[CONTRIBUTION FROM THE DEPARTMENT OF PATHOLOGY, THE GEORGETOWN UNIVERSITY MEDICAL CENTER]

Hypotensive Agents. X. 3-Azabicyclo[3.3.0]octane Derivatives[1,2]

LEONARD M. RICE[3] AND CHARLES H. GROGAN[4]

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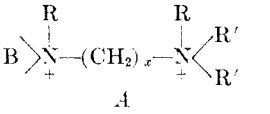
The investigation of various nitrogen heterocycles for use in the formation of symmetrical and unsymmetrical bis-ammonium salts for screening as hypotensive agents demonstrated dramatic changes in activity with variation in ring bridging. In the bicyclic rings derived from cyclohexane a change in the points of attachment of the second ring from the 1,2 positions (isoindole or 2-azabicyclo[4.3.0]nonane) to the 1,3 positions (3-azabicyclo[3.3.1]nonane) resulted in almost complete loss of hypotensive activity. Accordingly, we have investigated the effect of changing the points of attachment to the cyclopentane ring. *N*-Alkyl and *N*-dialkylaminoalkyl imides have been synthesized from *cis*-1,2-cyclopentane dicarboxylic anhydride and reduced to 3-azabicyclo[3.3.0]octanes. On comparison of the bis-quaternary salts with previously reported derivatives of the 1,3-anhydride, 3-azabicyclo[3.2.1]octane, which were very potent as hypotensive agents, significant loss of activity was not observed in the present series.

For many years we have been concerned with the synthesis of fused ring bi-and tricyclic nitrogen heterocycles for use as one or both of the terminal groups in the preparation of *alpha, omega* symmetrical and unsymmetrical bis-ammonium salts for screening in our hypertension chemotherapy program. Among the theories and methods advocated for the chemotherapeutic treatment of hypertensive disease the two most important have involved, at the extremes, emphasis on ganglioplegic agents and centrally acting agents. The pendulum of emphasis on the most desirable of either type of these agents for such use has vacillated frequently in the past decade. This shifting emphasis has probably been largely influenced by the therapeutic limitations of the chemical agents available from time to time.

In surveys of large groups of symmetrical[5-7] and unsymmetrical[8] bis-ammonium salts hypotensive activity has been encountered widely in both types. In the symmetrical types maximal therapeutic effectiveness has been generally attained when the terminal groups are small aliphatic radicals (hexamethonium hexane-1,6-bistrimethylammonium cation) or small heterocycles (pentolinium, pentane-1,5-bis-*N*-methyl pyrrolidinium cation) in which the onium centers are separated by a 5 or 6 membered polymethylene chain.

On the contrary, extensive studies by Cavallito and associates[8-11] as well as our group[12] have shown that the most desirable structure therapeutically, known at this time, in the unsymmetrical bis-ammonium type is represented by structure A.



A

In this general structure optimal effectiveness (as judged by the criteria of therapeutic ratio, potency, toxicity, duration of action, and therapeutic effectiveness *in vivo*) was usually encountered when x was 2 or 3, R methyl or ethyl (generally preferably methyl), R′ methyl or ethyl or small heterocycles such as morpholine, pyrrolidine, or piperidine (again generally preferably methyl), and B the residue of a small mono, di or tricyclic ring (in most cases preferably saturated).

While this simple structure-activity correlation is generally true, subtle and therapeutically significant differences in effectiveness have been brought about by relatively small changes in the basic structure. Changes in structure that affect therapeutic effectiveness comprise substitution on the ring, changing the bridging in the ring (thus changing its size and shape), length of the side chain, size and shape of end groups and quaternizing group.

In all of our past investigations concerning modification of the size and ring bridging (shape) of the terminal amine, hypotensive activity from moderate to excellent, in animals and man, was found in the following ring systems: 3-azabicyclo[3.2.1]-octane,[13] I, 1,8,8-trimethyl-3-azabicyclo[3.2.1]-

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(3) Present address: Wyeth Institute for Medical Research, Radnor, Pa.

(4) Present address: National Institutes of Health, Bethesda, Md.

(5) W. D. M. Paton and E. J. Zaimis, *Brit. J. Pharmacol*, **4**, 381 (1949).

(6) C. J. Cavallito, A. P. Gray, and E. E. Spinner, *J. Am. Chem. Soc.*, **76**, 1862 (1954).

(7) L. M. Rice, C. H. Grogan, and E. E. Reid, *J. Am. Chem. Soc.*, **77**, 616 (1955).

(8) C. J. Cavallito, A. P. Gray, and T. B. O'Dell, *Arch. intern. pharmacodynamie*, **101**, 38 (1955).

(9) W. L. Archer, E. E. Spinner, D. C. Schlieper, and C. J. Cavallito, *J. Am. Chem. Soc.*, **78**, 1227 (1957).

(10) A. P. Gray, E. E. Spinner, D. C. Schlieper, and C. J. Cavallito, *J. Am. Chem. Soc.* **77**, 3533 (1955).

(11) A. P. Gray, W. L. Archer, D. C. Schlieper, E. E. Spinner, and C. J. Cavallito, *J. Am. Chem. Soc.* **77**, 3536 (1955).

(12) L. M. Rice, C. H. Grogan, and E. E. Reid, *J. Am. Chem. Soc.*, **75**, 4911 (1953).

(13) C. H. Grogan and L. M. Rice, *J. Org. Chem.*, **22**, 1223 (1957).

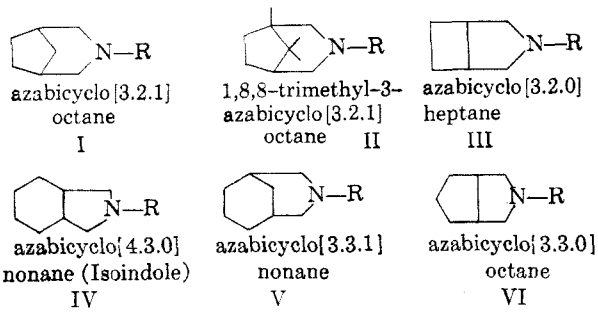
TABLE I

N-ALKYL-3-AZABICYCLO[3.3.0]OCTANE-2,4-DIONES

Alkyl	Formula	B.P., °C.	Mm.	Analyses, %[b] Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	n_D^{20}
Methyl	$C_8H_{11}NO_2$	144–148	29.0	62.73	62.95	7.24	7.05	9.15	9.22	1.5026[a]
Ethyl	$C_9H_{13}NO_2$	68–72	0.1	64.65	64.73	7.84	7.71	8.38	8.09	1.4933
Propyl	$C_{10}H_{15}NO_2$	68–72	0.05	66.27	66.60	8.34	8.10	7.73	7.79	1.4917
Butyl	$C_{11}H_{17}NO_2$	84–88	0.1	67.66	67.83	8.78	8.63	7.17	7.10	1.4875
Amyl	$C_{12}H_{19}NO_2$	86–94	0.08	68.86	68.80	9.15	8.98	6.69	6.54	1.4864
Hexyl	$C_{13}H_{21}NO_2$	90–95	0.05	69.92	69.89	9.48	9.30	6.27	6.37	1.4855
Heptyl	$C_{14}H_{23}NO_2$	103–107	0.07	70.85	70.99	9.77	9.80	5.90	5.77	1.4840
Octyl	$C_{15}H_{25}NO_2$	104–107	0.04	71.67	71.85	10.03	10.19	5.57	5.76	1.4830
Nonyl	$C_{16}H_{27}NO_2$	135–138	0.1	72.41	72.51	10.25	10.26	5.28	5.12	1.4802
Decyl	$C_{17}H_{29}NO_2$	136–140	0.1	73.07	73.17	10.46	10.29	5.01	5.06	1.4798

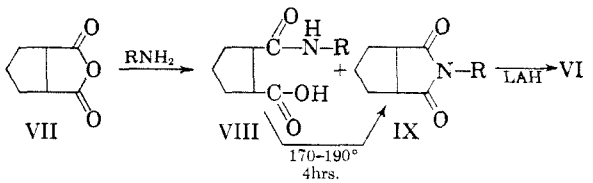
[a] All refractive indices are values obtained for the analytical samples. [b] Carbon, hydrogen, and nitrogen analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Ionic halogen analyses were performed by one of us (C. H. G.) by methods previously described, Grogan, C. H., Rice, L. M., and Reid, E. E., *J. Org. Chem.*, **20**, 50 (1955).

octane,[14] II, 3-azabicyclo[3.2.0] heptane,[15] III, various modifications including ring bridging and substitution of the isoindole, 2-azabicyclo[4.3.0]-nonane nucleus,[12] IV.



We recently reported the synthesis and properties[16] of unsymmetrical bis-ammonium salts employing the 3-azabicyclo[3.3.1]nonane nucleus, V. These compounds were either inactive or possessed a low degree of activity. This was quite surprising since activity was encountered in all modifications of the azabicyclo[4.3.0]nonanes that we investigated. Since this change amounted to changing the position of attachment on the cyclohexane ring from 1,2 to 1,3 (azabicyclo[4.3.0]-nonane to azabicyclo[3.3.1]nonane), we were prompted to investigate the effects of changing the attachment on the cyclopentane ring from 1,3 to 1,2 (azabicyclo[3.2.1]octane to azabicyclo-[3.3.0]octane). This led to the synthesis and screening of *N*-substituted derivatives of this nucleus, VI, which are reported herein.

As in past series reported, the desired *N*-dialkylaminoalkyl bases were most readily accessible by a two step process from the appropriate anhydride. In the present series of compounds the key anhydride was *cis*-1,2-cyclopentane dicarboxylic anhydride. This anhydride was prepared according to the procedure of Fuson and Cole.[17]



The anhydride, VII, was allowed to react with either primary alkyl amines, dialkylaminoalkylamines, or heterocyclicalkylamines to yield a mixture of the amic acid, VIII, and imide, IX. This mixture was then heated at 170–190° for several hours to convert all amic acid to the imide. Products from all three types of amines were isolated by distillation *in vacuo* in an excellent state of purity and in yields of from 70–90% on runs of 10–25 grams.

Table I lists several *N*-alkyl imides thus prepared together with pertinent physical data. Table III lists some *N*-dialkylaminoalkyl imides similarly prepared, their hydrochlorides and methiodides, with pertinent physical data. All of the imides in each series were stable colorless oily liquids.

Reduction of the imides to the corresponding bicyclic bases, VI, was accomplished in all cases by adding the imide dissolved in anhydrous ether to an ethereal solution of lithium aluminum hydride. The reaction was clean and proceeded in the expected manner to give the desired bases in an excellent state of purity and in yields ranging from 70–90% on runs of 10–25 grams. These tertiary amine heterocycles were all stable colorless liquids with typical amine properties. Representative *N*-alkyl-substituted 3-azabicyclo[3.3.0]octanes were reduced and are listed in Table II together with their hydrochlorides, methiodides, and pic-

(14) L. M. Rice and C. H. Grogan, *J. Org. Chem.*, **22**, 185 (1957).

(15) L. M. Rice and C. H. Grogan, *J. Org. Chem.*, **22**, 1100 (1957).

(16) L. M. Rice and C. H. Grogan, *J. Org. Chem.*, **23**, 844 (1958).

(17) R. C. Fuson and W. Cole, *J. Am. Chem. Soc.*, **60**, 1237 (1938).

TABLE II

N-ALKYL-3-AZABICYCLO[3.3.0]OCTANES

Alkyl	Formula	B.P., °C.	Mm.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	n_D^{26}
Methyl	$C_8H_{15}N$	69–70	48.0	76.74	76.88	12.07	11.94	11.19	11.48	1.4648
Butyl	$C_{11}H_{21}N$	120–123	52.0	78.97	79.02	12.65	12.52	8.37	8.56	1.4655
Hexyl	$C_{13}H_{25}N$	108–112	10.0	79.93	79.56	12.90	12.77	7.17	7.40	1.4665
Decyl	$C_{17}H_{33}N$	87–94	0.1	81.20	81.26	13.23	13.18	5.57	5.74	1.4670

Derivatives of Above Bases

Hydrochloride[a] Formula	M.P., °C.	Chlorine, % Calcd.	Chlorine, % Found	Methiodide[a] Formula	M.P., °C.	Iodine, % Calcd.	Iodine, % Found	Picrate[a] Formula	M.P., °C.	Nitrogen, % Calcd.	Nitrogen, % Found
$C_8H_{16}ClN$	150–151	21.93	21.91	$C_9H_{18}IN$	205–206	47.51	47.69	$C_{14}H_{18}N_4O_7$	226–227	15.81	15.59
$C_{11}H_{22}ClN$	237–239	17.40	17.33	$C_{12}H_{24}IN$	150–151	41.04	41.38	$C_{17}H_{24}N_4O_7$	175–176	14.14	14.12
$C_{13}H_{26}ClN$	210–211	15.30	15.19	$C_{14}H_{28}IN$	123–125	37.63	37.25	$C_{19}H_{28}N_4O_7$	135–136	13.20	13.44
$C_{17}H_{34}ClN$	192–194	12.31	12.60	$C_{18}H_{36}IN$	177–179	32.26	32.51	.....	.....	...	...

[a] All hydrochlorides, methiodides, dihydrochlorides, and bis-methiodides were recrystallized from isopropyl alcohol–ether mixtures; picrates were recrystallized from water-ethanol. Usually one or two recrystallizations yielded constant melting derivatives. All melting and boiling points are uncorrected.

TABLE III

N-DIALKYLAMINOALKYL-3-AZABICYCLO[3.3.0]OCTANE-2,4-DIONES

	Substituent	Formula	B.P., °C.	MM.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	n_D^{20}
1	Dimethylaminoethyl	$C_{11}H_{18}N_2O_2$	93–98	0.3	62.83	63.07	8.63	8.85	13.32	13.40	1.4958
2	Dimethylaminopropyl	$C_{12}H_{20}N_2O_2$	87–90	0.1	64.25	64.48	8.99	9.13	12.49	12.22	1.4940
3	Diethylaminoethyl	$C_{13}H_{22}N_2O_2$	105–115	0.3	65.51	65.56	9.31	9.55	11.76	11.51	1.4930
4	Diethylaminopropyl	$C_{14}H_{24}N_2O_2$	108–113	0.08	66.63	66.87	9.59	9.45	11.10	11.47	1.4908
5	Morpholinopropyl	$C_{14}H_{22}N_2O_3$	145–155	0.08	63.13	63.63	8.33	8.16	10.52	10.47	1.5115

Derivatives of Above Bases

	Hydrochloride Formula	M.P., °C.	Chlorine, % Calcd.	Chlorine, % Found	Methiodide Formula	M.P., °C.	Iodine, % Calcd.	Iodine, % Found
1	$C_{17}H_{19}ClN_2O_2$	199–200	14.37	14.35	$C_{12}H_{21}IN_2O_2$	180–181	36.03	36.07
2	$C_{12}H_{21}ClN_2O_2$	173–174	13.60	13.70	$C_{13}H_{23}IN_2O_2$	230–231	34.65	34.61
3	$C_{13}H_{23}ClN_2O_2$	183–185	12.90	13.23	$C_{14}H_{25}IN_2O_2$	.....	...	...
4	$C_{14}H_{25}ClN_2O_2$	123–124	12.28	12.56	$C_{15}H_{27}IN_2O_2$	114–115	32.18	32.02
5	$C_{14}H_{23}ClN_2O_3$	184–185	11.71	11.66	$C_{15}H_{25}IN_2O_3$	201–202	31.08	30.97

rates. Representative *N*-dialkylaminoalkyl substituted 3-azabicyclo[3.3.0]octanes thus prepared are listed in Table IV together with their dihydrochloride and bis-methonium salts.

Members from each series were screened for hypotensive activity in dogs by techniques previously described.[18] As expected from previous series, the methiodides of the *N*-alkyl bases, hydrochlorides and methiodides of the *N*-dialkylaminoalkylimides, and hydrochlorides of the bicyclic bases were inactive. However, conversion of the *N*-dialkylaminoalkyl bicyclic bases to bis-quaternary methonium salts resulted in compounds with good activity. Hence it has been shown that in the cyclopentane-derived bicyclic structure a change in position of attachment of the second ring bearing the nitrogen atom from 1,3, I, II, to 1,2, VI, did not result in loss of activity. In the cyclohexane derived structures, on the other hand, a change in bridging from 1,2 (IV) to 1,3 (V) resulted in almost complete loss of activity.

In summary of our extensive work on the type of compound illustrated by formula A, we should like to emphasize that even though broad general hypotensive activity was encountered in both symmetrical and unsymmetrical permutations of this structure, subtle differences in toxicity, potency, duration of effect, therapeutic ratio, and therapeutic effectiveness were noted. For example some of the compounds from II and IV, although in both cases nearly equally potent by injection, have a ratio of activity on oral administration of about 2 to 1. That II is more effective orally, although similar in over-all structural characteristics, has

(18) W. E. O'Malley, G. Winkler, L. M. Rice, and C. F. Geschickter, *J. Am. Pharm. Assoc. Sci Ed.*, **46**, 346 (1957).

TABLE IV

N-DIALKYLAMINOALKYL-3-AZABICYCLO[3.3.0]OCTANES

	Substituent	Formula	B.P., °C.	MM.	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	n_D^{20}
1	Dimethylaminoethyl	$C_{11}H_{22}N_2$	93–95	10.0	72.47	72.41	12.16	12.32	15.37	15.43	1.4755
2	Dimethylaminopropyl	$C_{12}H_{24}N_2$	102–104	5.0	73.41	73.70	12.32	12.28	14.27	14.46	1.4742
3	Diethylaminoethyl	$C_{13}N_{26}N_2$	59–63	0.08	74.22	74.55	12.46	12.16	13.32	13.54	1.4757
4	Diethylaminopropyl	$C_{14}H_{28}N_2$	99–103	1.2	74.94	74.92	12.58	12.48	12.49	12.54	1.4746
5	Morpholinopropyl	$C_{14}H_{26}N_2O$	94–98	0.1	70.54	70.74	10.99	11.01	11.75	11.99	1.4957

Derivatives of Above Bases

	Dihydrochloride Formula	M.P., °C.	Chlorine, % Calcd.	Chlorine, % Found	Dimethiodide Formula	M.P., °C.	Iodine, % Calcd.	Iodine, % Found
1	$C_{11}H_{24}Cl_2N_2$	296–298	27.78	27.78	$C_{13}H_{28}I_2N_2$	236–238	54.45	54.28
2	$C_{12}H_{26}Cl_2N_2$	261–262	26.33	26.20	$C_{14}H_{30}I_2N_2$	256–257	52.85	52.97
3	$C_{13}H_{28}Cl_2N_2$	210–211	25.03	24.83	$C_{15}H_{32}I_2N_2$	213–214	51.35	51.11
4	$C_{14}H_{30}Cl_2N_2$	204–205	23.85	23.53	$C_{16}H_{34}I_2N_2$	209–210	49.94	49.88
5	$C_{14}H_{28}Cl_2N_2O$	265–267	22.78	22.59	$C_{16}H_{32}I_2N_2O$	234–236	48.60	48.48

been demonstrated clinically. The bis-methionium derivative of the *N*-dimethylaminopropyl base derived from II has been under clinical trials by Wyeth Laboratories (Wy-1395)[19] for over a year and is believed to possess centrally acting components in addition to its ganglioplegic properties.

Of all the compounds prepared in the various series, the compounds derived from IV with an additional oxygen bridge in the cyclohexane ring and bearing methyl substituents on this ring were the finest examples of almost ideal agents.[20] The bis-methonium salts of the compound, *N*-dimethylaminoethyl - 4 - methyl - 4,7 - endoxyperhydroisoindole (Wy-1263)[18,21] possessed an extremely low toxicity in rats, dogs, and humans. Yet it was a very potent hypotensive agent of long duration of action in parenterally administered doses of a fraction of a mg./kg. In addition it had a therapeutic plateau effect which makes it the safest drug we have ever seen for lowering blood pressure. This is particularly important from a safety point of view when such agents are used to titrate patients with severe or malignant hypertension. In addition to this use in more critical cases, it can be employed orally and can satisfactorily control mild and severe types of hypertension.

EXPERIMENTAL

The general synthesizing procedures employed in the present series of compounds are illustrated by the following examples.

3-Methyl-3-azabicyclo[3.3.0]octane-2,4-dione. cis-1,2-Cyclopentane dicarboxylic anhydride, 49 g. (0.35 mole) was placed in a 200-ml. round bottom flask and 96 g. (0.386 mole) of a 12.5% aqueous solution of methylamine was added. The mixture was stirred vigorously during the addition of the amine and cooled as needed to prevent loss of amine. When the initial reaction had subsided, the solution was heated to boiling. After all water had been boiled off, the temperature was slowly raised to 220°. The crude product was allowed to cool and then distilled *in vacuo* to yield 43 g., 80%, of product with b.p. 144–148°/29 mm., n_D^{20} 1.5026.

3-Azabicyclo[3.3.0]octane-2,4-dione. The simple imide of *cis*-1,2-cyclopentane dicarboxylic anhydride was readily formed in a manner analogous to that employed to synthesize the *N*-methyl imide when concentrated aqueous ammonia was used instead of methyl amine. The crude product was recrystallized from water and melted at 85–87°.

Anal. Calcd. for $C_7H_9NO_2$: C, 60.41; H, 6.52; N, 10.07. Found: C, 60.60; H, 6.56; N, 10.07.

3-Methyl-3-azabicyclo[3.3.0]octane. A solution of 15 g. (excess) of lithium aluminum hydride was dissolved in 800 ml. of anhydrous ether in a 2-liter, 3-necked, reaction flask equipped with Hershberg stirrer, dropping funnel, reflux condenser, and drying tube. A solution of 36 g. (0.235 mole) of 3-methyl-3-azabicyclo [3.3.0]octane-2,4-dione in 400 ml. of anhydrous ether was added dropwise with stirring at such a rate as to just maintain reflux of the ether. When addition was complete, the mixture was stirred for 2 hr. and then decomposed by the dropwise addition of water. This was added so as to just maintain reflux of the ether; and then a 5-ml. excess was added. The inorganic solid precipitate was filtered off with rapid suction, pressed tightly, and washed three times with 100-ml. portions of ether. The filtrate and washings were combined and dried over anhydrous sodium sulfate. The ether was stripped off and the residue distilled under reduced pressure to yield the base, 21 g. 71%, b.p. 69–70°/48 mm., n_D^{20} 1.4648.

The *hydrochloride* was prepared in isopropyl alcohol with excess alcoholic HCl and precipitated with ether. On recrystallization from isopropyl alcohol–ether, it melted at 150–151°.

The *methiodide* was prepared in isopropyl alcohol with a slight excess of methyl iodide at room temperature and recrystallized from isopropyl alcohol–ether, m.p. 205–206°.

The *picrate* was prepared in the usual way, m.p. 226–227°.

3-Dimethylaminopropyl-3-azabicyclo[3.3.0]octane-2,4-dione was prepared by causing to react 8 g. (0.078) mole of dimethylaminopropylamine with 10.0 g. (0.071 mole) of *cis*-1,2-cyclopentane dicarboxylic anhydride and heating the resulting homogeneous reaction mixture at 170–190° for

(19) W. E. O'Malley, G. W. Haemmerli, L. M. Rice, and C. F. Geschickter, *J. Am. Pharm. Assoc. Sci. Ed.*, **47**, 263 (1958).

(20) C. H. Grogan and L. M. Rice, U. S. Patent **2,784,199**, March 5, 1957.

(21) G. Winkler, W. E. O'Malley, L. M. Rice, and C. F. Geschickter, *J. Am. Pharm. Assoc. Sci. Ed.*, **47**, 620 (1958).

2 hr. The imide was isolated by distillation *in vacuo* to yield 12.4 g., 78%, of product, b.p. 87–90°/0.1 mm., n_D^{20} 1.4940. The *hydrochloride* was prepared as described, m.p. 173–174°. The *methiodide* was prepared as described, m.p. 230–231°.

3-Dimethylaminopropyl-3-azabicyclo[3.3.0]octane. The *N*-dimethylaminopropyl base was obtained in a manner analogous to that of the simple *N*-methyl base on reduction of the *N*-dimethylaminopropyl imide with lithium aluminum hydride in anhydrous ether. From 18 g. (0.080 mole) of the imide there was obtained 14 g., 89% of the base with b.p. 102–104°/5 mm., n_D^{20} 1.4742. The *dihydrochloride* was prepared in alcohol in the usual way and on recrystallization melted at 261–262°. The *bis-methiodide* was prepared by refluxing the base with a 10% excess of methyl iodide in methanol for 1 hr. The bis-methonium salt was collected on cooling and recrystallized from methanol-ether, m.p. 256–257°.

WASHINGTON 7, D. C.

[CONTRIBUTION FROM THE PHYSIOLOGY DEPARTMENT, TUFTS UNIVERSITY SCHOOL OF MEDICINE]

Chemistry of Pyrimidines. I. The Reaction of Bromine with Uracils[1-3]

SHIH YI WANG

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Uracils react with bromine in aqueous solution to form 5-bromo-6-hydroxyhydro derivatives. Upon dehydration in solution the latter form 5-bromo derivatives, which in turn with excess bromine, form 5,5-dibromo-6-hydroxyhydro derivatives. The mechanism of the formation of the 5-bromo derivatives from the latter has been elucidated.

In our study of the effects of ultraviolet irradiation on nucleic acids, we have used a number of pyrimidine derivatives as model compounds. Several common reactions have been studied and certain inconsistencies in the literature have been noted. It was the purpose of the present work to clarify these inconsistencies.

In 1907, Wheeler and Johnson[4] first observed the colored reaction product obtained by the action of bromine on uracil and cytosine and since then this reaction has become a well known color test for uracils and cytosines. More recently, a colorimetric method for the determination of uracil and cytosine[5] has been based upon this bromine reaction. The reaction also played an important part in the structural determination of pyrimidine nucleosides and the preparation of derivatives.[6] In 1940. Johnson *et al.* concluded that "5,5-dibromo-4(6)-hydroxyhydrouracil (IVA) decomposed spontaneously and quantitatively to 5-bromouracil and HOBr."[7] In order to prove their statement that HOBr was formed and subsequently served as an oxidizing agent, Johnson *et al.* allowed the dibromo compound to react with thiourea, ethylenethiourea, malonic acid, and barbituric acid and ketene.[7,8] Some interesting observations on the reaction of bromine with pyrimidine compounds have also been reported by Cohn.[9] In the present report we wish to propose a possible mechanism for this bromine reaction.

RESULTS AND DISCUSSION

The addition of one mole equivalent of bromine to uracil (IA) or 1,3-dimethyluracil (IB) in aqueous solution resulted in the formation of 5-bromo-6-hydroxyhydro derivatives (IIA or IIB). The existence of (IIA) or (IIB) was indicated by the loss of the ultraviolet spectrum of IA or IB, the isolation of 6-hydroxyhydro-1,3-dimethyluracil[10] by hydrogenolysis and the formation of 5-bromo-derivatives (IIIA or IIIB). The dehydration of IIA or IIB to form IIIA or IIIB proceeded spontaneously and quantitatively and thus provided an excellent method for the preparation of III. Therefore, it seemed that Johnson's statements[7] to the effect that IVA decomposed spontaneously and quantitatively to III could actually hold for II. A thorough study of IV was made in order to clarify these different views. IV was prepared by the addition of one mole equivalent of bromine to 5-bromo- derivatives (III) in aqueous solution. However, the bromine analysis of the product obtained after repeated crystallization from hot water were low (IVA, found: 54.05, 54.38; IVB, found: 49.15)

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(3) A preliminary report has been published in *Nature*, **180**, 91 (1957).

(4) H. L. Wheeler and T. B. Johnson, *J. Biol. Chem.*, **3**, *Am. Chem. J.*, **42**, 30 (1909); H. L. Wheeler and L. M. Liddle, *J. Am. Chem. Soc.*, **30**, 1152 (1908).

(5) M. Soodak, A. Pircio, and L. R. Cerecedo, *J. Biol. Chem.*, **181**, 712 (1949).

(6) P. A. Levene, *J. Biol. Chem.*, **63**, 653 (1925); "Nucleic Acids", ACS Monograph No. 56, pp. 54 and pp. 148 (Chemical Catalog Co., Inc., New York, 1931).

(7) T. B. Johnson, *J. Am. Chem. Soc.*, **62**, 2269 (1940); also G. E. Hilbert and E. F. Jansen, *J. Am. Chem. Soc.*, **56**, 136 (1934).

(8) T. B. Johnson and C. O. Edens, *J. Am. Chem. Soc.*, **63**, 1058 (1941); T. B. Johnson and M. J. Winton, *J. Am. Chem. Soc.*, **63**, 2379 (1941); M. Fytelson and T. B. Johnson, *J. Am. Chem. Soc.*, **64**, 306 (1942).

(9) W. E. Cohn, *Biochem. J.*, **64**, 28P (1956).

(10) S. Y. Wang, M. A. Apicella, and B. R. Stone, *J. Am. Chem. Soc.*, **78**, 4180 (1956).