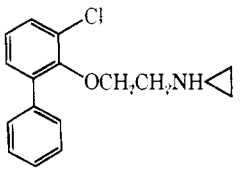
resided in the primary amines.[2] For that reason the present study was designed to assess the effect of N substitution on the activity of the primary amine **2c**. Three model substrates were chosen (a) propoxyphene, an excellent substrate that is difficult to inhibit, (b) diphenyldimethylaminoethane, also a very active substrate but more easily inhibited, and (c) butynamine, a less active substrate that is very readily inhibited. The results summarized in Table I show that, indeed, substitution on N does diminish activity in accord with the earlier studies.[2a,b] There was, however, one conspicuous exception, that of the *N*-cyclopropyl analog, **2b** which was substantially more active than **2c**.

The activity of **2b** was further investigated by a comparative study with 2,4-dichloro-6-phenylphenoxyethylamine (**1**), the most active compound in the earlier study. The relative activities of the two inhibitors in the presence of a number of substrates are shown in Table II. In the rat liver microsomal system, **2b** was clearly a more effective inhibitor than was **1**. It also appeared to be more effective in the liver slice incubation. In guinea pig microsomes, however, **2b** was a less active inhibitor.

Preliminary *in vivo* studies with **2b** were also conducted. In order to insure a maximum effect **2b** was given by ip injection 5 min prior to administration of substrate. These studies showed **2b** to be less effective than **1** both in prolonging hexobarbital sleeping time in mice and in inhibiting the *in vivo* demethylation of imipramine (see McMahon, *et al.*,[2a] for a description of *in vivo* methodology). Because of these results, further studies of **2b** in the intact animal did not appear warranted. However, its activity in the rat liver microsomal system suggests that it may be a very valuable tool in the study of the mechanism by which these interesting oxygenases oxidize drugs.

The effect of cyclopropyl substitution in the phenoxyethylamine series was also investigated. Thus *N*-cyclopropyl-2-chloro-6-phenylphenoxyethylamine (**3**) was synthesized, and its activity was compared with that of the corresponding primary amine. Compound **3** was found to be significantly more active than the corresponding primary amine in rat liver microsomes. However, it appeared to have very little *in vivo* activity.



3

The basis for the effect of cyclopropyl substitution in the two series of microsomal inhibitors remains obscure. The effect is not simply the effect of branching on the α-C since the *i*-Pr compound is no more active than *n*-Pr. The electron-attracting properties of cyclopropyl would be expected to lower the pK_a of the amine. However, this effect alone cannot explain the unique effect of cyclopropyl since other electron-withdrawing groups such as benzyl, allyl, and dimethylpropargyl[8] have a very minimal effect. A number of other workers[9] have investigated the effect of small ring alkyl substituents but as yet no substantive clues to their unique effects have emerged.

Acknowledgment.—We wish to thank Mr. W. M. Miller and Mrs. Catherine Cobb for expert technical assistance. We also thank Dr. W. Lacefield for advice on the chemical aspects of the study.

(8) R. E. McMahon and N. R. Easton, *J. Med. Pharm. Chem.*, **4**, 437 (1961).

(9) A. Burger, R. T. Standridge, and E. J. Ariens, *ibid.*, **6**, 221 (1963); J. Mills, R. Kattau, I. H. Slater, and R. W. Fuller, *ibid.*, **11**, 95 (1968).

Pyrazine Diuretics. VIII. *N*-Amidino-3-aminopyrazinecarboxamide 4-Oxides

JAMES H. JONES AND EDWARD J. CRAGOE, JR.

Merck Sharp and Dohme Research Laboratories, Division of Merck & Co., Inc. West Point, Pennsylvania 19486

Received April 9, 1970

The interesting diuretic–saluretic properties of the *N*-amidino-3-amino-pyrazinecarboxamides[1] prompted the preparation of some corresponding *N*-amidino-3-aminopyrazinecarboxamide 4-oxides (IIIa, IIIb, V). These compounds were prepared by the reaction of the corresponding pyrazine ester with guanidine according to the method previously reported[2] (see Scheme I). The methyl 3-aminopyrazinecarboxylate 4-oxides (IIa, b) were obtained from the appropriate pyrazine esters by treatment with *m*-chloroperbenzoic acid. The position of the oxide function was established by the preparation of IV and VII from IIa and IIb by treatment with $POCl_3$. Thus, our experiences with py-

TABLE I

BIOLOGICAL RESULTS

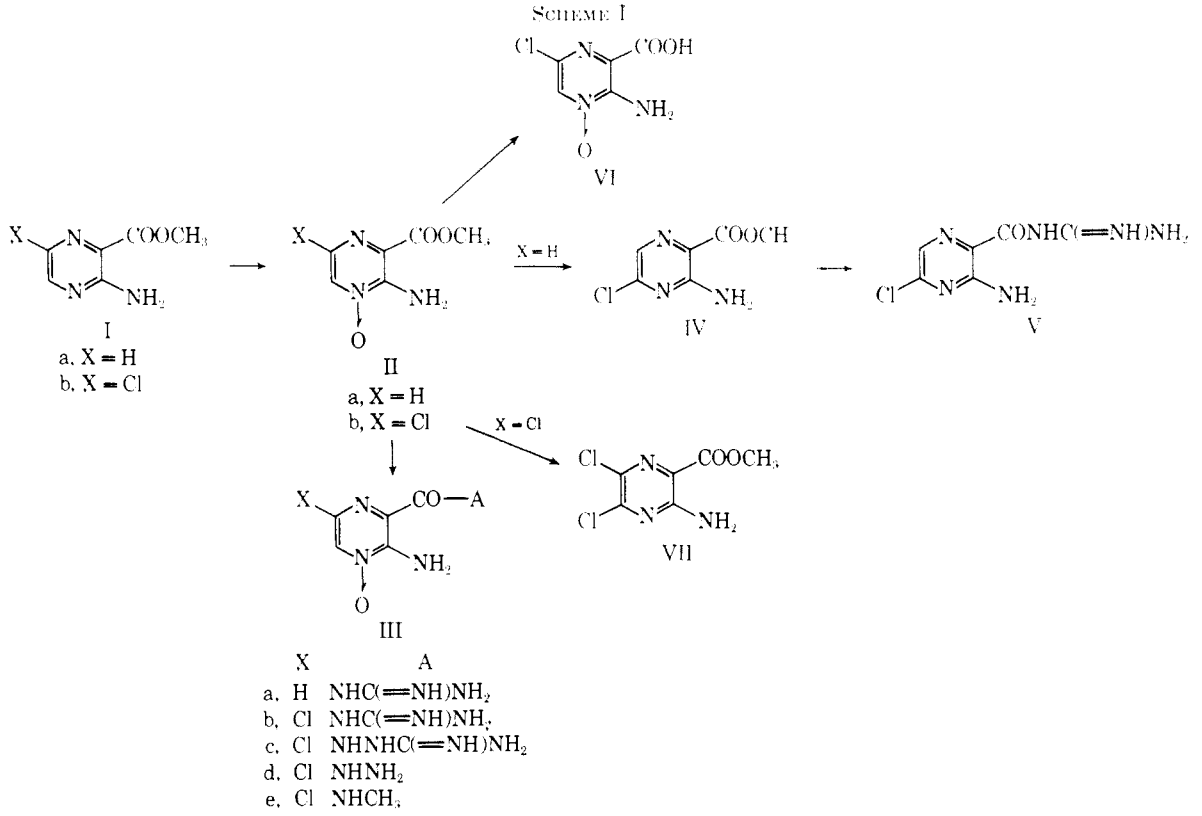
Compd	Rat DOCA-inhibn[a] score[c]	Normal rat[b] score[d]
IIIa	+1	+1
IIIb	±	±
IIIc	±	+2
IV	0	+2
VIIIa[e]	0	+2
VIIIb[f]	+3	+3
VIIIc[g]	+3	+2

[a] Drs. M. S. Glitzer and S. L. Steelman and their associates supplied part of this data; the remainder was supplied by Dr. J. E. Baer and his associates. [b] Dr. J. E. Baer and his associates supplied these data. [c] The DOCA-inhibition score[1] is the dose producing reversal of the DOAC Na/K effect: +3 = 10–50 μg/rat; +2 = 51–100; +1 = 101–800; ± = >800; 0 = inactive at any dose. [d] Activity [E. J. Cragoe, Jr., O. W. Woltersdorf, Jr., J. E. Baer, and J. M. Sprague, *J. Med. Chem.*, **5**, 896 (1962)] is based upon increase in urinary electrolyte and volume over control values referred to standards: +3 = activity of 100 mg/kg hydrochlorothiazide; +2 = activity of 100 mg/kg of chlorothiazide, 0 = controls. Compounds with activities between chlorothiazide and controls are scored +1 or ±. [e] J. B. Bicking, C. M. Robb, S. F. Kwong, and E. J. Cragoe, Jr., *J. Med. Chem.*, **10**, 598 (1967). [f] See ref 1. [g] K. L. Shepard, J. W. Mason, O. W. Woltersdorf, Jr., J. H. Jones, and E. J. Cragoe, Jr., *J. Med. Chem.*, **12**, 280 (1969).

(1) J. B. Bicking, J. W. Mason, O. W. Woltersdorf, Jr., J. H. Jones, S. F. Kwong, C. M. Robb, and E. J. Cragoe, Jr., *J. Med. Chem.*, **8**, 638 (1965).

(2) E. J. Cragoe, Jr., O. W. Woltersdorf, Jr., J. B. Bicking, S. F. Kwong, and J. H. Jones, *ibid.*, **10**, 66 (1967).

SCHEME I

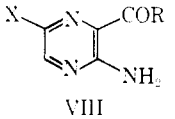


Compd[a]	X	Y	A	Synthetic method[b]	Recrystn solvent	% yield	Mp, °C
IIa	H	II		1	CH_3CN	73	235–237 dec
b	Cl	II		1	MeOH	75	200–202
IIIa	H	H	$NHC(=NH)NH_2$	2	HCl–NaOH	92	>300
b	Cl	II	$NHC(=NH)NH_2$	2	HCl–NaOH	78	260 dec
c	Cl	H	$NHNHC(=NH)NH_2$	2	HCl–NaOH	68	205 dec
d	Cl	H	$NHNH_2$	2	Dioxane	67	228–230 dec
e	Cl	H	$NHCH_3$	2	EtOH	53	170–172
IV				3	EtOH	45	185–187
V				2	HCl–NaOH	32	250 dec
VI				4	NaOH–HCl	72	240 dec

[a] All compounds were analyzed for C, H and N and found to be within ±0.4% of the theoretical value. [b] See the Experimental Section for the method that corresponds to each number.

razine *N*-oxides agree with those of B. Klein, *et al.*,[3–5] who have carried out extensive investigations in this area.

Structure–Activity Relationships.—As seen in Table I, *N*-amidino-3-aminopyrazinecarboxamide 4-oxide (IIIa) exhibited modest activity in both the rat DOAC-inhibition and normal rat assays. It is more active than the corresponding deoxy compound (VIIIa)



VIII

VIII	X	R
a	H	$NHC(=NH)NH_2$
b	Cl	$NHC(=NH)NH_2$
c	Cl	$NHNHC(=NH)NH_2$

in the DOCA-inhibition assay but less active in the normal rat test. Introduction of Cl in the 6 position (to produce IIIb) resulted in a decrease in activity both assays. This is contrary to the marked increase in activity produced by the corresponding change in the deoxy series (VIIIb).

(3-Amino-6-chloropyrazinecarboxamido)guanidine 4-oxide (IIIc) is equipotent to IIIb in the DOCA-inhibition test but much more active in the normal rat assay. It is much less active in the former test than the deoxy compound (VIIIc) but equipotent in the normal rat test.

It is interesting to note that the 5-chloro ester (IV), although inactive in the DOCA assay, is moderately diuretic as measured by the normal rat test.

Experimental Section[7]

Details of the synthesis of the new compounds are presented. Where several compounds of one type have been prepared by a

(3) B. Klein and J. Berkowitz, *J. Amer. Chem. Soc.*, **81**, 5160 (1959).
(4) B. Klein, J. Berkowitz, and N. E. Hetman, *J. Org. Chem.*, **26**, 127 (1961).
(5) B. Klein and P. E. Spoerri, *J. Amer. Chem. Soc.*, **73**, 2951 (1951).
(6) B. Klein, E. O'Donnell, and J. M. Gordon, *J. Org. Chem.*, **29**, 2623 (1964).

(7) Mr. K. B. Streeter, Mr. Y. C. Lee, and their staff have provided the analytical data. The melting points are corrected (open capillaries).

particular method, only one example is given. Pertinent data regarding each compound is recorded in Table II.

1. Methyl 3-Amino-6-chloropyrazinecarboxylate 4-Oxide (IIb).—A suspension of Ib (3.8 g, 0.02 mole) and *m*-chloroperbenzoic acid (3.4 g, 0.02 mole) in $CHCl_3$ (50 ml) was stirred at room temperature until the materials dissolved. This solution was refluxed for 1 hr and then chilled and the solid that separated was recovered by filtration. This solid was a mixture of the product and *m*-chlorobenzoic acid which upon recrystallization yielded the pure product.

2. The preparation of the ***N*-amidinopyrazinecarboxamides** IIIa, b, and V were carried out as described previously.[2] Compounds IIId and IIIe were prepared by treatment of IIb with the appropriate amine in EtOH, IIIc was made by treatment of IIb with aminoguanidine in MeOH.

3. Methyl 3-Amino-5-chloropyrazinecarboxylate (IV).—To a stirred suspension of IIa (2.5 g, 0.015 mole) in DMF (25 ml), $POCl_3$ (5 ml) was added in one portion. The reaction temperature rose to 90° and was maintained there for 10 min, then the mixture was poured into ice–water (100 ml). The clear solution was allowed to stand for 20 hr during which time the product separated.

4. 3-Amino-6-chloropyrazinecarboxylic Acid 4-Oxide (VI).—A suspension of IIb (3.0 g, 0.015 mole) in 5% NaOH (50 ml) was stirred at room temperature until a clear solution was obtained (1 hr). Addition of 20% NaOH (20 ml) precipitated the Na salt of the product which was recovered by filtration. This salt was dissolved in H_2O (50 ml), filtered, and the filtrate acidified (HCl) to obtain the pure product.

Modification of Recovered Dominant Lethal Mutations Induced by Heteroaromatic Aziridines in Stored Housefly Sperm

J. GEORGE POMONIS, DAVID T. NORTH, AND RICHARD G. ZAYLSKIE

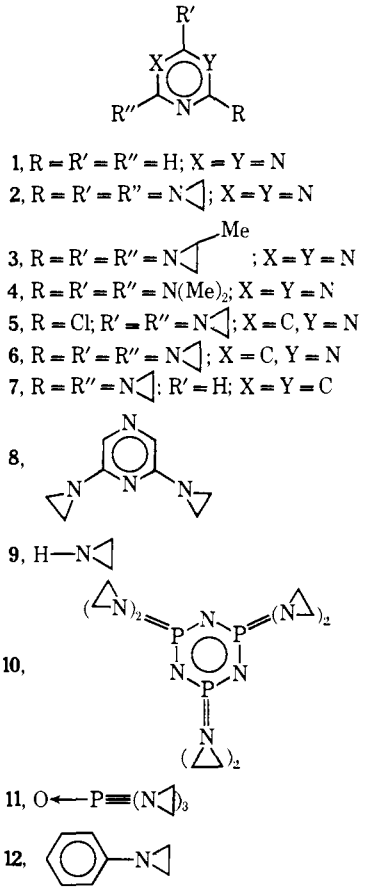
Metabolism and Radiation Research Laboratory, Agricultural Research Service, United States Department of Agriculture, Fargo, North Dakota 58102

Received February 6, 1970

The induction of genetic damage in mature sperm by biological alkylating agents has been well established.[1] For example, genetic damage induced in mature sperm of *Drosophila* increased as a function of time when it was stored in the female after treatment of the male with 2,4,6-tris(1-aziridinyl)-*s*-triazine (**2**)[2a–c] (Chart I). However, in the housefly, *Musca domestica* L., the number of recovered dominant lethal mutations decreased when sperm that had been treated with **2** or with some of its analogs and homologs were stored in the female for 7 days.[3] Furthermore, this decrease did not occur when the housefly was similarly treated with **10** and **11**.[3]

We therefore synthesized and tested a small number of aziridinyl-substituted heteroaromatic compounds to explore further this difference in storage effect between the house fly and *Drosophila*. We also compared the dominant lethal mutations induced by **2** or **4** with those induced by **10** or **11** to determine whether the heteroaromatic portion of the molecule was a factor in the decrease of dominant lethal mutations recovered from stored housefly sperm.

CHART I



Chemistry.—The synthesis of the title compounds was generally accomplished in a straight-forward manner by using the methods of Bestian[4] and Gilman, *et al.*[5] We also found that the method of Gilman, *et al.*[5] in which Li aziridine was used was of general utility in the synthesis of new compounds **7** and **8** as well as of **6**.[6] In general, the new compounds had the instability typical of this class and tended to polymerize or decompose on exposure to light or acidic conditions.

Biological Activity.—For each dose, 20 adult male houseflies (3 days old) were injected, held for 24 hr at 26°, and then allowed to mate. Each mating was individually observed. The mated females were held for 24 hr and then allowed to oviposit. The eggs were plated and counted; after 24 hr, the unhatched eggs were counted. In 7 days, the females were allowed to oviposit again. Dominant lethality is a function of unhatched fertilized eggs.[3]

Discussion

The genetic basis and time of expression of dominant lethal mutations in sperm have been discussed in detail.[1,3] The female housefly normally mates only once, but she stores the sperm in the spermatheca to use for many ovipositions. Moreover, she oviposits only when a suitable substrate is available such as the CSMA housefly medium. Thus, she is

(1) L. E. LaChance, D. T. North, and W. Klassen in "Principle of Insect Chemosterilization," Chapter 4, G. C. LaBrecque and C. N. Smith, Ed., Appleton-Century-Crofts, New York, N. Y., 1968, pp 99–157.

(2) (a) I. H. Herskowitz, *Genetics*, **40**, 574 (1955); (b) I. H. Herskowitz, *ibid.*, **41**, 605 (1956); (c) L. A. Snyder, *Z. Verbungslehre*, **94**, 182 (1963).

(3) D. T. North, *Mutat. Res.*, **4**, 225 (1967).

(4) H. Bestian, *Justus Liebigs Ann. Chem.*, **566**, 210 (1950).

(5) H. Gilman, N. N. Crouse, S. P. Massie, Jr., R. A. Benkeser, and S. M. Spatz, *J. Amer. Chem. Soc.*, **67**, 2106 (1945).

(6) Y. I. Bogodist and L. D. Protsenko, *Ukr. Khim. Zh.*, **32**, 1094 (1966); *Chem. Abstr.*, **66**, 94988 (1967).