane, whose derivatives show, as known, interesting pharmacological properties.



I

Chemistry.—A first group of products prepared is listed in Table I and concerns cinnamyl and benzhydryl derivatives and esters of 3-hydroxyethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane [III (I, R = CH_2CH_2OH)] with representative aromatic acids. Most of these products were obtained by conventional methods. The compounds IV–IX (Table I) were prepared by refluxing in benzene two moles of II with one mole of the appropriate aralkyl chlorides. Cinnamyl chloride is commercially available; the other substituted cinnamyl chlorides, which previously were unknown, were synthesized by alkaline condensation of acetaldehyde with substituted benzaldehydes to yield the corresponding cinnamaldehydes,[3] which were reduced, as described,[4] with sodium borohydride to substituted cinnamyl alcohols and eventually transformed with thionyl chloride in chloroform solution to the desired aralkyl chlorides. The ultraviolet spectra of these cinnamyl chlorides, as well as the starting cinnamyl alcohols, show a λ_{max} near 250 mμ, thus demonstrating the retention of the cinnamyl structure during the reaction with thionyl chloride. The known *o*- and *p*-chlorobenzhydryl chlorides[5] were obtained through the intermediate *o*- and *p*-chlorobenzhydrols prepared from *o*- and *p*-chlorobenzaldehyde by a Grignard reaction with phenylmagnesium bromide. The synthesis of compounds X (I, R = *p*-$O_2NC_6H_4COOCH_2CH_2$–) and XII [I, R = $(C_6H_5)_2CHCOOCH_2CH_2$–] started from III which was prepared by refluxing II with ethylene oxide in methanol. The product obtained (III) finally was condensed with *p*-nitrobenzoyl and diphenylacetyl chlorides in the presence of triethylamine yielding X and XII, respectively. Compound X was reduced catalytically with Pd/C to the corresponding amino compound XI.

A series of 3-N-acyl derivatives of 8-methyl-3,8-diazabicyclo[3.2.1]octane also was prepared; they are listed in Table II. The synthesis of these compounds generally was carried out by condensing II with the appropriate acid anhydride or chloride in aqueous alkaline medium by the Schotten–Baumann method. The 3-N-tropoyl and α-methyltropoyl derivatives of II (XVI–XIX) were synthesized by the sequence shown.

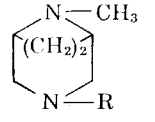


d,l-α-Methyltropic acid was prepared according to the method of Testa, *et al.*,[6] by diazotization of β-amino-α-methyl-α-phenylpropionic acid, and alkaline hydrolysis of the resulting lactone. The resolution of *d,l*-α-methyltropic acid was carried out[7] by fractional crystallization of its quinine salt in acetone; this procedure was a considerable improvement over an earlier method.[8] The tropic acids in the racemic and optically active

(1) G. Cignarella and G. G. Nathansohn, *J. Org. Chem.*, **26**, 1500 (1961).
(2) G. Cignarella, G. G. Nathansohn and E. Occelli, *J. Org. Chem.*, **26**, 2747 (1961).
(3) S. G. Waley, *J. Chem. Soc.*, 2008 (1948).
(4) G. Carrara, E. Ettorre, F. Fava, G. Rolland, E. Testa and A. Vecchi, *J. Am. Chem. Soc.*, **76**, 4391 (1954).
(5) J. F. Norris and C. Banta, *J. Am. Chem. Soc.*, **50**, 1807 (1928).
(6) E. Testa, L. Fontanella, G. F. Cristiani and F. Fava, *Ann. Chem.*, **619**, 47 (1958).
(7) We are gratefully indebted to Mr. G. F. Cristiani for the development of this procedure.
(8) G. Melone, A. Vecchi, G. Pagani and E. Testa, *J. Org. Chem.*, **25**, 859 (1960).

TABLE I

3-ARALKYL-8-METHYL-3,8-DIAZABICYCLO[3.2.1]OCTANES

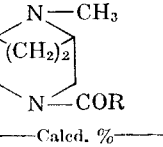


Cpd.	R	B.p. or m.p. °C.	(mm.)	Cryst. solvent	Yield %	Formula	Calcd., % C	H	N	Cl	Found, % C	H	N	Cl
III	$HOCH_2CH_2$	104–105	1	...	94.5	$C_9H_{18}N_2O$	63.48	10.65	16.45		63.58	10.90	16.25	
IV	$C_6H_5CH{=}CHCH_2$	120	0.6	...	68	$C_{16}H_{22}N_2$	79.29	9.15	11.56		79.40	9.40	11.70	
V	o-$ClC_6H_4CH{=}CHCH_2$	209–211	...	i-PrOH	45	$C_{16}H_{21}ClN_2{\cdot}2HCl$			8.01	30.41			7.88	30.37
VI	p-$ClC_6H_4CH{=}CHCH_2$	240 dec.	...	EtOH	51	$C_{16}H_{21}ClN_2{\cdot}2HCl$			8.01	30.41			7.76	30.19
VII	$(C_6H_5)_2CH$	265 dec.	...	EtOH	50	$C_{20}H_{24}N_2{\cdot}2HCl$			7.66	19.41			7.80	19.27
VIII	o-$ClC_6H_4CH(C_6H_5)$	283 dec.	...	EtOH–Et_2O	35	$C_{20}H_{23}ClN_2{\cdot}2HCl$	60.08	6.30	7.00	26.60	59.98	6.64	6.97	26.64
IX	p-$ClC_6H_4CH(C_6H_5)$	176–179	...	EtOH–Et_2O	60	$C_{20}H_{23}ClN_2{\cdot}2HCl$	60.08	6.30	7.00	26.60	60.01	6.51	7.12	26.14
X	p-$O_2NC_6H_4COOCH_2CH_2$	56–60	...	Petr. ether	87	$C_{16}H_{21}N_3O_4$	60.17	6.62	13.15		60.50	6.90	13.25	
XI	p-$H_2NC_6H_4COOCH_2CH_2$	219–221	...	EtOH	70.4[a]	$C_{16}H_{23}N_3O_2{\cdot}2HCl$			11.59	19.57			11.40	19.35
XII	$(C_6H_5)_2CHCOOCH_2CH_2$	162–165[b]	...	...	67	$C_{23}H_{28}N_2O_2{\cdot}2HCl$			6.40	16.21			6.75	16.42

[a] From X. [b] Obtained in a pure state.

TABLE II

3-ACYL-8-METHYL-3,8-DIAZABICYCLO[3.2.1]OCTANES

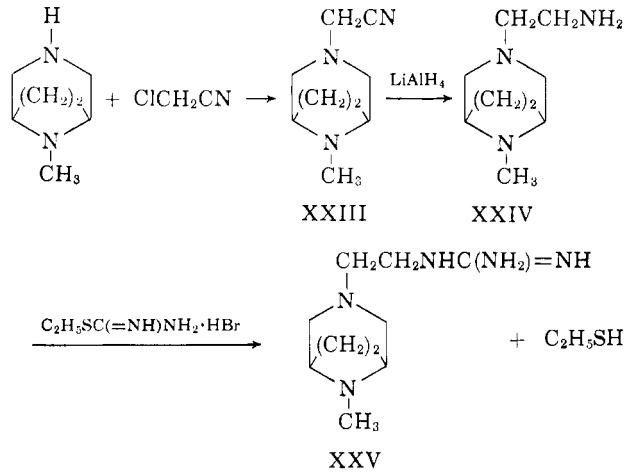


Cpd.	R	B.p. or m.p. °C.	(mm.)	Cryst. solvent	Yield. %	Formula	Calcd. % C	H	N	Cl	Found, % C	H	N	Cl
XIII	C_2H_5	110–113	0.8		91.4	$C_{10}H_{18}N_2O$	65.89	9.95	15.37		65.65	10.01	15.70	
XIV	C_6H_5	158–160	1		68	$C_{14}H_{18}N_2O$	73.01	7.87	12.16		72.96	8.00	11.90	
XV	$C_6H_5CH{=}CH$	120–121		EtOH	95	$C_{16}H_{20}N_2O$	74.96	7.86	10.92		74.50	8.01	10.65	
XVIa	$C_6H_5CH(CH_2OH)$	78–80[a]			54	$C_{16}H_{22}N_2O_2$			10.21				10.10	
XVIb		195–197[a]				$C_{16}H_{22}N_2O_2{\cdot}HCl$			9.01	11.40			8.90	11.40
XVII	dl-$C_6H_5C(CH_3)CH_2OH$	95		H_2O	58	$C_{17}H_{24}N_2O_2{\cdot}H_2O$	66.63	8.55	9.14		66.70	8.71	8.95	
XVIII	d-$C_6H_5C(CH_3)CH_2OH$	76–78		H_2O	60	$C_{17}H_{24}N_2O_2{\cdot}H_2O$	66.63	8.55	9.14		66.45	8.66	8.93	
XIX	l-$C_6H_5C(CH_3)CH_2OH$	177–180[a]			57	$C_{17}H_{24}N_2O_2{\cdot}HCl$			8.62	10.91			8.60	10.73

[a] Obtained in a pure state.

forms were converted to their O-acetyl chlorides, and allowed to react with II. Partial hydrolysis (O-deacetylation) of the resulting esters/amides yielded the products, XVI–XIX.

Eleven other 8-methyl-3,8-diazabicyclo[3.2.1]octane derivatives also were synthesized (see Table III). The Mannich bases XX–XXII were obtained as usual by refluxing equimolar amounts of II with formaldehyde and an appropriate imide in ethanol. 3-Guanidinoethyl - 8 - methyl - 3,8 - diazabicyclo[3.2.1]octane (XXV) was prepared starting from XXIII which was obtained by condensation of II with chloroacetonitrile in benzene. Reduction of XXIII with $LiAlH_4$ in tetrahydrofuran yielded XXIV, which condensed in chloroform with S-ethylisothiuronium bromide.[9] A mixture XXV of mono- and dihydrobromides was thus obtained.



Diazotization of II led to the 3-nitroso derivative (XXVI), which by reduction with $LiAlH_4$ in tetrahydro-furan, gave the corresponding 3-amino compound (XXVII). Finally, the 3-carbethoxy- (XXVIII), 3-N-diethylcarbamoyl- (XXIX) and 3-phenylcarbamoyl- (XXX) derivatives of II were prepared by standard procedures.

Pharmacology.—With the exception of some analogies with known compounds the majority of the 3-substituted 8-methyl-3,8-diazabicyclo[3.2.1]octanes could not be correlated with any particular pharmacological activity. Therefore extensive preliminary screening was carried out concerning acute toxicity, behavioral effects and anticonvulsant, analgesic, local anesthetic, anticholinergic, spasmolytic, antihistaminic and diuretic actions. Also, their systemic effects on blood pressure and respiration were investigated, as was their ability to inhibit certain enzyme systems *in vitro*.

Materials and Methods.—The approximate acute toxicity and the effects on behavior were studied in mice by intraperitoneal administration of the drugs at doses of 10, 30, 60, 100, 300 and 1000 mg./kg. Three animals were used for each dose level. The LD_{50} was estimated by the method of Litchfield and Wilcoxon.[10] The anticonvulsant action of the compounds was determined in mice by the technique of Swinyard, *et al.*,[11] The drugs were administered intraperitoneally to 5 animals for each dose. The analgesic activity was measured in rats according to the technique of Randall, *et al.*[12]; the pain threshold was measured as the amount of pressure in mm. required to provoke the flight reaction when applied to the foot. The drugs were injected intraperitoneally to 5 animals for each dose.

Local anesthesia was tested in rabbits by the usual cornea method of Régnier.[13] Drugs were instilled in the conjunctival sac at various concentrations and at pH values of 6–7. The evaluation of local anesthetic potency was based on the number of mechanical stimuli necessary to provoke the wink reflex. In order to avoid corneal lesions a cut-off of 30 stimuli was fixed. Anticholinergic and antispasmodic activities were investigated *in vitro* by studying the inhibitory action of the drugs on acetylcholine- and $BaCl_2$-induced spasm of strips of rat ileum; antihistaminic action was determined similarly on isolated guinea pig ileum. Each compound was tested at least on two preparations, the sensitivity of which to the standards was ascertained at the beginning and at the end of the experiment. The effective concentrations in μg./ml., reported in Table V, are those able to reduce by 50% the response to acetylcholine, $BaCl_2$ and histamine, respectively.

The diuretic action was studied on rats according to the technique of Lipschitz, *et al.*[14]; each dose was given to 6 animals. The urine was measured volumetrically, and the sodium concentration was determined by flame photometry. Respiratory and cardiovascular effects were studied in mongrel dogs anesthetized with pentobarbital sodium (35 mg./kg. i.v.).

Arterial blood pressure was recorded from the carotid artery by a mercury manometer. The respiratory movements were recorded by the respirometer of Anderson[15] connected with a tracheal cannula or by a pneumometer fixed to the thorax of the animal. The peripheral stump of the vagus nerve was stimulated by rectangular pulses (0.5 msec., 10 v., 300/sec. for 0.5 sec.). Carotid occlusion was performed manually using a hemostat. The drugs were administered intravenously, and changes in arterial blood pressure and respiratory frequency were measured, just as those of the vascular responses to temporary occlusion of the carotid artery (c.a.), to vagal stimulation and to the intravenous administration of norepinephrine, acetylcholine and histamine.

The inhibiting action against the activity of various enzyme systems (carbonic anhydrase, acetylcholinesterase, pseudocholinesterase and monoamine oxidase) was investigated *in vitro* by incubating the drugs with purified preparations of these enzymes.

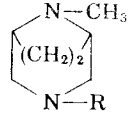
Effects on Behavior in Mice.—Changes in the behavior of mice produced by 3-substituted diazabicyclooctanes appeared to be almost completely unspecific and were found only after the administration of nearly lethal doses. Most of the compounds produced effects that could be classified as CNS excitement, like increase in spontaneous motor activity, tremors, Straub tail and convulsions. Usually these manifestations were followed by death, and therefore have little pharmacological significance. They were observed in a different degree, after the administration of compounds III–IX, XII–XIV, XVII, XXa, XXVIIa–XXX. Signs of depression, such as a decrease in spontaneous motility, and in body muscle tonus and impairment of righting reflex were observed after the administration of XXII, XXVI, and especially of XXI (Table III). The action of the latter compound may be correlated easily with the known hypnotic action of azetidindiones.[16]

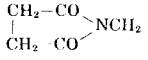
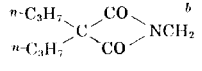
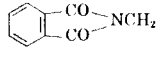
Anticonvulsant and Analgesic Activities, and Inhibition of Enzymes *in Vitro*.—None of the compounds protected mice from the tonic phase of maximal

(9) E. Brand and F. C. Brand, *Org. Synth.* Coll. Vol. III, 440 (1955).
(10) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).
(11) E. A. Swinyard, W. C. Brown and L. S. Goodman, *J. Pharmacol. Exptl. Therap.*, **106**, 319 (1952).
(12) L. O. Randall and J. J. Selitto, *Arch. Int. Pharmacodyn.*, **111**, 409 (1957).
(13) J. Régnier, *Compt. Rend. Acad. Sci.*, **177**, 558 (1923).
(14) W. L. Lipschitz, Z. Hadidian and A. Kerpesar, *J. Pharmacol. Exptl. Therap.*, **79**, 97 (1943).
(15) F. F. Anderson, *Arch. Int. Pharmacodyn.*, **94**, 460 (1953).
(16) G. Maffii, unpublished data.

TABLE III

MISCELLANEOUS 3-SUBSTITUTED-8-METHYL-3,8-DIAZABICYCLO[3.2.1]OCTANES



Cpd.	R	B.p. or m.p. °C. (mm.)	Crystn. solvent	Yield, %	Formula	Calcd., % C	H	N	Cl	Found, % C	H	N	Cl
XXa		206–207[a]		71	$C_{12}H_{19}N_3O_2 \cdot HCl$			15.35	12.95			15.20	13.30
XXb		191–192[a]			$C_{12}H_{19}N_3O_2 \cdot 2HCl$			13.54	22.85			13.47	22.70
XXI		88–90	Ligroin	90	$C_{17}H_{29}N_3O_2$	66.41	9.50	13.66		66.50	9.60	13.60	
XXII		120–123	EtOH	85	$C_{16}H_{19}N_3O_2$	67.34	6.71	14.72		67.01	6.69	14.52	
XXIII	$NCCH_2$	100	0.7	67	$C_9H_{15}N_3$	65.41	9.15	25.43		65.40	9.27	25.23	
XXIV	$H_2NCH_2CH_2$	97–99	2	69[c]	$C_9H_{19}N_3$	63.85	11.31	24.82		64.10	11.45	24.60	
XXVa	$H_2NC(=NH)NHCH_2CH_2$	189–191	iso-PrOH	76[d]	$C_{10}H_{21}N_5 \cdot HBr$			23.96	27.34 (Br)			24.01	27.56 (Br)
XXVb		230–233	iso-PrOH		$C_{10}H_{21}N_5 \cdot 2HBr$			18.76	42.83 (Br)			18.94	42.47 (Br)
XXVI	NO	130–135	10	93	$C_7H_{13}N_3O$	54.16	8.44	27.07		53.87	8.38	26.89	
XXVIIa	NH_2	110–115	20	82[e]	$C_7H_{15}N_3$	59.53	10.70	29.75		59.63	11.00	29.49	
XXVIIb		230–233	EtOH		$C_7H_{15}N_3 2HCl$			19.62	33.11			19.49	33.02
XXVIII	$COOC_2H_5$	114–115	0.6	79	$C_{10}H_{18}N_2O_2$	60.57	9.15	14.13		60.83	9.20	14.01	
XXIXa	$(C_2H_5)_2NCO$	135–138	2.5	73	$C_{12}H_{23}N_3O$			18.65				18.41	
XXIXb		155–157	EtOH		$C_{12}H_{23}N_3O \cdot C_6H_8O_7$[f]	51.78	7.48	10.06		52.00	7.60	10.05	
XXX	C_6H_5NHCO	131–134	EtOH	63	$C_{14}H_{19}N_3O$	68.54	7.80	17.13		68.75	7.75	17.05	

[a] Attempts at crystallization were unsuccessful because of instability of the product on heating. [b] The starting 3,3-di-*n*-propylazetidine-2,4-dione was prepared according to the method of E. Testa, L. Fontanella, G. F. Cristiani and L. Mariani, *Helv. Chim. Acta*, **42**, 2370 (1959). [c] Calculated from XXIII. [d] Calculated as base: from XXIV. [e] Calcd. from XXVI. [f] Citric acid.

TABLE IV

LOCAL ANESTHETIC ACTION

Table	Comp.	Concn., %	No. of stimuli	Duration of action, min.	Effective concn., %	Other actions	Approximate LD_{50} mg./kg.i.p., mouse
I	IV	1	>30	30	0.25	Antihistaminic, diuretic	70
		0.5	>30	27			
		0.25	>30	22			
		0.2	15	7			
I	V	1	>30	15	0.25	Slight anticholinergic	80
		0.5	>30	9			
		0.25	26	12			
I	VI	1	>30	15	0.8	Irritating	200
		0.5	1				
I	VII	1	>30	15	0.5	Spasmolytic	80
		0.5	29	15			
		0.25	13	10			
I	VIII	1	>30	25	0.125	Anticholinergic	40
		0.5	>30	25			
		0.2	25	27			
		0.1	21	21			
I	IX	1	>30	>30	0.125	Irritating, anticholinergic	200
		0.5	>30	>30			
		0.25	>30	>30			
		0.1	13	12			
I	XI	1	21	25	0.125		100
		0.5	16	20			
		0.25	20	6			
		0.125	16	10			
I	XII	1	>30	>30	0.125	Anticholinergic	80
		0.5	>30	25			
		0.25	>30	>30			
		0.125	>30	25			
		0.06	1				
	Procaine	2	22	15	1.0		123.8[a]
		1	22	10			
		0.5	3	6			
	Lidocaine	1	>30	>27	0.5		135
		0.5	24	10			
		0.25	16	9			

[a] J. R. Schamp, H. M. Schamp and M. L. Tainter, *Anaesthesiology*, **3**, 398 (1942).

TABLE V

ANTICHOLINERGIC, SPASMOLYTIC AND ANTIHISTAMINIC ACTION

Table	Compd.	Effective concn. μg./ml. against spasm produced by Acetylcholine	$BaCl_2$	Histamine	Approximate LD_{50} mg./kg.i.p., mouse
I	IV	5	1.5	2	70
I	V	8	10	10	80
I	VII	0.6	0.4	≥10	80
I	VIII	0.2–0.3	0.6	3	40
I	IX	1	1	1	200
I	XII	0.4	6.8	15	80
II	XVIa	0.18	≥20	4	124 (104–149)
II	XVII	0.04	Inact.	20	252 (220–288)
II	XVIII	10–20	Inact.	Inact.	434 (379–496)
II	XIX	0.01–0.03	≥15	Inact.	220 (192–252)
III	XXVIII	≥18	≥20	Inact.	100
III	XXIX	≥18	Inact.	Inact.	150
Atropine sulfate		0.016			222 (192–248)
Adiphenine		0.47	5.4		200
Promethazine·HCl				0.02	(175–200)[a]

[a] H. Friebel, H. Flict and C. Reichle, *Arzneimittel-Forsch.*, **4**, 171 (1954).

electroshock seizures when administered intraperitoneally in doses as high as 1/10 of the approximate LD_{50}. No significant change in the pain threshold of rats was observed after i.p. treatment. None of the materials produced significant changes in the activity of the following enzymatic systems: carbonic anhydrase, acetylcholinesterase, pseudocholinesterase and monoamine oxidase.

Local Anesthetic Action.—The results listed in Table IV indicate possible relations of structure and activity. Local anesthetic action is bound to the presence of an aromatic group in the substituent in position 3; substitution of the aromatic ring with chlorine produces in one case an increase in local anesthetic potency [*e.g.*, VIII (*o*-chlorobenzhydryl) and IX (*p*-chlorobenzhydryl), as compared to VII (benzhydryl)]. While the position of the chlorine does not seem to be important for the local anesthetic action of VIII and IX, the *o*-chlorocinnamyl derivative V is much more potent than the *p*-chlorocinnamyl derivative VI. Both *p*-chloro derivatives (VI, IX) are less toxic than the *o*-chloro isomers (V, VIII). The Mannich bases (XXa, XXI and XXII) (Table III) and all the acyl derivatives (Table II) are inactive as local anesthetics. Finally, other pharmacological actions than local anesthesia were noticed; practically only one of the local anesthe-

TABLE VI

ANTICHOLINERGIC ACTIVITY *in Vivo*

Table	Compd.	Dose, mg./kg.i.v.	No. of animals	Arterial blood pressure	Respiration	Vascular response[b] to: Vagal stimulation	Occlusion of carotid artery	i.v. administration of: Norepinephrine	Acetylcholine	Histamine
I	VII	5	3	d[a]	i[a]	0.1	0.8	2.0	0.7	0.8
I	VIII	5	1	i	u[a]	0	1.0	1.65	0	1.0
I	IX	10	1	d	u	0.2	1.0	2.0	0.35	1.0
I	XII	10	1	d	u	0.2	1.0	1.0	0	0.5
		5	1	u	i	0.6	1.0	1.5	0.5	1.0
II	XVIa	3	1	d	u	0	1.0	1.3	0	1.0
II	XVII	5	2	u	u	0	1.0	1.0	0	0.5
II	XIX	5	1	d	d	1.0	1.0	1.1	1.0	1.0
		0.5	1	u	u	1.0	1.0	1.0	0	1.0
		0.2	1	u	u	1.0	1.0	1.0	0.5	1.0
		0.1	3	u	u	0.8	1.0	1.0	0.75	1.0
	Atropine	0.2	2			0	1.0	1.0	0.5	1.0
		0.1	2			0	1.0	1.0	0.5	1.0

[a] d = decrease; i = increased; u = unchanged. [b] norm. = 1.0.

ties (XI) was without any pharmacological side-effects, namely, a β-aminoethyl *p*-aminobenzoate ester.

Anticholinergic, Spasmolytic and Antihistaminic Action (Tables V–VI).—Compounds VII (benzhydryl), VIII (*o*-chlorobenzhydryl) and XII (β-ethyl diphenylacetate) showed anticholinergic activity. The activity is enhanced by *o*-substitution, and is decreased by *p*-substitution in the benzhydryl group. By contrast, both *o*- and *p*-substitutions favored local anesthetic action.

The inhibitory action of these compounds on histamine-induced spasm of isolated guinea pig ileum is not significant and presumably little specific, whereas the spasmolytic action of VII is worthy of more detailed investigation. The action of the analog of adiphenine (XII) on $BaCl_2$-induced spasm is slightly inferior to that of adiphenine, whereas the compounds VII and IX which may be compared to cyclizine[17] and chlorocyclizine,[18,19] respectively, were much less potent as antihistaminics. The tropoyl (XVI) and *dl*-methyltropoyl (XVII) derivatives were the most active test compounds as anticholinergic agents; the activity of XVII was found to reside largely in the *l*-isomer (XIX). Investigations *in vivo* on these compounds confirmed the anticholinergic potency for compounds VII, VIII, IX, XII, XVIa, XVII and XIX (see Table VI).

Diuretic Action (Table VII).—The diuretic activity in the rat was tested on compounds III–IX, XIII–XV, XVII, XXVI–XXX, but only the products recorded in Table VII produced significant changes in water and in Na^+ excretion. Chloro substitution in the benzhydryl group (VIII, IX) enhanced the activity; in the case of the cinnamyl group, only the unsubstituted derivative (IV) showed diuretic activity.

The structural analogies between some of the 3-substituted 8-methyldiazabicycloöctanes and corresponding pharmacologically active compounds, allow some conclusion about the biological significance of this new structure. Comparison of XI (I, R = p-$H_2NC_6H_4COOCH_2CH_2$–) and procaine, and of XII (I, R = $(C_6H_5)_2CHCOOCH_2CH_2$–) and adiphenine, demonstrated that 8-methyldiazabicycloöctane may be substituted for diethylamine in the two aminoethyl esters without qualitative changes in the pharmacological activity. But, while spasmolytic activity is little decreased *in vitro*, local anesthetic activity tested on the rabbit cornea is enhanced many times in the diazabicycloöctane corresponding to procaine.

The substitution of an N-methylpiperazine group by 8-methyldiazabicycloöctane produces, in compound IX, very slight antihistaminic activity as compared with N-methyl-N′-(4-chlorobenzhydryl)-piperazine (chlorocyclizine).[18,19] Compound VIII appears less active as a parasympatholytic than N-methyl-N′-(2-chlorobenzhydryl)-piperazine.[19,20] These results suggest that N-methylpiperazine cannot be replaced by 8-methyldiazabicycloöctane without significant decrease of specific pharmacological activity. However, the "quality" of the action is maintained. As concerns anticholinergic activity, the analogy of 8-methyl-3,8-diazabicyclo[3.2.1]octane with tropane, may be still valid. In fact, compounds XVI and XIX (Table II), although less active *in vitro*, showed *in vivo*, and especially by the oral route, an activity comparable with that of atropine and *l*-tropine α-methyltropate,

TABLE VII

DIURETIC ACTION

Table	Compd.	Dose, mg./kg. oral	Increase of excretion, % Urine	Sodium	Approximate LD_{50} mg./kg. i.p., mouse
I	IV	25	133	178	70
		10	161	115	
		5	38	75	
I	VIII	50	152	130	40
		25	100	87	
		10	32	29	
I	IX	50	78	131	200
		25	24	48	
		10	1.5	37	
III	XXVI	50	152	118	200
		25	152	33	
		10	−36	−37	
Chlorothiazide-HCl		50	126	176	
		25	109	140	618
		10	105	112	(473–806)

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respectively.[21] Compound XXV (Table III) which was synthesized in view of a partial analogy with guanethidine[22] was found inactive.

Experimental

Cinnamaldehydes were obtained by the method of Waley.[3] *o*-Chlorocinnamaldehyde,[23] m.p. 60–61° (ether/petroleum ether); yield, 57%. *p*-Chlorocinnamaldehyde,[24] m.p. 64–65° (ether/petroleum ether); yield 53%.

Cinnamyl Alcohols.—The method described by Carrara, *et al.*,[4] was employed.

***o*-Chlorocinnamyl alcohol,** b.p. 108–110° (0.5 mm.); yield 82%. *Anal.* Calcd. for C_9H_9ClO: C, 64.05; H, 5.38; Cl, 21.15. Found: C, 63.91; H, 5.50; Cl, 21.38.

***p*-Chlorocinnamyl alcohol,**[25] b.p. 114–115° (0.4 mm.); m.p. 50–53°; yield 75%.

Cinnamyl Chlorides. General Procedure.—The appropriate cinnamyl alcohol (0.1 mole) was suspended in 150 ml. of chloroform and treated dropwise, with stirring at room temperature, with 0.15 mole of thionyl chloride. The reaction mixture was stirred for 1 hr. at room temperature and 3 hr. at 50° until gas evolution ceased. The solvent and the excess of thionyl chloride were evaporated; the oily residue was distilled under reduced pressure.

***o*-Chlorocinnamyl Chloride,** b.p. 90–92° (0.6 mm.); yield 72%. *Anal.* Calcd. for $C_9H_8Cl_2$: C, 57.76; H, 4.27; Cl, 37.96. Found: C, 58.00; H, 4.40; Cl, 37.59.

***p*-Chlorocinnamyl chloride,** b.p. 98–100° (0.6 mm.); m.p. 47–49° (from ligroin); yield, 80%. *Anal.* Calcd. for $C_9H_8Cl_2$: C, 57.76; H, 4.27; Cl, 37.96. Found: C, 57.64; H, 4.50; Cl, 37.38.

Benzhydryl Chlorides.—The *o*- and *p*-chlorobenzhydryl chlorides were prepared according to the method of Norris and Banta[5] by saturating with dry hydrogen chloride an ether solution of *o*- and *p*-chlorobenzhydrol, respectively.

3-Aralkyl-8-methyl-3,8-diazabicyclo[3.2.1]octanes (IV–IX). General Procedure.—A mixture of 0.01 mole of the appropriate chloride, 0.02 mole of 8-methyl-3,8-diazabicyclo[3.2.1]octane (II) and 15 ml. of benzene was refluxed for 14–24 hr. Sometimes during the heating separation of crystals occurred. After cooling, the precipitate (identified as II·hydrochloride) was filtered and washed with benzene. The filtrate was collected and washed with water until neutral. The organic layer was dried over sodium sulfate and the solvent evaporated. The oily residue was dissolved in anhydrous ether and treated with dry HCl. The hydrochloride separated mostly in a viscous oil, which after decanting the acid solution and scratching in ether solidified. Crystallization from appropriate solvents gave the pure products.

3-Hydroxyethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (III).—To a solution of 16 g. (0.128 mole) of II in 50 ml. of methanol was added at room temperature with stirring a solution of 16 g. (0.36 mole) of ethylene oxide in 50 ml. of methanol. The reaction mixture was refluxed 90 min., the solvent evaporated and the oily residue distilled.

Esters of 3-Hydroxymethyl-8-methyl-3,8-diazabicyclo[3.2.1]-octane (X, XII). General Procedure.—To a mixture of 0.02 mole of III, 0.022 mole of triethylamine and 50 ml. of ether, 0.022 mole of the acid chloride in 50 ml. of dry ether was added dropwise, with stirring and cooling. The reaction mixture was kept overnight at room temperature, 10% sodium bicarbonate was added and the organic layer was dried over sodium sulfate. After removing the solvent and triethylamine by distillation, the residue was purified by crystallization (X) or through the hydrochloride (XII).

3-[β-Ethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane] *p*-Aminobenzoate Dihydrochloride (XI).—Five grams of X was hydrogenated at normal pressure in 100 ml. of ethanol in the presence of 2 g. of 10% palladium-on-charcoal catalyst. After 1 hr. the gas absorption ceased, the catalyst was filtered off, the filtrate concentrated to a small volume and treated with dry HCl. The precipitate was collected and crystallized from ethanol.

β-Acetoxy-α-phenylpropionyl Chloride.—The method of Toomey and Riegel[26] was modified as follows. A mixture of 10 g. of tropic acid and 33 ml. of acetic anhydride was refluxed for 1 hr., excess acetic anhydride was distilled off under reduced pressure, the oily residue was treated with 40 ml. of thionyl chloride and refluxed for 2 hr. The excess of thionyl chloride was distilled off, the residue diluted with benzene, refluxed 5 min. with the addition of charcoal, filtered and the filtrate evaporated. The crude acetoxy chloride, after drying at 50° (1 mm.), weighed 10.1 g. and was sufficiently pure for the condensation with II. The same procedure was used for obtaining these acid chlorides: (±)-acetoxy-α-methyl-α-phenylpropionyl chloride, b.p. 108–110° (0.6 mm.); yield, 71%; (+) isomer, b.p. 113° (0.6 mm.): yield 52%; (−) isomer: b.p. 115–118° (0.6 mm.), yield 62%.

Optical Resolution of α-Methyltropic Acid.—A solution of 70 g. of (±)-α-methyltropic acid in 315 ml. of acetone was added rapidly to a refluxing solution of 174 g. of quinine in 2200 ml. of acetone and the mixture was allowed to stay at room temperature for 4 hr. The crystals which separated were filtered and washed with 200 ml. of acetone. The filtrates (A) were collected and stored for recovery of the (+)-antipode. The solid material, after drying at 50° *in vacuo*, yielded 127 g. of crude quinine (−)α-methyltropate (m.p. 180–181°), which was recrystallized from 4400 ml. of water to give 110 g. of quinine (−)-α-methyltropate (m.p. 183–184°), $[\alpha]^{20}D$ −120° (c = 2, ethanol). This salt was suspended in 125 ml. of water, acidified with 10% sulfuric acid to pH 3 with stirring and cooling, and the oil which separated was extracted three times with 300 ml. of ether. The extracts were collected, dried over sodium sulfate and evaporated to give an oil which solidified by scratching. After trituration with petroleum ether and filtration, 24.5 g. of (−)-α-methyltropic acid was obtained, m.p. 85–86°, $[\alpha]^{20}D$ −23.8°. Recrystallization from benzene–petroleum ether (1:1) yielded 20 g. (57%) of (−)-α-methyltropic acid, m.p. 88–89° $[\alpha]^{20}D$ −28° (c = 2, ethanol). The (+)-isomer was isolated by evaporating the filtrates A, suspending the crystalline residue in water and acidifying to pH 3 with 10% sulfuric acid. The separated oil was thoroughly extracted with ether, dried and the solvent evaporated to give 25.6 g. of crude (+)-α-methyltropic acid, $[\alpha]^{20}D$ +21° (c = 2, ethanol). The product was crystallized from benzene–petroleum ether to give 19.2 g. (55%) of (+)-α-methyltropic acid, m.p. 88–89°, $[\alpha]^{20}D$ +27° (c = 2%, ethanol).

3-Acyl-8-methyl-3,8-diazabicyclo[3.2.1]octanes. Method A (from Acid Anhydride).—Example: Preparation of XIII.—To 7 ml. of propionic anhydride was added dropwise at 0° 2.52 g. (0.02 mole) of II. The mixture was heated 1 hr. at 100°, cooled and poured with stirring into ice water. Sodium hydroxide (50%, 5 ml.) then was added and the mixture stirred 1 hr. at room temperature to decompose the excess of propionic anhydride. The mixture was extracted with ether, the organic layer dried over sodium sulfate, the solvent evaporated and the residue distilled.

Method B (from Acid Chloride). Example: Preparation of XVII.—To a solution of 3 g. (0.0238 mole) of II in 15 ml. of 2 *N* sodium hydroxide cooled at −10°, 7.2 g. (0.03 mole) (±)-β-acetoxy-α-methyl-α-phenylpropionyl chloride was added with stirring. The mixture was evaporated yielding 7 g. of crude O-acetoxy-α-methyltropyl derivative. This product was saponified by keeping it in 100 ml. of 5% aqueous-alcoholic (1:1) sodium hydroxide for 1 hr. at room temperature with occasional stirring. The mixture was adjusted at pH 7.5–8 with hydrochloric acid; a first crop of XVII, (3.1 g.), m.p. 90–94° was obtained. On concentration of the mother liquor and cooling, a second crop (2.5 g.) melting at 90–91° was recovered. Both crops were crystallized together from water yielding 4.3 g. (59%) of white crystals, m.p. 95°. Compound XVII crystallizes with 1 mole of water and loses its crystalline form when dried *in vacuo*.

Mannich Bases (XX–XXII). General Procedure.—A mixture of 0.02 mole of II, 0.02 mole of the appropriate imide (succinimide, 3,3-di-*n*-propylazetidine-2,4-dione, phthalimide) and 0.025 mole of 38% formaldehyde solution in 15 ml. of ethanol was refluxed 90 min. After evaporation of the solvent and cooling a solid crop was collected and crystallized. In the case of the Mannich base with succinimide the crude compound XX was dissolved in ethanol and converted with a slight excess of dry HCl in ether into the corresponding dihydrochloride, m.p. 191–192°. This product decomposed on crystallization.

3-Cyanomethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXIII).

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—A mixture of 4 g. (0.032 mole) of II and 2.65 g. (0.035 mole) of chloroacetonitrile in 20 ml. of benzene was refluxed for 90 min. The separated hydrochloride was collected, the base liberated with 10% sodium hydroxide, extracted with ether and distilled.

3-β-Aminoethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXIV).—To a stirred suspension of 2.3 g. (0.06 mole) of lithium aluminum hydride in 150 ml. of dry tetrahydrofuran at 0°, 3.2 g. (0.0197 mole) of XXIII diluted with 10 ml. of tetrahydrofuran was added. The reaction mixture was refluxed for 3 hr., cooled to −5° and cautiously decomposed with 8 ml. of water. After stirring for 1 hr. at room temperature, the inorganic material was filtered and thoroughly washed with tetrahydrofuran. The filtrate was dried over sodium sulfate, the solvent evaporated and the residue distilled.

3-Guanidinoethyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXV).—A stirred mixture of 2.2 g. (0.013 mole) of XXIV, 4.8 g. (0.026 mole) of S-ethylisothiuronium bromide and 30 ml. of chloroform was refluxed for 5 hr., under an efficient hood. A viscous oil separated and solidified on cooling. The chloroform solution was decanted and stored. The solid crop (4 g.) after washing with ether was crystallized twice from isopropyl alcohol (200 ml.) to give 1.7 g. (35%) of the dihydrobromide of XXV, m.p. 230–233°. The chloroform solution was evaporated and the oily residue was crystallized from isopropyl alcohol to give 1.7 g. (45%) of XXV monohydrobromide.

8-Methyl-3-nitroso-3,8-diazabicyclo[3.2.1]octane (XXVI).—To a stirred solution at 0° of 7.3 g. (0.058 mole) of II in 29 ml. of 2 *N* hydrochloric acid, was added dropwise a solution of 4.5 g. (0.065 mole) of sodium nitrite in 10 ml. of water. The reaction mixture was kept at room temperature for 2 hr., cooled, made alkaline with 50% sodium hydroxide solution and extracted with ether. The extract was dried over sodium sulfate, the solvent evaporated and the residue distilled.

3-Amino-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXVII).—To a stirred suspension of 3.8 g. (0.1 mole) of lithium aluminum hydride in 200 ml. of dry tetrahydrofuran, a solution of 7.6 g. (0.049 mole) of XXVI in 40 ml. of tetrahydrofuran was added at such a rate that the temperature was kept at 40–45°. At the end of the addition, the mixture was refluxed for 6 hr., then cooled to −5° and cautiously decomposed with 13 ml. of water. After stirring for 1 hr. at room temperature, the reaction mass was filtered, washed with ether, the filtrate was collected and dried over sodium sulfate. The solvent was evaporated, the oily residue diluted with 20 ml. ether and dried again over sodium hydroxide. After evaporation of the solvent, the oil was distilled to yield 5.5 g. (82%) of XXVII, b.p. 110–115° (20 mm.). The product was highly hygroscopic. The *dihydrochloride* was obtained by adding the base to alcoholic hydrogen chloride. By mixing an ether solution of the base with an ethanol solution of *p*-nitrobenzaldehyde, yellow crystals of the *3-p-nitrobenzalamino derivative* separated, m.p. 96–98°. It was transformed to the corresponding *hydrochloride*, m.p. 250–252° (ethanol).

Anal. Calcd. for $C_{14}H_{19}ClN_4O_2$: N, 18.05; Cl, 11.45. Found: N, 17.99; Cl, 11.40.

3-Carbethoxy-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXVIII).—To a mixture of 2.1 g. (0.0166 mole) of II and 10 ml. of 2 *N* sodium hydroxide stirred at 0°, 2.2 g. (0.02 mole) of ethyl chlorocarbonate was added dropwise. The mixture was stirred for 2 hr. at room temperature, the reaction product was extracted with ether, dried and distilled.

3-Diethylcarbamyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXIX).—A suspension of 3.65 g. (0.029 mole) of II in 15 ml. of 2 *N* sodium hydroxide was treated dropwise and with stirring at 0°, with 4 g. (0.03 mole) of diethyl chloroformamide.[27] The temperature was allowed to rise to 40° and after 1 hr. the mixture was extracted with ether. The extract was dried over solid sodium hydroxide and distilled. *The citrate* (XXIXb) was prepared by mixing an ethanolic solution of citric acid and an ether solution of the base.

3-Phenylcarbamyl-8-methyl-3,8-diazabicyclo[3.2.1]octane (XXX).—A mixture of 2.52 g. (0.02 mole) of II, 2.4 g. (0.02 mole) of phenyl isocyanate and 10 ml. of benzene was refluxed for 30 min. After cooling the solid product was collected and crystallized from ethanol.

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The Synthesis and Antineoplastic Properties of Selenoguanine, Selenocytosine and Related Compounds[1]

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Selenoguanine, selenocytosine, diselenothymine, and 5-methylselenocytosine have been synthesized. In several experimental murine neoplasms selenoguanine and thioguanine showed comparable antitumor activities; however, some differences were noted. These compounds exhibited cross-resistance.

During a course of study of selenium analogs of physiologically active sulfur compounds, 2-selenouracil, 2-selenothymine, and 6-selenopurine (I) were synthesized.[2] As an inhibitor of the growth of the mouse lymphoma L-1210, the activity of (I) was equivalent to that of 6-mercaptopurine,[3] a clinically useful antileukemic agent.[4] In the other mouse tumors studied[3] (I) was less active than mercaptopurine. On the other hand, (I) had greater antibacterial activity[5] and greater ability to inhibit the incorporation of formate into purines[5] than did its sulfur analog.

Selenopurine is rather unstable, its half-life in pH 7 phosphate-citrate buffer being only 6 hours.[6] 6-Selenopurine-9-β-D-ribonucleoside has a half-life of only 1 hour under these conditions.[6]

The synthesis of 2-amino-6-selenopurine (selenoguanine) (II) was undertaken in the hope that resonance

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