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-

Table I

RNHYX

No.	R	X	Y	Mp or bp (mm), °C	Formula[a]	Form	Yield, %	MIC, μg/ml: *P. ovale* strains 1	2	3	*P. orbiculare* strains 8	14
1	C_8H_{17}	CO_2Et	$(CH_2)_2$	134-136 (0.8)	$C_{13}H_{27}NO_2$	Base	48	>1000	>1000	>1000	128	64
2	$C_{10}H_{21}$	CO_2Et	$(CH_2)_2$	179-180	$C_{15}H_{32}ClNO_2$	HCl Salt	56	125	125	125	125	125
3	$C_{16}H_{33}$	$CONH_2$	$(CH_2)_2$	78-80	$C_{19}H_{40}N_2O$	Base	26	500	500	500	>1000	>1000
4	$C_{16}H_{33}$	CN	$(CH_2)_2$	38-40	$C_{19}H_{38}N_2$	Base	81	500	500	500	500	500
5	$C_{16}H_{33}$	CN	$(CH_2)_2$	64-65	$C_{21}H_{40}N_2O$	Acetyl deriv of 4	55	>1000	>1000	>1000	>1000	>1000
6	$C_{12}H_{25}$	CO_2Et	$(CH_2)_2$	171-172	$C_{17}H_{36}BrNO_2$	HBr salt	50	31	31	31	125	125
7	$C_{12}H_{25}$	CO_2Et	$(CH_2)_2$	3-5	$C_{19}H_{37}NO_3$	Acetyl deriv of 6	50	>1000	>1000	>1000	>1000	>1000
8	C_8H_{17}	CO_2Et	$(CH_2)_2$	200 (11)	$C_{15}H_{29}NO_3$	Acetyl deriv of 1	55	500	500	500	>1000	>1000
9	$C_{12}H_{25}$	CO_2Et	$CH_2CH(CH_3)$	135-136	$C_{18}H_{38}BrNO_2$	HBr salt	16	125	125	125	256	256[b]
10	$C_{12}H_{25}$	CO_2Me	$(CH_2)_2$	182-184	$C_{16}H_{34}BrNO_2$	HBr salt	30	62.5	62.5	62.5	>1000	>1000
11	$C_{12}H_{25}$	CO_2Et	CH_2	180 (0.5)	$C_{16}H_{33}NO_2$	Base	45	500	500	500	>1000	>1000
12	$C_{11}H_{23}$	CO_2Et	$(CH_2)_2$	176 (0.8)	$C_{16}H_{33}NO_2$	Base	64	31	31	31	64	64
13	$C_{15}H_{31}$	CO_2Et	$(CH_2)_2$	220 (0.8)	$C_{20}H_{41}NO_2$	Base	63	31	31	31	128	128
14	$C_{16}H_{33}$	CO_2Et	$(CH_2)_2$	231-232	$C_{21}H_{44}ClNO_2$	HCl salt	25	31	31	31	500	1000
15	$C_{12}H_{25}$			245-247	$C_{12}H_{28}BrN$	HBr salt	80	62.5	125	62.5	>1000	>1000
Zinc pyrithione as a control								16	16	16	8	8

[a] All compds analyzed for C, H, N. [b] Partial inhibition at 128 μg/ml.

5-oxo-3-pyrroline-3-carboxylates have moderate activity against *P. ovale* and *P. orbiculare*.[5] The object of the present work is to investigate the antipityrosporum activity of smaller molecules than those described before[5] and to indicate some structure-activity relationship with regard to *P. ovale* and *P. orbiculare*.

Examination of Table I shows that amines (**1**) have modest activity against *P. ovale* and *P. orbiculare*. *P. orbiculare* is a much less vigorous organism in artificial culture and more exacting in its growth requirements than is *P. ovale*. The selectivity of **1**, **10**, and **14** does tend to justify the classification of these yeasts as distinct species. In the case of *P. ovale* there is some correlation between the tests on malt extract Tween agar medium (without added fatty acids or bile salts) and the suspension assay technique.

Optimal activity against *P. ovale* of amines **1** on malt extract Tween agar medium was observed in those compounds where R was C_{10} or more. The nature of the functional group, X, was significant, X = CO_2Et showing maximal inhibition, X = CO_2Me being slightly less active, with X = $CONH_2$ much less active, and X = CN having minimal activity of the groups tested.

The presence of a secondary amino group was found to be essential for the activity of amines **1** in both assays. The nature of the side chain Y had considerable influence on the activity of the compounds against *P. ovale* in the malt extract Tween agar medium assay (**11** and **9** being less active than **6**), but possibly less effect on activity when tested by the suspension assay.

With regard to *P. orbiculare* (Table I) there is no indication that the length of the aliphatic chain R is critical for inhibitory action. The secondary amino group appears to be essential, since acetylation abolishes activity (**7** and **8**). Apparently optimal activity is found when the side chain, Y, is $(CH_2)_2$, **6** being more active than **9** and much more active than **11**. The nature of the functional group, X, influences the activity of amines **1** against *P. orbiculare*, X = CO_2Et being the most active and X = $CONH_2$ being the least active, with X = CN having an intermediate level of activity amongst the groups tested, in contrast with the results of tests on *P. ovale*. The latter observation adds some weight to these yeasts being regarded as separate species.

Experimental Section

Uv, ir, and nmr spectra were measured for all compds and were as expected. Melting points were taken on a Büchi apparatus and are uncorrected.

Alkyl *N*-Substituted-3-aminopropionates (1). Primary amines (0.1 mole) and ethyl or methyl acrylate, or ethyl methacrylate (0.1 mole) were heated under reflux in abs EtOH (100 ml) for 1 hr, and the product was isolated by the method described by Sugden[6] and characterized as the base or converted into an appropriate salt. Acetyl derivatives were prepd by conventional methods and recrystd from petr ether, bp 60–80° (see Table I).

***N*-Hexadecyl-3-aminopropionitrile** was prepd by the method of Caldo[7] from hexadecylamine (24.2 g, 0.1 mole) and acrylonitrile (5.3 g, 0.1 mole) and recrystd from petr ether: bp 40–60°; mp 39–40°; yield, 12.8 g (81%).

Acetamido-*N*-hexadecylamino-3-propionitrile was prepd from *N*-hexadecyl-3-aminopropionitrile (12.1 g, 0.08 mole) in Ac_2O (20 ml) by a conventional method: mp 64–65°; yield, 13.5 g (50%). *Anal.* ($C_{21}H_{40}N_2O$) C, H, N.

***N*-Hexadecyl-3-aminopropionamide** was prepd from hexadecylamine (12.1 g, 0.05 mole) and acrylamide (3.5 g, 0.05 mole) and catalyzed by Triton B soln (0.05 ml) in the usual manner: mp 78–80°; yield, 4.0 g (26%). *Anal.* ($C_{19}H_{40}N_2O$) C, H, N.

Ethyl *N*-Dodecylaminoacetate. *n*-Dodecylamine (18.0 g, 0.1 mole), and ethyl chloroacetate (12.2 g, 0.1 mole), and anhyd Na_2CO_3 (10.6 g, 0.1 mole) were heated in abs EtOH (100 ml) for 18 hr, and the product was isolated in the usual way: bp 180–190° (0.5–0.7 mm); yield, 6.0 g (22%). *Anal.* ($C_{16}H_{33}NO_2$) C, H, N.

Microbiological Testing. Minimum Inhibitory Concentration. The technique for detg the MIC involved making serial dilns of the compd in agar and surface inoculating the growth medium with test organisms. The growth medium was Dixon's formula[2] without modification for tests against *P. orbiculare* but for studies with *P. ovale*, the ox bile and glyceryl monooleate were omitted since these appeared to inactivate the test materials and were not essential for the growth of *P. ovale*, except under condns of primary isolation; they are, however, needed for *P. orbiculare*.

After dispersing the compd in 2% Tween 40, two-fold dilns were prepd in measured amt of liquefied culture medium.[2] The final concn of compd tested in agar was within the range 1000–8

μg/ml. The medium was poured into petri dishes and left to harden overnight at room temp. The surface of the agar was then inoculated with the test organisms (0.02 ml of standard suspension). The inoculated plates together with the appropriate organism controls were incubated for 3 days at 37° in the case of *P. ovale* and up to 5 days in the case of *P. orbiculare*. MIC's were detd by observing the lowest concn which inhibited growth under the prescribed condns.

Suspension Technique. Each test compd (0.1 g) was dissolved or suspended in Tween 40 (2 ml) and the vol made up to 100 ml with sterile dist H_2O. A sample was inoculated with *P. ovale* (0.1 ml of standard suspension contg 10^6 organisms/ml) and stored at room temp for 1 hr. The test samples and appropriate controls were plated out on petri dishes of Dixon's medium[2] and incubated at 37° for 3 days. Activity of the compds was assessed on the growth observed.

References

(1) J. Lodder, "The Yeasts; A taxonomic study," North Holland Publishing Co., Amsterdam, 1970, pp. 1166-1186.
(2) N. J. Van Abbé, *J. Soc. Cosmet. Chem.*, 15, 609 (1964).
(3) T. H. Sternberg and F. M. Keddie, *Arch. Dermatol.*, 84, 999 (1961).
(4) R. C. Burke, *J. Invest. Dermatol.*, 36, 389 (1961).
(5) J. E. Hogan, Ph.D. Thesis, University of London, 1970.
(6) J. K. Sugden, Ph.D. Thesis, University of London, 1964.
(7) C. Caldo, *Chim. Ind. (Milan)*, 44, 753 (1962).

New Compounds

A Rapid, Convenient Preparative Procedure for Phenethylamines

Edgar F. Kiefer

Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822.

Received August 6, 1971

In view of the very broad pharmacological utility of substituted 2-phenylethylamines, we wish to contribute a synthetic procedure which, because of its versatility and convenience, may find considerable use. Although based entirely on standard synthetic methods, the overall scheme is specifically tailored to the properties of the benzylic intermediates involved, and eliminates the need for isolation of intermediates and other time-consuming operations. The procedure is described for the *p*-methoxy derivative; it is also applicable without substantive modification to other ring alkoxy-, alkyl-, and halogen-substituted phenethylamines.

Experimental Section

4-Methoxyphenylethylamine Hydrochloride. *p*-Anisyl alcohol (100 g, 0.725 mole) was shaken with 500 ml of concd HCl for 2 min. The org phase was washed with H_2O, 5% $NaHCO_3$, and H_2O, then added over 40 min to a stirred slurry of 49 g (1.0 mole) of NaCN in 400 ml of DMSO,[1] with ice-water cooling to maintain the temp at 35–40°. After addn was complete, the cooling bath was removed, the mixt was stirred for 90 min and then added to 300 ml of H_2O, and the small upper phase sepd. The aq DMSO layer was extd with two 100-ml portions of Et_2O, which were combined with the product layer, and the whole was washed once with H_2O and dried ($MgSO_4$).

A dry flask was charged with *ca.* 600 ml of abs Et_2O and chilled in ice as 80 g (0.6 mole) of anhyd $AlCl_3$ was added portionwise, followed by 23 g (0.6 mole) of LAH.[2]† The dried Et_2O soln of crude *p*-methoxyphenylacetonitrile was added at such a rate as to maintain gentle reflux without external heat (*ca.* 1 hr). The mixt was stirred for 2 hr, then chilled in ice, and treated dropwise with 25 ml of H_2O followed by 250 ml of 20% of aq NaOH, with periodic addn of Et_2O through the condenser to replenish losses and facilitate stirring. The resulting voluminous, granular ppt of NaCl and LiCl and aluminate was removed by filtration, washed well with Et_2O, and discarded. The filtrate was mixed with one-third its vol of abs EtOH and 60 ml of concd HCl was added slowly with continuous swirling and ice cooling. After chilling to 0°, the cryst amine hydrochloride was collected, 101 g, mp 212–214°, identified by mass spectroscopy [*m/e* 122, 30, 121, 28, 151 (M^+)]. The overall yield was 75% from anisyl alcohol. The hydrochloride may be recrystd from Et_2O–EtOH or *i*-PrOH.

†LAH alone and other metal hydride reagents are unsatisfactory for the reduction of benzylic nitriles to amines.

***N*-Methyl-*p*-methoxyphenylethylamine Hydrochloride.** *p*-Methoxyphenethylamine, generated from 100 g (0.536 mole) of the hydrochloride by stirring with concd aq NaOH, was treated with 100 ml of PhH and 70 g (0.66 mole) of PhCHO. A mildly exothermic reaction began at once. The mixt was heated under reflux until no more H_2O was present in the condensate (*ca.* 1 hr), then, without cooling, an attached Dean-Stark trap was removed and a soln of 82 g (0.65 mole) of Me_2SO_4[3] in 200 ml of PhH was added through the condenser at such a rate as to maintain reflux (15 min). The 2-phase mixt was heated for 90 min on the steam bath, cooled slightly, treated with 200 ml of H_2O, and heated for an addl 20 min. After cooling in ice, the aq layer was washed twice with Et_2O to remove unreacted PhCHO and made strongly basic with 50% aq NaOH. Two Et_2O exts of the basic aq phase were added to the amine layer which sepd, and the resulting soln was evacd at the aspirator for 30 min, leaving 90 g (102%) of crude *N*-methyl-*p*-methoxyphenethylamine. This material was dissolved in 500 ml of 20% abs EtOH–Et_2O and treated with 50 ml of concd HCl with swirling and cooling to yield the white, cryst hydrochloride, which was washed thoroughly with ice-cold 20% EtOH–Et_2O and dried, mp 185.5–186.5°, identified by mass spectroscopy [*m/e* 121, 44, 165 (M^+)]. The yield was 83 g (77%).

References

(1) R. A. Smiley and C. Arnold, *J. Org. Chem.*, 25, 257 (1960).
(2) R. F. Nystrom, *J. Amer. Chem. Soc.*, 77, 2544 (1955).
(3) J. J. Lucier, A. D. Harris, and P. S. Korosec, *Org. Syn.*, 44, 72 (1964).

Synthesis of 2-Fluoro-9-β-D-ribofuranosylpurine (2-Fluoronebularine)

Masajiro Kawana, Robert J. Rousseau,* and Roland K. Robins

ICN Nucleic Acid Research Institute, Irvine, California 92664.

Received August 16, 1971

The antibiotic nebularine (9-β-D-ribofuranosylpurine) has shown tuberculostatic,[1] antimitotic,[2] and anticancer activity.[2,3] The mode of action has been proposed to be in the purine biosynthetic pathway.[4,5] It has limited usefulness because of its high toxicity.[2,6,7]

We wish to report the synthesis of 2-fluoronebularine (2a). Synthesis of the title compound 2a was accomplished by removal of the benzylthio group from 6-benzylthio-2-fluoronebularine (1)[8] with Raney Ni.