Hepatocarcinogenicity of the Trimethyl Homologs of 4-Dimethylaminazobenzene

Ellis V. Brown* and Alice Kruegel

Department of Chemistry, University of Kentucky, Lexington, Kentucky 40506. *Received August 11, 1971*

Definite to strong carcinogenic activity has been shown by some monosubstituted and disubstituted derivatives of 4-dimethylaminoazobenzene (DAB).[1-3] The only active trisubstituted-DAB tested has been the 2',4',6'-trifluoro derivative.[4] It seemed of interest to synthesize and test for rat hepatocarcinogenic activity all of the trimethyl homologs of DAB with Me groups in the primed positions only. These are all new compounds and can be prepared by the diazotization of the proper trimethylanilines followed by coupling with $PhNMe_2$. The new azo compounds are listed in Table I.

Experimental Section

All melting points were detd on a Fisher-Johns apparatus and are uncorrected. The C, H, N analyses were performed in this department on an F and M Model 185 analyzer by Mr. Daryl Sharp. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

Trimethylanilines. Mesidine,[5] bp 224–228°, was prepd from nitromesitylene[6] by reduction with Sn–HCl. 5-Aminopseudocumene,[7] mp 62–63°, was prepd from 5-nitropseudocumene[8] in the same way. 4-Aminohemimellitene,[8] mp 29–27°, was obtd from 5-nitrohemimellitene,[8] 6-aminopseudocumene[9] was prepd from 6-nitropseudocumene,[10] and 5-aminohemimellitene,[11] mp 75–78°, was prepared from 5-nitrohemimellitene[8] by reduction (Fe–AcOH). 3-Aminopseudocumene[9] was produced by Fe–AcOH reduction of 3-nitropseudocumene which in turn was produced by the hypophosphorus acid reduction of the diazonium salt from 3-nitro-6-aminopseudocumene.[12]

2',4',6'-Trimethyl-DAB. Mesidine (60 g) was dissolved in a mixt of 113 ml of concd HCl and 376 ml of H_2O and diazotized at 0° using 30.6 g of $NaNO_2$ in 150 ml of H_2O. One-half hr after the final addn, a soln of 54 g of $C_6H_5NMe_2$, 552 ml of 95% EtOH, 264 ml of H_2O, and 109 g of NaOAc was added, and the soln was stirred for 24 hr. Extn with PhH and evapn of PhH left a semisolid material which was submitted to column chromatog over alumina in toluene–heptane soln. The first orange band was eluted, and the solvent was removed to give 19.9 g of the dye as bright orange needles which were recrystd from abs EtOH, mp 104.5–106° (see Table I).

Biological Properties. Young male rats of the Sprague–Dawley strain, approximately 8 weeks old and weighing 150–200 g, were distributed as equally as possible in initial body weight into groups of 10 animals each. Each group was fed a diet, patterned after the "low protein, low riboflavin" diet of Miller[1] to which had been added one of the azo compounds at a level of 0.06%. The composition of the basal diet per kilogram was as follows: crude casein, 120 g; Cerelose, 770 g; Osborne and Mendel salt mixt, 40 g; corn oil, 50 g; Vitab (rice bran concentrate, from Charles Bowman Co.), 20 g; riboflavin, 0.5 mg; vitamin A palmitate, 67,500 IU.

A group received DAB at the 0.06% level while the control group received only the basal diet. All the rats were kept individually in screen-bottomed cages and were offered food and water *ad libitum*. Laparotomies were performed at the indicated times and microscopic examinations were made whenever an animal died or at the end of the experiment.

Table I. Trimethyl-4-dimethylaminoazobenzenes

Compd	Mp, °C	Yield[a] recryst %	Formula[b]
2',4',6'-Trimethyl-DAB	104.5–106	17	$C_{17}H_{21}N_3$
2',4',5'-Trimethyl-DAB	145–146.5	55	$C_{17}H_{21}N_3$
2',3',4'-Trimethyl-DAB	147–151	26	$C_{17}H_{21}N_3$
2',3',5'-Trimethyl-DAB	101.5–103	25	$C_{17}H_{21}N_3$
3',4',5'-Trimethyl-DAB	131.5–133	57	$C_{17}H_{21}N_3$
2',3',6'-Trimethyl-DAB	132.5–134	8	$C_{17}H_{21}N_3$

[a] All samples recrystd from 95% EtOH after chromatog on alumina from toluene–heptane. [b] All compds were analyzed for C, H, N.

Results

DAB gave tumor incidences of 6/10 at 4 months and 9/10 at 6 months, 3',4',5'-DAB gave 9/10 at 4 months and 10/10 at 6 months. None of the other trimethyl-DAB homologs produced tumors at 9 months at which time the experiment was terminated. 3',4',5-Trimethyl-DAB appears to be about as hepatocarcinogenic as DAB.

Acknowledgment. The authors are indebted to Dr. Daniel L. Weiss and Dr. T. Yoneyama, Department of Pathology, University of Kentucky College of Medicine, for the microscopic evaluation of the tumors.

References

(1) J. A. Miller and E. C. Miller, *Advan. Cancer Res.*, 1, 339 (1953).
(2) E. V. Brown and A. A. Hamdan, *J. Nat. Cancer Inst.*, **27**, 663 (1961).
(3) E. V. Brown, *J. Med. Chem.*, 11, 1234 (1968).
(4) J. A. Miller, E. C. Miller, and R. W. Sapp, *Cancer Res.*, 11, 269 (1951).
(5) A. Landenburg, *Justus Liebigs Ann. Chem.*, **179**, 163 (1875).
(6) J. E. Purvis, *J. Chem. Soc.*, **97**, 1546 (1910).
(7) M. Dolinsky, J. H. Jones, C. D. Ritchie, R. L. Yates, and M. A. Hall, *J. Ass. Offic. Agr. Chem.*, **42**, 709 (1959).
(8) C. H. Fisher and C. T. Walling, *J. Amer. Chem. Soc.*, **57**, 1700 (1935).
(9) K. Sato, Y. Fujima, and A. Yamada, *Bull. Chem. Soc. Jap.*, **41**, 442 (1968).
(10) H. Hock and H. Kropf, *Chem. Ber.*, **89**, 2436 (1956).
(11) F. M. Beringer and I. Ugelow, *J. Amer. Chem. Soc.*, **75**, 2635 (1953).
(12) A. Huender, *Recl. Trav. Chim. Pays-Bas.*, **34**, 1 (1915).

Some Aliphatic Amines as Antipityrosporum Agents

Harold Dixon, Norman J. Van Abbé,

Beecham Products (U. K.), Brentford, England

and John K. Sugden*

School of Pharmacy, City of Leicester Polytechnic, Leicester, England. *Received May 26, 1971*

Pityrosporum ovale and *orbiculare*, budding yeasts of the family *Cryptococcacae*[1] are not usually regarded as pathogens. Van Abbé[2] has discussed the possible relationship between dandruff and the presence of *P. ovale* on the scalp. Tinea versicolor was formerly attributed to the presence of *Malassazia furfur*,[3] but more recent work has shown that *M. furfur* and *P. orbiculare* are probably the same organisms in different phases of growth.[4] It is claimed that *P. ovale* and *P. orbiculare* are only pathogenic in susceptible persons. In spite of this limited pathogenicity or perhaps limited virulence, these yeasts do seem to be implicated in the causation of skin disorders and their suppression is clinically desirable. Much of the doubt as to the pathogenicity of *Pityrosporum* species has arisen from the difficulties encountered in artificial culture, since isolation and maintainance *in vitro* of these lipophilic organisms are markedly influenced by variations in the constituents of the media. This sensitivity makes assessment of antipityrosporum agents difficult and earlier reports of measurements suspect. Developments in the cultural technique[1,2] have much reduced the problems of assessment of this class of compound. Recent work in pyrrolidine chemistry has shown that ethyl *N*-alkyl-4-hydroxy-