Carcinogenic Activity of Some Heterocyclic Analogs of p-Dimethylaminoazobenzene*

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Miller and Baumann (4), in a study of the relationship of the chemical structure of p-dimethylaminoazobenzene (DAB) to its potency in inducing hepatoma formation in rats, prepared a series of methylated derivatives of the parent compound. These workers found that 3'-methyl-DAB was not only more active than the parent compound but was the most active of all the azo dyes investigated up to that time (2). The carcinogenic action of DAB and its substitution products has been recently reviewed and discussed (3, 5).

In the present study, it seemed of interest to investigate the effect of changes in the nucleus or skeletal structure of DAB on the potency of this hepatic carcinogen. Accordingly, a series of heterocyclic analogs of DAB, prepared by Faessinger and Brown (1), in which the pyridine or thiazole nucleus was substituted for one of the benzene rings, was tested for carcinogenic activity. Owing to the presence of the hetero atom or atoms in these rings, position isomerism becomes an important factor. Therefore, the various position isomers of the analogs of DAB were synthesized and tested in the hope of obtaining a correlation of chemical structure and carcinogenic activity. It also seemed of interest to prepare1 and compare the effect of the so-called N- or 1-oxides of the pyridineazo-p-dimethylanilines.

MATERIALS AND METHODS

The series of analogs of DAB which was tested in this study is listed in Table 1, together with the code name by which each compound was designated. Young male rats of the Sprague-Dawley strain, approximately 8 weeks of age and weighing 150—200 gm., were distributed as equally as possible with regard to initial body weights into eight groups of ten animals each. Each group was fed a diet patterned after the "low protein, low ribo-

flavin" diet of Miller et al. (5) to which had been added one of the test substances at a level of 0.06 per cent. The composition of the basal diet on a kilogram basis was as follows: crude casein, 120 gm.; cerelose, 770 gm.; salt mixture (Osborne and Mendel), 40 gm.; corn oil, 50 gm.; Vitab (rice bran concentrate), 20 gm.; riboflavin, 0.5 mg.; vitamin A concentrate, 67,500 I. U. A ninth group of rats receiving the basal diet alone served as the control. The fact that the control group ate well, grew normally, and remained healthy throughout the experiment attested to the adequacy of this diet in supplying the necessary nutrients. All the rats were kept individually in screen-bottomed cages and were offered food and water ad libitum. Weight gains, mortality rates, food consumption, and liver weights were recorded. Animals from each group were sacrificed at the end of 4 months of feeding and at 2-month intervals thereafter. Gross and microscopic examinations were carried out on the extirpated livers. The response elicited from rats consuming DAB at the same level as those receiving the test substances and maintained under identical experimental conditions was used as a standard of comparison in ascertaining the potency of our test compounds.

RESULTS AND DISCUSSION

The effect of the various pyridine analogs and of the thiazole analog on the gross appearance of the livers of rats sacrificed at the stated intervals is compared to that of DAB in Table 1. The data indicate that P-2, P-3, T-2, and PO2 do not cause tumors by the end of a year. P-4 is active but probably somewhat less so than DAB, while PO4 seems more active than DAB.

Sections were made of the livers of rats from the various groups and were examined by Dr. George T. Hoffman of St. Vincent's Hospital of the City of New York. A summary of his findings is given in Table 2. It would appear, according to Dr. Hoffman's data, that the compounds P-2, P-3, T-2, and

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1 Faessinger, Malloy, and Brown, unpublished work.

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* Obtained from the Nopco Chemical Co.
PO	extsubscript{04} cause initial fatty changes which disappear without tumor formation and that P-4 produces tumors at 6 months, while PO	extsubscript{4} produces tumors within 4 months. Variations in tumor patterns are probably of no significance.

Because the carcinogenic activity of PO	extsubscript{4} was so much more pronounced than that of DAB, its potency in inducing hepatoma formation was compared to that of S'-methyl-DAB. The latter was chosen as a standard of comparison, because it is considered to be as active as any of the azo dyes of the DAB series tested and reported to date. A group of five animals was fed the diet containing PO	extsubscript{4}, while another group of five rats was fed the basal diet containing S'-methyl-DAB at the same level. Because of the high toxicity of PO	extsubscript{4}, it was necessary about once each week for the first 6 weeks to give some of the basal control diet to all animals in order to insure their survival. The control diet was also substituted for the S'-methyl-DAB diet at the same times in order to insure an optimum basis of comparison of the relative carcinogenicity of the two compounds. The results of this experiment are summarized in Table 3.

The data indicate that PO	extsubscript{4} is more active than S'-methyl-DAB, although the intermittent feeding and the small number of animals tested make an exact quantitative comparison impossible. Further

### Table 1

**Tumor Incidence* among the Various Groups of Rats Receiving Heterocyclic Analogs of DAB**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Code</th>
<th>4 mo.</th>
<th>6 mo.</th>
<th>8 mo.</th>
<th>10 mo.</th>
<th>18 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyridine-2-azo-p-dimethylaniline</td>
<td>P-2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Pyridine-3-azo-p-dimethylaniline</td>
<td>P-3</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Pyridine-4-azo-p-dimethylaniline</td>
<td>P-4</td>
<td>0/2</td>
<td>2/2</td>
<td>4/4</td>
<td>no survivors</td>
<td></td>
</tr>
<tr>
<td>Thiazole-2-azo-p-dimethylaniline</td>
<td>T-2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Pyridine-1-oxide-2-azo-p-dimethylaniline</td>
<td>P02</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Pyridine-1-oxide-4-azo-p-dimethylaniline</td>
<td>P04</td>
<td>5/5</td>
<td>no survivors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Dimethylaminoaazobenzene</td>
<td>DAB</td>
<td>1/5</td>
<td>2/5</td>
<td>2/2</td>
<td>2/2</td>
<td>no survivors</td>
</tr>
</tbody>
</table>

* Tumor incidence = number of rats with hepatic tumors/number of rats sacrificed.

### Table 2

**Summary of Histological Data Obtained from the Livers of Rats Receiving Heterocyclic Analogs of DAB**

<table>
<thead>
<tr>
<th>Code</th>
<th>4 months</th>
<th>6 months</th>
<th>8 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-2</td>
<td>Diffuse fatty changes to normal</td>
<td>Moderate fatty changes</td>
<td>Moderate fatty changes</td>
</tr>
<tr>
<td>P-3</td>
<td>Moderate fatty changes, less than P-2</td>
<td>Normal</td>
<td>Slight irregularity of lobular pattern, some large or double nuclei</td>
</tr>
<tr>
<td>P-4</td>
<td>Slight fatty changes</td>
<td>Nodular tumors; two kinds of neoplasm-hepatoma and papillary adenocarcinoma arising from bile ducts</td>
<td>Large nodules of necrotic tumor of liver cell type</td>
</tr>
<tr>
<td>T-2</td>
<td>Marked fatty changes</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>PO	extsubscript{2}</td>
<td>Normal</td>
<td>Moderate fatty changes</td>
<td>Normal</td>
</tr>
<tr>
<td>PO	extsubscript{4}</td>
<td>Liver entirely replaced by papillary-type tumor, fibrous tissue reaction, acute inflammatory necrosis</td>
<td>No survivors</td>
<td></td>
</tr>
<tr>
<td>DAB</td>
<td>One animal with multiple tumor nodules, fibrous tissue reaction, inflammation, fatty changes; two animals, livers normal</td>
<td>Two animals with tumor masses of liver cell type surrounded by slight fatty changes; one animal liver normal</td>
<td>No survivors</td>
</tr>
<tr>
<td>Control</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

### Table 3

**Responses of Rats Receiving PO	extsubscript{4} and S'-Methyl-DAB**

<table>
<thead>
<tr>
<th>Survival (4 months)</th>
<th>PO	extsubscript{4}</th>
<th>5'-Me-DAB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Average body weight of survivors</td>
<td>145 gm.</td>
<td>230 gm.</td>
</tr>
<tr>
<td>Weight change (3 months)</td>
<td>19 gm.</td>
<td>32 gm.</td>
</tr>
<tr>
<td>Liver weight as per cent of body weight</td>
<td>15 per cent</td>
<td>9 per cent</td>
</tr>
<tr>
<td>Food consumption (gm/day)</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>Tumor formation (4 months)</td>
<td>4/5 had massive tumor formation; 1/5 had nodulation and small tumors</td>
<td>3/5 had extensive nodulation to definite tumors; 1/5 had slight nodulation; 1/5 normal</td>
</tr>
</tbody>
</table>
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studies on the effect of substitution in the pyridine ring of this potent analog of DAB on its carcino
genic activity are now in progress.

SUMMARY

1. A series of compounds in which the pyridine, pyridine-1-oxide, or thiazole rings were substituted
for the benzene nucleus of p-dimethylaminoozobenzene (DAB) was tested on male rats of the
Sprague-Dawley strain.
2. These compounds were pyridine-2-azo-p-dimethylaniline, pyridine-3-azo-p-dimethylaniline,
pyridine-4-azo-p-dimethylaniline, thiazole-2-azo-p-dimethylaniline, pyridine-1-oxide-2-azo-p-di-
methylaniline, and pyridine-1-oxide-4-azo-p-dimethylaniline (PO4).
3. The pyridine-2-, pyridine-3-, thiazole-2-, and pyridine-1-oxide-2-azo analogs showed no
carcinogenic action after 12 months of feeding. The pyridine-4- and the pyridine-1-oxide-4-azo
analogs were carcinogenic.
4. In an experiment designed to test the relative potencies of PO4 and 3'-methyl-DAB, one of
the most active compounds of the DAB series, PO4 appeared to possess a greater degree of car-
cinogenic activity.

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. George T. Hoff-
man of St. Vincent's Hospital of the City of New York for the
histological data listed in Table 2.

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