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Short communication

MUTAGENIC ACTIVITY OF SOME CENTRALLY ACTIVE AROMATIC AMINES IN *SALMONELLA TYPHIMURIUM* *

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Safrole (4-allyl-1,2-methylenedioxybenzene), a natural product present in some foods, is a hepatocarcinogen in rats and mice [12]. The derivatives 1'-hydroxysafrole and 1'-acetoxysafrole are also hepatocarcinogens in these species and the latter compound is mutagenic in the Salmonella/microsome test system [10—12]. Several methylenedioxyphenyl compounds are used as synergists for insecticides [8]; other derivatives have been useful in studies of structure-activity relationships among psychotropic drugs [17,19]. The purpose of the present study was to test a series of methylenedioxyphenyl compounds and structurally related, centrally-active aromatic amines for mutagenicity in the Salmonella/microsome system.

The names, chemical structures and sources of the compounds tested are listed in Table 1. The compounds listed in Table 1 are either centrally-active amines, or precursors, metabolites or analogs of centrally-active amines. Each compound was pure by the criteria of melting point and elemental analysis. Dimethyl sulfoxide (DMSO, spectrophotometric grade) was purchased from Schwarz/Mann. All other chemical were reagent grade.

Salmonella typhimurium tester strains TA 1537, TA 1538, TA 98 (TA 1538/pKM101) and TA 100 (TA 1535/pKM101) were a gift of Dr. Bruce Ames of the University of California, Berkeley.

The method of testing for mutagenic activity was that of Ames et al. [1]. The bacterial tester strains have mutations in the histidine operon which result in a nutritional requirement for histidine. Strain TA 100 can be reverted to prototrophy by DNA base pair substitutions whereas strains TA 1537, TA 98 and TA 1538 are revertible by frameshift mutations.

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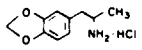
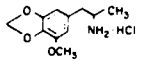
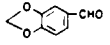
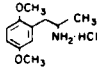
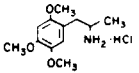
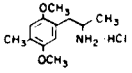
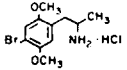
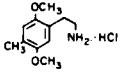
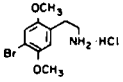
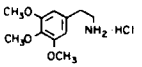
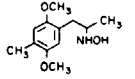
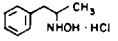
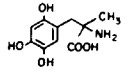
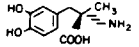
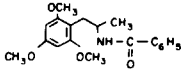
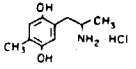
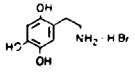
TABLE I

NAME, CHEMICAL STRUCTURE AND REFERENCE FOR THE COMPOUNDS TESTED FOR MUTAGENICITY

Number	Name	Structure	Reference
1	1-(3,4-methylenedioxyphenyl)-2-aminopropane · HCl		(9)
2	1-(3,4-methylenedioxy-5-methoxyphenyl)-2-aminopropane · HCl		(15)
3	3,4-(methylenedioxy) benzaldehyde		d
4	1,2-methylenedioxybenzene		d
5	1-(2,5-dimethoxyphenyl)-2-aminopropane · HCl		(7)
6	1-(2,4,5-trimethoxyphenyl)-2-aminopropane · HCl		(3)
7	1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane · HCl		(16)
8	1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane · HCl		(17)
9	2-(2,5-dimethoxy-4-methylphenyl)-ethylamine · HCl		(7)
10	2-(2,5-dimethoxy-4-bromophenyl)-ethylamine · HCl		(20)
11	2-(3,4,5-trimethoxyphenyl)ethylamine · HCl [a]		e
12	1-(2,5-dimethoxy-4-methylphenyl)-2-hydroxyaminopropane		(6)
13	1-phenyl-2-hydroxyaminopropane·HCl		(2)
14	2-methyl-3-(2,4,5-trihydroxyphenyl) alanine		(21)
15	5,6-dihydroxyindoline		(13)
16	(S)-2-methyl-3-(3,4-dihydroxyphenyl) alanine [b]		(14)
17	1-(2,4,6-trimethoxyphenyl)-2-benzoylaminopropane		f
18	2-thio-4,5-dihydroxytoluene		(4)
19	1-(2,5-dihydroxy-4-methyl)phenyl-2-aminopropane · HCl		(23)
20	2-(2,4,5-trihydroxyphenyl)ethylamine · HBr [c]		d

[a] Mescaline · HCl Chemical Abstracts Service (CAS) number 54-04-6
[b] L-α-Methyldopa CAS number 555-30-6
[c] 6-Hydroxydopamine · HBr
[d] Aldrich Chemical Co.
[e] Sigma Chemical Co.
[f] Jacob, P. III, A.T. Shulgin, N. Castagnoli Jr., unpublished results

For activation of compounds by liver homogenate, 50 μl of the 9000 *g* liver homogenate fraction (S-9) prepared from rats treated with phenobarbital, Aroclor 1254 or 3-methyl-cholanthrene [1] was incorporated into the top agar. Compounds 1—20 were tested with liver homogenates prepared from rats treated with each of the inducers.

Freshly prepared solutions of compounds 1—11 in DMSO were tested at 0.02, 0.2, 2.0, 20.0, 200, 1000 and 5000 μg/plate with bacterial strains TA 1537, TA 98 and TA 100 with and without a rat liver microsomal activation system. Compounds 12—20 were dissolved in DMSO and tested at 0.2, 2.0, 20.0 and 200 μg/plate using bacterial strains TA 98 and TA 100 with and without liver homogenate. Revertant *his*$^+$ colonies were counted after incubation in the dark at 37° C for 48 h.

No difference in colony counts could be detected over a dose range of 0.02—5000 μg when compounds 1—11 and control plates were compared in strains TA 1537, TA 98 and TA 100. Microscopic examination of the plates revealed that an adequate bacterial lawn was present. Compounds 9 and 10 were toxic to the bacteria at the levels of 1 and 5 mg. Typical numbers of spontaneous revertants in the presence of DMSO ± S-9 were as follows: TA 1537 (10); TA 1538 (7); TA 98 (28); TA 100 (153). Sterility controls of S-9 Mix, DMSO and compounds 1—20 were negative. The positive control substances 9-aminoacridine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine and daunomycin did produce substantial increases in the number of revertants per plate in strains TA 1537, TA 100 and TA 98, respectively, in the absence of liver homogenate. The activity of the S-9 preparation was demonstrated by an increase in the mutagenic activity of benzidine (50 μg) or 2,5-diaminoanisole (50 μg) in strain TA 1538.

No difference in colony counts could be detected over a dose range of 0.2—200 μg when compounds 12—20 were tested using strains TA 98 and TA 100 with and without liver homogenate. In spot tests using 0.5 mg of material, compounds 14—20 were toxic to the bacteria and gave kill zones of 1—3 cm in diameter.

A weak mutagenic activity was detected when 5 mg of compound 1, 3,4-methylenedioxyamphetamine and 10 mg of compound 5, 2,5-dimethoxyamphetamine were spot tested using strain TA 100 without liver homogenate. Three separate spot tests gave approximately 450 revertants and 200—400 revertants above background for compounds 1 and 5 respectively. The kill zone with compound 5 was approximately 2.5 cm in diameter. Plate incorporation tests of 10 mg of compounds 1 and 5 using strain TA 100 did not increase the number of revertants. Although no mutagenic activity was detected in plate incorporation tests over a dose range of 0.02—10,000 μg/plate, the positive spot tests with 3,4-methylenedioxyamphetamine and 2,5-dimethoxyamphetamine should not be discounted. The mutagenic activity detected was extremely weak, however, and the presence of mutagenic impurities cannot be ruled out when such high dose levels are tested.

In spite of the sensitivity of the test method and the presence of the liver microsomal activation system no mutagenic activity could be detected in most of the compounds tested. This may be due to the absence of the appropriate metabolic activity, since a urinary metabolite of safrole is mutagenic in the Salmonella test [11] whereas safrole (with or without Aroclor-induced rat liver

homogenate) is not mutagenic. Although safrole may be activated to a carcinogen by liver microsomal enzymes of the type induced by 3-methylcholanthrene [5], compounds 1—20 were not activated to mutagens by liver homogenates prepared from rats induced with phenobarbital, Aroclor or 3-methyl-cholanthrene.

Note. No mutagenic activity was detected with piperonyl butoxide, a widely used insecticide synergist, in spot tests on strains TA 1537, TA 98 and TA 100, with and without liver homogenate.

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References

1 Ames, B.N., J. McCann and E. Yamasaki, Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutation Res., 31 (1975) 347—364.
2 Borch, R.F., M.D. Berstein and H.D. Durst, The cyanohydridoborate anion as a selective reducing agent, J. Am. Chem. Soc., 93 (1971) 2897—2904.
3 Bruckner, V., Uber das Pseudonitrosit des Asarons, J. Prakt. Chem., 138 (1933) 268—274.
4 Daneke, J., U. Jahnke, B. Pankow and H-W. Wanzlick, Einfache Darstellung empfindlicher Mercaptane, Tetrahedron Lett., 15 (1970) 1271—1272.
5 Elcombe, C.R., J.W. Bridges, T.J.B. Gray, R.H. Nimmo-Smith and K.J. Netter, Studies on the interaction of safrole with rat hepatic microsomes, Biochem. Pharmacol., 24 (1975) 1427—1433.
6 Gal, J., L.D. Gruenke, N. Castagnoli Jr., *N*-Hydroxylation of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane by rabbit liver microsomes, J. Med. Chem., 18 (1975) 683—688.
7 Ho, B.T., L.W. Tansey, R.L. Balster, R. An, W.M. McIsaac and R.T. Harris, Amphetamine analogs II. Methylated phenethylamines, J. Med. Chem., 13 (1970) 134—135.
8 Kamienski, F.X. and J.E. Casida, Importance of demethylation in the metabolism in vivo and in vitro of methylenedioxyphenyl synergists and related compounds in mammals, Biochem. Pharmacol., 19 (1970) 91—112.
9 Mannich, C. and W. Jacobsohn, Uber Oxyphenylalkylamine und Dioxyphenylalkylamine, Chem. Ber., 43 (1910) 189—197.
10 McCann, J., E. Choi, E. Yamasaki and B.N. Ames, Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals, Proc. Natl. Acad. Sci. (US), 72 (1975) 5135—5139.
11 McCann, J. and B.N. Ames, Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals — Discussion, Proc. Natl. Acad. Sci. (US), 73 (1976) 950—954.
12 Miller, J.A. and E.C. Miller, The metabolic activation of chemical carcinogens — Recent results with aromatic amines, safrole and aflatoxin B_1, R. Montesano, H. Bartsch and L. Tomatis (eds.), Screening Tests in Chemical Carcinogenesis, International Agency for Research on Cancer, Lyon, 1976, pp. 153—176.
13 Oriente, G.P., S. Sciuto and M. Piattelli, Una sintesi semplice della 5,6-diossindolina, Gaz. Chim. Italiana, 100 (1970) 693—696.
14 Pfister and Stein, U.S. Patent No. 2,868,818 (1959 Merck and Co., Inc.).
15 Shulgin, A.T., 3-Methoxy-4,5-methylenedioxy amphetamine — A new psychotomimetic agent, Nature, 201 (1964) 1120—1121.
16 Shulgin, A.T., U.S. Patent No. 3,547,999 (1970).
17 Shulgin, A.T., T. Sargent and C. Naranjo, Structure-activity relationships of one-ring psychotomimetics, Nature, 221 (1969) 537—541.
18 Shulgin, A.T., T. Sargent and C. Naranjo, 4-Bromo-2,5-dimethoxyphenylisopropylamine — A new centrally active amphetamine analog, Pharmacology, 5 (1971) 103—107.
19 Shulgin, A.T., T. Sargent and C. Naranjo, Animal pharmacology and human psychopharmacology of 3-methoxy-4,5-methylenedioxyphenylisopropylamine (MMDA), Pharmacology, 10 (1973) 12—18.
20 Shulgin, A.T. and M.F. Carter, Centrally active phenethylamines, Psychopharm. Commun., 1 (1975) 93—98.
21 Sletzinger, M. and F.W. Bollinger, U.S. Patent No. 944990 (Merck and Co., Inc.).
22 Spath, E., Uber die Anhaloniumalkaloide. I. Anhalin und Mezcalin, Monatsch. Chem., 40 (1919) 129—155.
23 Zweig, J.S. and N. Castagnoli Jr., Chemical conversion of the psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane to 5-hydroxy-2,6-dimethylindole, J. Med. Chem., 17 (1974) 747—749.