Carcinogenic Activity of Some Heterocyclic Analogs of p-Dimethylaminoazobenzene*  

II. Effects of Methyl Groups in the Pyridine Ring  

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The first paper of this series (1) reported the results of tests of the carcinogenic activity of certain analogs of p-dimethylaminoazobenzene (DAB) in which a pyridine ring or a thiazole ring replaced one of the benzene rings of DAB. Due to the presence of the hetero atom or atoms, position isomers are possible in the heterocyclic analogs, and some of these isomers were synthesized and tested.

The wide variations in activity exhibited by isomers in the pyridine and pyridine-1-oxide series suggested the synthesis and testing of certain additional analogs, some of which are reported here. In the pyridine series, pyridine-2-azo-p-dimethylaniline (P2) and pyridine-3-azo-p-dimethylaniline (P5) produced no tumors in the test animals during the period of observation, whereas pyridine-4-azo-p-dimethylaniline (P4) was effective in inducing tumors. In the pyridine-1-oxide series, the variation in activity was much more striking, since pyridine-1-oxide-2-azo-p-dimethylaniline (PO2) was found to be inactive, whereas pyridine-1-oxide-4-azo-p-dimethylaniline (PO4) was found to be extremely active.

One of the interesting findings of J. A. Miller and co-workers (4) in their extensive studies on the carcinogenicity of derivatives of DAB was that the introduction of substituents into the unsubstituted benzene ring produced varying effects depending upon the position of the substituent relative to the azo linkage. The effect of a given substituent may be to increase or decrease the activity relative to the parent compound, but, as a general rule, the activity of the derivatives is in the order m' > o' > p'. For example, where the substituent is the methyl group, m'-methyl-DAB is not only more active than the o' and p' derivatives, but is about twice as active as DAB.

To investigate further the wide variations in activity reported in the previous paper (1) and to evaluate the effect of m'-methyl substitution, analogs of DAB in which methyl groups were appropriately positioned in the pyridine ring were synthesized (2,3) and tested for carcinogenic activity. In the case of P2 and PO2, there are two isomers possible with methyl groups meta to the azo linkage. In the case of P4 and PO4, only one such isomer is possible. All these derivatives were synthesized and tested. The maximum variation in activity of isomers observed had been in the pyridine-1-oxide series between PO2 and PO4. In the work reported here, pyridine-1-oxide-3-azo-p-dimethylaniline (PO3) is included to complete this series. To determine whether the activity of PO4 could be diminished by substitution of methyl groups in positions other than meta, o'-methyl PO4 and di-m'-methyl PO4 (designated 3-Me PO4 and 2,6-diMe-PO4, respectively) were synthesized and tested.

MATERIALS AND METHODS

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 riboflavin” diet of Miller et al. (1,4), to which had been added one of the test substances at a level of 0.06 per cent. Vitamin A was supplied in the form of a corn oil solution of Vitamin A Palmitate.1

The methods employed were the same through-

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1 We are grateful to Chas. Pfizer & Co., Inc., for a generous supply of the Vitamin A Palmitate.
out except in cases where toxicity and/or carcinogenic activity of the test compounds was too great to allow the feeding of the usual concentration of 0.06 per cent in the diet. Then the concentrations of the test compounds and, in some cases, the time intervals between sacrifices of the test animals were reduced.

RESULTS AND DISCUSSION

The tumor incidences for the rats fed the various pyridine analogs and sacrificed at the stated level are shown in Table I. The data obtained with DAB under the same conditions are included in the table.

The two isomeric m'-methyl derivatives of P2, 4-Me-P2 and 6-Me-P2, were found to be inactive, no tumors being observed in the 10-month interval. Similarly, the two isomeric m'-methyl derivatives of PO2, 4-Me-PO2 and 6-Me-PO2, were found to be inactive, no tumors being observed in the same period. In these cases, then, the introduction of methyl groups, suitably positioned by analogy with the DAB series, does not cause a noticeable increase in the activity of the parent compounds. The m'-methyl derivative of P4, 2-Me-P4, exhibited activity greater than that of the parent compound and approximately equivalent to that of DAB both in the time required for tumors to appear and also in extent of tumor growth. PO3, the structural isomer of the inactive PO2 and the highly active PO4, exhibited activity intermediate between that of the two isomers, being approximately equal to DAB in time required for tumors to appear.

All the derivatives of PO4, the most active of the compounds previously reported, exhibited high activity. The compound containing a methyl group meta to the azo linkage, 2-Me-PO4, was more active than PO4 itself; the compound with a methyl group ortho to the azo group, 3-Me-PO4, was slightly less active than the parent compound.

When there were two methyl groups meta to the azo linkage, as in 2,6-diMe-PO4, the compound was very active but also quite toxic, so that it was necessary to reduce the level in the diet. Under these conditions, 2,6-diMe-PO4 appeared to be the most active compound tested, but paired feeding of 2,6-diMe-PO4 and 2-Me-PO4 will be necessary to establish this last point. Even at the level of 0.02 per cent, 2-Me-PO4 was seemingly active, causing earlier and more extensive cirrhosis than the parent compound. In the case of 2,6-diMe-PO4, none of the animals survived the first month of administration of the carcinogen at the usual level of 0.06 per cent. A second group of animals was fed at the 0.02 per cent level for 1 month, after which the level was increased to 0.03 per cent. All the animals receiving 2-Me-PO4 and 2,6-diMe-PO4, even at the reduced levels, for 1 month exhibited cirrhosis, and tumor growth was extensive in the

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### TABLE 1

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>CODE</th>
<th>LEVEL IN DIET (per cent)</th>
<th>1 mo.</th>
<th>2 mo.</th>
<th>3 mo.</th>
<th>4 mo.</th>
<th>5 mo.</th>
<th>6 mo.</th>
<th>8 mo.</th>
<th>10 mo.</th>
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</thead>
<tbody>
<tr>
<td>4-Methylpyridine-2-azo-p-dimethyl-aniline</td>
<td>4-Me-P2</td>
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<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<tr>
<td>6-Methylpyridine-2-azo-p-dimethyl-aniline</td>
<td>6-Me-P2</td>
<td>a</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<tr>
<td>4-Methylpyridine-1-oxide-2-azo-p-dimethylaniline</td>
<td>4-Me-PO2</td>
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<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<td>Pyridine-1-oxide-8-azo-p-dimethyl-aniline</td>
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<td>0/2</td>
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<td>2/3</td>
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<td>3-Methylpyridine-1-oxide-4-azo-p-dimethylaniline</td>
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<tr>
<td>p-Dimethylaminoazo benzene</td>
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<td>a</td>
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<td>1/3</td>
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<td>2-Methylpyridine-1-oxide-4-azo-p-dimethylaniline</td>
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<td>0.02</td>
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<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<td>2,6-diMe-PO4</td>
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</tr>
</tbody>
</table>

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

† At the end of the first month the level was increased to 0.08 per cent.

§ Diet level was reduced by intermittent use of carcinogen-free diet. The number of days on and off the carcinogenic diet are indicated by the following sequence where numbers in parentheses are days on basal diet with n-carbonogen. 11-(8)-6-(1)-1-(2)-11-(8)-5O-(4)-34.

† These rats, while having no tumors, did show advanced cirrhosis.

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animals fed 2,6-diMe-PO4 at 4 months. 2-Me-PO4 and m'-methyl-DAB were compared in closely controlled paired-feeding experiments as had been done with PO4 in the previous work (1). The results are shown in Table 2. On the basis of these

### Table 2

<table>
<thead>
<tr>
<th>RESPONSES OF RATS RECEIVING 2-ME-PO4 AND m'-METHYL-DAB*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-ME-PO4</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Survival at 4 months</td>
</tr>
<tr>
<td>Av. body weight of survivors</td>
</tr>
<tr>
<td>Av. weight change at 4 months</td>
</tr>
<tr>
<td>Av. liver weight as per cent of body weight</td>
</tr>
<tr>
<td>Tumor formation at 4 months</td>
</tr>
</tbody>
</table>

* The carcinogens at the 0.06 per cent level were fed intermittently to both groups in identical manner. The number of days on and off the carcinogenic diet are indicated by the following sequence where numbers in parentheses are days on basal diet with no carcinogen. 11-(8)-6-(1)-1-(8)-11-(4)-30-(4)=34.

results, 2-Me-PO4 seems far more active than m'-methyl-DAB and more active than PO4.

Sections were made of the livers of rats from the various groups and were examined by Dr. George T. Hoffmann of St. Vincent's Hospital of the City of New York. His examination indicated that the tumors were of the same type reported in our first article (1) and that where no tumors were present the livers were either normal or cirrhotic (Table 1).

**SUMMARY**

1. A new series of compounds in which the pyridine and pynidine-1-oxide rings were substituted for a benzene ring of p-dimethylaminoazobenzene (DAB) were prepared and tested for ability to induce rat tumors.
2. The compounds tested were 4-methylpyridine-2-azo-p-dimethylaniline (4-Me-P2), 6-methylpyridine-2-azo-p-dimethylaniline (6-Me-P2), 4-methylpyridine-1-oxide-2-azo-p-dimethylaniline (4-Me-P02), 6-methylpyridine-1-oxide-2-azo-p-dimethylaniline (6-Me-P02), 2-methylpyridine-4-azo-p-dimethylaniline (2-Me-P4), pyridine-1-oxide-3-azo-p-dimethylaniline (PO3), 2-methylpyridine-1-oxide-4-azo-p-dimethylaniline (2-Me-PO4), 3-methylpyridine-1-oxide-4-azo-p-dimethylaniline (3-Me-PO4), and 2,6-dimethylpyridine-1-oxide-4-azo-p-dimethylaniline (2,6-diMe-PO4).
3. Over a period of 10 months' administration of the test compounds, 4-Me-P2, 6-Me-P2, 4-Me-P02, and 6-Me-P02 were ineffective in tumor production. 2-Me-P4 was observed to be more effective than P4 and about equivalent to DAB. PO3 was observed to be less active than P04 and about as active as DAB. 3-Me-P04 seemed to be more active than DAB and about equal to m'-Me-DAB. 2-Me-PO4 and 2,6-diMe-PO4 both appeared far more active than m'-Me-DAB and both are more active than PO4, which was the most potent of the compounds previously tested.

**ACKNOWLEDGMENTS**

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**REFERENCES**

Carcinogenic Activity of Some Heterocyclic Analogs of p-Dimethylaminoazobenzene: II. Effects of Methyl Groups in the Pyidine Ring

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