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.

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

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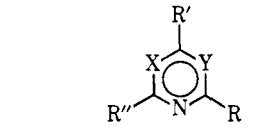
*Received February 6, 1970*

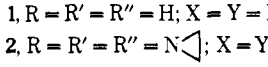
The induction of genetic damage in mature sperm by biological alkylating agents has been well established.[1] For example, genetic damage induced in mature sperm of *Drosophila* increased as a function of time when it was stored in the female after treatment of the male with 2,4,6-tris(1-aziridinyl)-*s*-triazine (**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.

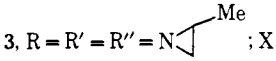
CHART I



1, R = R′ = R″ = H; X = Y = N

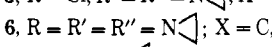


2, R = R′ = R″ = N; X = Y = N



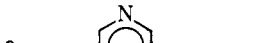
3, R = R′ = R″ = N; X = Y = N

4, R = R′ = R″ = N(Me)$_2$; X = Y = N

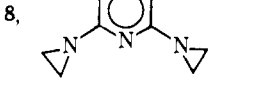


5, R = Cl; R′ = R″ = N; X = C, Y = N

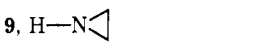
6, R = R′ = R″ = N; X = C, Y = N



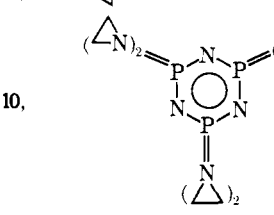
7, R = R″ = N; R′ = H; X = Y = C



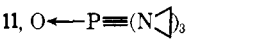
8,



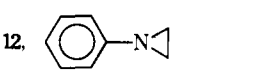
9, H—N



10,



11, O←P≡(N)$_3$



12,

**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).

TABLE I

EFFECTS OF STORAGE AT 27.5° ON THE FREQUENCY OF RECOVERED DOMINANT LETHAL MUTATIONS INDUCED IN MATURE SPERM OF MALE HOUSEFLIES WITH 1 μL OF VARIOUS CONCENTRATIONS OF ALKYLATING AGENTS

| Compd | Dose[a] (μg/fly) | % dom letal mutations at 24 hr | % dom lethal mutations at 7 days |
|---|---|---|---|
| 1 | 0.0 | 0.0 | 0.0 |
| | 0.81 | 0.8 | 0.0 |
| | 4.86 | 9.97 | 0.0 |
| | 4.86 | 8.05 | 0.0 |
| | 24.30 | 14.0 | 0.0 |
| 2[b] | 0.0 | 0.0 | 0.0 |
| | 0.01 | 25.8 | 4.9 |
| | 0.02 | 54.5 | 15.0 |
| | 0.03 | 73.3 | 20.4 |
| | 0.05 | 78.9 | 43.8 |
| | 0.06 | 90.2 | 50.5 |
| | 0.09 | 95.2 | 64.2 |
| | 0.12 | 94.4 | 88.8 |
| 3 | 0.0 | 0.0 | 0.0 |
| | 0.02 | 2.0 | 1.5 |
| | 0.04 | 0.2 | 8.8 |
| | 0.07 | 23.0 | 17.4 |
| | 0.15 | 24.0 | 38.0 |
| | 0.30 | 31.0 | 40.0 |
| | 0.58 | 69.0 | 55.0 |
| 4 | 0.0 | 0.0 | 0.0 |
| | 1.10 | 4.0 | 0.0 |
| | 1.58 | 18.0 | 0.0 |
| | 1.58 | 41.0 | 21.0 |
| | 2.10 | 57.0 | 17.0 |
| | 3.70 | 68.0 | 11.0 |
| | 4.10 | 96.2 | 58.0 |
| | 6.70 | 98.0 | |
| | 7.90 | 95.5 | 73.0 |
| | 10.50 | 100.0 | |
| | 13.40 | 98.0 | |
| 5[b] | 0.0 | 0.0 | 0.0 |
| | 0.02 | 13.4 | 5.8 |
| | 0.04 | 33.6 | 13.7 |
| | 0.06 | 54.0 | 25.5 |
| | 0.10 | 68.1 | 46.0 |
| | 0.20 | 75.6 | 51.3 |
| | 0.40 | 93.2 | 72.0 |
| 6 | 0.0 | 0.0 | 0.0 |
| | 0.00 | 0.0 | 10.0 |
| | 0.02 | 24.0 | 7.4 |
| | 0.03 | 54.5 | 14.0 |
| | 0.04 | 89.4 | 33.7 |
| | 0.06 | 95.2 | 54.3 |
| | 0.08 | 99.4 | 86.9 |
| | 0.12 | 99.5 | 90.9 |
| 7 | 0.0 | 0.0 | 0.0 |
| | 0.02 | 13.1 | 8.0 |
| | 0.03 | 15.4 | 0.0 |
| | 0.04 | 22.4 | 25.7 |
| | 0.16 | 97.4 | 57.4 |
| | 0.32 | 97.8 | 63.5 |
| 8 | 0.0 | 0.0 | 0.0 |
| | 0.01 | 41.0 | |
| | 0.02 | 51.7 | 45.8 |
| | 0.03 | 58.2 | 39.6 |
| | 0.05 | 28.7 | 69.9 |
| | 0.06 | 87.7 | 3.2 |
| | 0.16 | 98.0 | 67.8 |
| | 0.32 | 99.0 | 95.1 |
| | 0.65 | 99.9 | 98.9 |
| | 0.97 | 99.9 | 97.3 |
| | 1.30 | 99.9 | 98.6 |
| 1, plus 2[c,d] | 0.0 | 0.0 | 0.0 |
| | 0.024[e] | 0.0 | 11.3 |
| | 0.01 | 4.2 | 9.5 |
| | 0.015 | 12.3 | 5.3 |
| | 0.02 | 17.3 | 15.7 |
| | 0.03 | | 48.4 |
| | 0.04 | 60.9 | 39.0 |
| | 0.06 | 55.1 | 61.7 |
| | 0.08 | | 47.6 |
| | 0.12 | | 86.6 |

[a] Injected. [b] Reference 4. [c] Solutions containing 0.024 μg/μl of *s*-triazine were used to dissolve **2** in concentrations shown. [d] Saline solutions. [e] 0.024 μg/μl of *s*-triazine.

an ideal test organism for our studies. Also, since fertilization of housefly eggs occurs at the time of oviposition, such a biological system allows for (a) the removal of the sperm from prolonged influence of the test compound in the treated male, (b) sampling of the treated sperm at desired intervals, and (c) the opportunity to observe any effects on the rate of modification of dominant lethal mutations that might be caused by storing the inseminated female at lower temperatures.

In our studies the sperm were sampled at 24 hr and at 7 days after insemination. Table I compares the effects of the various compounds. All the substituted heteroaromatic compounds demonstrated a recovery effect, that is, fewer dominant lethal mutations were obtained when the sperm were stored for 7 days than when they were used 24 hr after insemination. However, aziridine (**9**) induced dominant lethal mutations but did not allow recovery; phenylaziridine (**12**) was very toxic and at low doses was ineffective,[7] and *s*-triazine (**1**) showed little mutagenic effect, even at relatively high doses. It was of particular interest that when mixtures of **1** (constant concentration) and **2** (graded concentrations) were injected, the frequency of recovered dominant lethal mutations was reduced at the initial sampling periods and apparently eliminated the storage effect at 7 days. (Compare this effect with that of **2** alone, Table I.) The result suggests possible competition of **1** with **2** for an unknown site or that **1** possibly inhibits a repair mechanism. There was also an absence of recovery effect when females storing treated sperm were kept for 7 days at a lower temperature (12.5°) before oviposition,[3] which suggests a possible enzymatic mechanism. However, the possibility that increased damage to

(7) Private communication from Ray F. Severson and Hollis M. Fine, Metabolism and Radiation Research Laboratory, ARS, USDA, Fargo, N. D.

the genetic material is occurring with time and that badly damaged sperm are being eliminated cannot now be discarded.

Sperm inactivation is another possible explanation for the decrease in frequency of mutation but it is rather difficult to show since the housefly is polyspermic.[3] However, **2** does not induce sperm inactivation in *Bracon*, even at doses greater than those needed to induce dominant lethal mutations in 100% of the sperm,[8] but with **11**, cytological examination established that the eggs were fertilized with the sperm from treated males.[3]

The data also suggest that mutagenic effectiveness is not necessarily directly related to the number of aziridinyl groups since compounds such as **5** and **7** were nearly as effective as **2**, and **8** was more active.

When the nonheteroaromatic compounds **9**, **10**, and **11** were tested for recovery, the number of recovered dominant lethal mutations did not decrease after storage for 7 days.

### Experimental Section[9,10]

Melting points were taken with a Thomas-Hoover apparatus and are corrected. Pmr spectra were obtained in $CDCl_3$ with $(CH_3)_4Si$ as an internal reference on a Varian A-60A spectrometer. The ir spectra were measured in KBr or CsI pellets on a Cary Model 14 spectrophotometer. All spectral correlations were as expected.[11] Elemental analyses were performed by Huffman Laboratories, Inc., Wheatride, Colo.

**Aziridinyllithium.**—The azyridinyllithium (AzLi) was generated at room temperature *in situ*.[5] The aziridine (Az) was diluted tenfold with solvent (either $C_6H_6$ or $Et_2O$), and drops of MeLi[12] (about 1.6 *M* in $Et_2O$) were added rapidly. Because of the low boiling point of Az, a 10% molar excess was used. All work with Li derivatives was done in flame-dried glassware under $N_2$.

**2-Chloro-4,6-bis(1-aziridinyl)pyrimidine (5).**[6]—A solution of 9 g of 2,4,6-trichloropyrimidine in 45 ml of $C_6H_6$ was added dropwise to a solution of 11 ml of Az and 14 ml of $Et_3N$ in 100 ml of $C_6H_6$ (in an ice bath). The ice bath was removed, and the solution was stirred for 4 hr. Charcoal was added to the mixture, and the suspension was filtered through Super Cel. The solution was concentrated *in vacuo* to 30 ml, and several portions of $C_6H_{14}$ were added; the crystals were allowed to grow between additions; yield 73%. The product was recrystallized from $C_6H_{14}$; mp 97° dec. *Anal.* ($C_8H_9ClN_4$) C, H, Cl, N.

**2,4,6-Tris(1-aziridinyl)pyrimidine (6).**[6] **Method A.**—Direct conversion of 2,4,6-trichloropyrimidine with AzLi yielded a mixture of partially substituted pyrimidine plus some trisaziridinyl product. Pmr data indicated the presence of both monoaziridinyldichloro isomers and **5**. Column chromatography on alumina did effect a separation, but the overall yield was poor. Compound **5** was therefore used as a starting material to improve the yield.

**Method B.**—AzLi (40 mmoles) was generated in 30 ml of $C_6H_6$. 2,4-Bis(1-aziridinyl)-6-chloropyrimidine (7.0 g) was added slowly (about 10 min) as a solid. After 3 hr of stirring, 1 ml of $H_2O$ and some charcoal were added to the mixture and the material was filtered. The solution was evaporated to near dryness, and 30 ml of $C_6H_{14}$ was added. The mixture was then triturated and the supernatant $C_6H_{14}$ was discarded. The residue was taken up in hot $C_6H_{14}$, charcoal was added, and the solution was allowed to cool after filtration. The compound was recrystallized from $C_6H_{14}$; yield 63%; mp 132–133° dec. *Anal.* ($C_{10}H_{13}N_5$) C, H, N.

**2,6-Bis(1-aziridinyl)pyridine (7).**—The compound was prepared in the same manner as **6**, method B, except that the quantity of AzLi was adjusted for the 2 equiv of Br to be replaced on 2,6-dibromopyridine. The product was recrystallized from $C_6H_{14}$; yield 65%; mp 98–100°. *Anal.* ($C_9H_{11}N_3$) C, H, N.

**2,6-Bis(1-aziridinyl)pyrazine (8).**—AzLi and 2,6-dichloropyrazine, when allowed to react by method B, produced the desired product in good yield. However, the reaction was somewhat more exothermic than the others reported; thus, the addition of the dichloro compound was slower. The product was recrystallized from $C_6H_{14}$; yield 75%; mp 57.5–59°. *Anal.* ($C_8H_{10}N_3$) C, H, N.

Compounds **1**, **2**, **3**, **4**, **9**, **10**, and **11** were prepared by methods described in the literature[4,5] or purchased from commerical sources. Compound **12** was prepared by the method of Heine, *et al.*[13]

**Acknowledgment.**—The authors express their gratitude for the technical assistance given by Gerald Holt of this laboratory.

(13) H. W. Heine, B. L. Kapur, and C. Mitch, *J. Amer. Chem. Soc.*, **76**, 1173 (1954).

(8) L. E. LaChance and A. P. Leverich, *Ann. Entomol. Soc. Amer.*, **61**, 164 (1968).

(9) Where analyses are indicated only by symbols of the elements or functions, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

(10) Mention of a proprietary product does not constitute a recommendation or an endorsement of this product by the U. S. Department of Agriculture.

(11) The ir assignments for azirdine were based on the paper by H. L. Spell, *Anal. Chem.*, **39**, 185 (1967).

(12) MeLi solution from Foote Mineral Co., was used directly.

## Potential Antineoplastics. V. Synthesis of Ethyl 2,3-Dioxobutyrate 2-Arylhydrazono-3-thiosemicarbazones

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*Received April 2, 1970*

Ever since the initial observation made by Brockman, *et al.*,[1] that 2-formylpyridine thiosemicarbazone possessed mild but definite antileukemic activity, chemists have tried to prepare several classes of thiosemicarbazones,[2-4] but virtually none of these appeared to be a clinically useful drug. In spite of this unpromising background we synthesized a few ethyl 2,3-dioxobutyrate 2-arylhydrazono-3-thiosemicarbazones, because the incorporation of an arylazo moiety in several cases resulted in the enhancement of the potency of candiate drugs.[5] Characteristics of the new ethyl 2,3-dioxobutyrate 2-arylhydrazono-3-thiosemicarbazones are summarized in Table I.

The synthetic route in all cases involved the coupling of aryldiazonium salts with ethyl 3-oxobutyrate 3-thiosemicarbazone.[6]

**Biological Activity.**—Shown in Table II are the data for antitumor activity against L-1210 lymphoid leukemia. From this primary screening it appears that the level of toxicity varied greatly in this group. Ethyl 2-(2,5-dimethylphenyl)hydrazono-, 2-(3,5-dimethylphenyl)hydrazono-, 2-(2,5-dimethoxyphenyl)hydrazono-, and 2-(2-methoxyphenyl)hydrozono-2,3-dioxobutyrate 3-thiosemicarbazones are somewhat more potent than other members in this series.

(1) R. W. Brockman, J. R. Thomson, M. J. Bell, and H. E. Skipper, *Cancer Res.*, **16**, 167 (1956).

(2) B. Prescott and C. P. Li, *J. Med. Chem.*, **7**, 383 (1964).

(3) A. B. Sen and R. N. Kapoor, *Indian J. Appl. Chem.*, **31**, 171 (1968).

(4) K. C. Agrawal and A. C. Sartorelli, *J. Med. Chem.*, **12**, 771 (1969), and earlier ref cited therein.

(5) R. E. Harmon, F. E. Dutton, and H. D. Warren, *ibid.*, **11**, 627 (1968).

(6) S. C. De and D. N. Dutt, *J. Indian Chem. Soc.*, **7**, 473 (1930).