either by fluorescence quenching or, in the case of 3,5-dimethylpyrazole, by spraying with a 1 M $CoCl_2$ solution. 3,5-Dimethylpyrazole is detected by this means as a dark blue spot. The following thin layer systems were employed most frequently: system A, ethyl acetate(saturated with water)–formic acid (99:1); and system B, ethyl acetate–dimethylformamide–water–formic acid (85:15:5:2). The R_f values of the compounds in system A were: I, 0.55; III, 0.40; in system B: I, 0.80; III, 0.65. The mobilities of the active metabolite were identical with III.

D. Paper Chromatography.—The following papergram systems were employed: BAW system, a descending system on dry Whatman No. 4 paper; the mobile phase is 1-butanol–acetic acid–water (2:1:1); BPW system, a descending system on dry Whatman No. 4 paper; the mobile phase is 1-butanol–piperidine–water (81:2:17). The R_f values of the compounds in BAW system were: I, 0.84; III, 0.78; in BPW system: I, 0.78; III, 0.37. The mobilities of the active metabolite were identical with III.

E. Gas Chromatographic Method for I.—The urine was adjusted to pH 8 and extracted with an equal volume of $CHCl_3$. The latter was then concentrated, and an aliquot was injected into an F and M Model 609 gas chromatograph, equipped with a polyester column (LAC-2-R446) and a hydrogen flame detector. With a column temperature of 175°, an injection port temperature of 250°, and a helium flow rate of 60 ml./min., I possessed a retention time of 7 min.

F. Brom Cresol Purple Procedure for I.—Five milliliters of a 10:1 or 100:1 dilution of urine was adjusted to pH 6.4 with 0.5 M phosphate buffer, extracted with 15.0 ml. of chloroform in a 35-ml. centrifuge tube, and centrifuged. Ten milliliters of the chloroform layer was pipetted into another centrifuge tube and shaken with 5.0 ml. of 0.1 N HCl. After centrifuging, 4 ml. of the aqueous layer was pipetted into a third centrifuge tube together with 4.0 ml. of 0.2 N NaOH and 10.0 ml. of chloroform. Seven milliliters of the latter was passed through Whatman No. 41 filter paper into a test tube. Exactly 3.0 ml. of the chloroform filtrate was added to 0.3 ml. of a chloroform solution of 0.05% brom cresol purple indicator. The absorbance at 410 mμ, after correction for the background response of control urine carried through the same procedure, is proportional to the content of I.

G. Thin Layer Chromatographic Procedure for I.—Since 3,5-dimethylpyrazole does not quench fluorescence, a spray was required to locate the zone. It was found that spraying with 1 M $CoCl_2$ could detect as little as 5 γ of I as a dark blue zone. The silica gel containing the zone was eluted with 5.0 ml. of absolute ethanol, and 3.0 ml. of this solution was added to exactly 1.0 ml. of water. This solution was then measured at 215 mμ. Known amounts of I were spotted, chromatographed, sprayed, eluted, and measured, yielding the following data: added (γ), 10.0, 20.0, 30.0, 40.0, 50.0; found (γ), 11.0, 18.6, 28.0, 40.6, 52.1.

When the zone, which had been sprayed with $CoCl_2$ solution, was eluted with absolute alcohol, the background was low, providing water was added just prior to ultraviolet measurement. The absolute alcohol eluate could not be measured directly in the ultraviolet because of the high background due to $CoCl_2$ in this nonaqueous solvent.

H. Quantitative Thin Layer Chromatographic Method for 5-Methylpyrazole-3-carboxylic Acid (III).—The 24-hr. urine collections from control and treated (I) rats were adjusted to pH 8.7–8.9 with 0.5 M Na_2CO_3 and extracted twice with twice its volume of $CHCl_3$. The urine was then adjusted to pH 1.5 with concentrated HCl and extracted twice with four times its volume of 1-butanol; the latter was then evaporated to dryness and taken up in 25 ml. of ethanol. Aliquots of the ethanol solutions were spotted on fluorescent, silica gel thin layer plates, and developed with system B. The silica gel corresponding to III was removed from the thin layer plate and eluted with 5.0 ml. of absolute methanol. The methanol eluate was measured at 215 mμ with a Cary spectrophotometer. Control urine was also subjected to this procedure, and the area of the thin layer plate corresponding to III was eluted and used as the blank. A calibration curve was prepared by spotting and eluting known amounts of III, followed by ultraviolet measurement. Calibration was found to be necessary because III could not be quantitatively eluted from silica gel even with polar solvents. Known amounts of III, subjected to this procedure, yielded the following data after correction for a recovery of 67.4%: added (γ), 5.7, 11.4, 17.1, 22.8, 28.5, 34.2; found (γ), 7.3, 9.8, 17.2, 19.8, 32.3, 35.2.

The Effects of Ring-Methoxyl Groups on Biological Deamination of Phenethylamines

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The oxidative deamination of all the possible ring-methoxylated β-phenethylamines by the amine oxidase systems present in rabbit liver has been investigated. The effects of the number and position of the methoxyl groups on ease of deamination of this series of mescaline congeners were determined.

Among the ever-increasing number of compounds which have been found to affect human mood and behavior, mescaline (3,4,5-trimethoxyphenethylamine) occupies a singular position in that it is a structurally simple molecule in comparison with many other psychotomimetic drugs. Early in our studies of the relation between chemical structure and psychotomimetic activity among ring-methoxylated β-phenethylamines, it had occurred to us that a series of mescaline analogs in which the number and ring positions of methoxyl groups in the phenethylamine moiety might be used to advantage in gaining further knowledge concerning the unique action of mescaline in biological systems. At one stage of this investigation, we demonstrated that methoxyl groups located in the 2- and 6-positions in phenethylamine exhibited a rather strong inhibitory effect on *in vitro* alkaline phosphatase; less enzyme inhibition was reported for those compounds in this series having methoxyl groups in the 3- and 2,3-positions.[1] Among several catabolic routes, oxidative deamination offers an obvious *in vivo* pathway for the detoxification of many biologically active amines.[2,3] Accordingly, it was thought worthwhile to examine the entire series of the nineteen possible ring-methoxylated phenethylamines, in order to determine which of these structures would undergo *in vitro* oxidative deamination in a biological system capable of deaminating both tyramine and mescaline. Compounds which did not undergo deamination were examined further as possible amine oxidase inhibitors, since they might either irreversibly occupy or modify receptor sites, thus preventing degradation of amines which would normally be deaminated.

With the exception of 2,6-dimethoxyphenethylamine, which is described here, all of the aforementioned ring-

(1) L. C. Clark, Jr., R. P. Fox, R. D. Morin, and F. Benington, *J. Nervous Mental Disease*, **124**, 466 (1956).
(2) H. Blaschko, *Pharmacol. Rev.*, **4**, 415 (1952).
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TABLE I

RELATIVE RATES OF DEAMINATION OF RING-METHOXYLATED PHENETHYLAMINES BY SOLUBLE AMINE OXIDASE FROM RABBIT LIVER

Substituent	M.p. of HCl Salt, °C.	Lit. ref.	% deamination of 0.005 *M* amine in 1 hr. Without semicarbazide	With semicarbazide
2-Methoxy	141–142	*a*	95	95
3-Methoxy	144–145	*b*	75	75
4-Methoxy	213–214	*c*	85	85
2,3-Dimethoxy	112–113	*d*	75	75
2,4-Dimethoxy	157–158	*e*	70	70
2,5-Dimethoxy	137–138	*f*	50	50
2,6-Dimethoxy	214–215	*g*	0	0
3,4-Dimethoxy	150–151	*c*	60	45
3,5-Dimethoxy	156–157	4	55	55
2,3,4-Trimethoxy	168–169	*c*	50	50
2,3,5-Trimethoxy	104–105	*h*	40	40
2,3,6-Trimethoxy	122–123	*h*	0	0
2,4,5-Trimethoxy	190–191	*i*	25	25
2,4,6-Trimethoxy	234–235	4	0	0
3,4,5-Trimethoxy	181–182	*c*	60	0
2,3,4,5-Tetramethoxy	167–168	4	0	0
2,3,4,6-Tetramethoxy	168–169	4	0	0
2,3,5,6-Tetramethoxy	136–137	4	0	0
2,3,4,5,6-Pentamethoxy	196–197	4	0	0
4-Hydroxy	269–270	*c*	95	95
None	217–218	*c*	80	80

[a] R. Pschorr and O. Einbeck, *Ber.*, **38**, 2076 (1905). [b] L. Helfer, *Helv. Chim. Acta*, **7**, 948 (1924). [c] Commercially available. [d] R. D. Haworth, *J. Chem. Soc.*, 2282 (1927). [e] M. Erne and F. Ramirez, *Helv. Chim. Acta*, **33**, 912 (1950). [f] R. Baltzly and J. S. Buck, *J. Am. Chem. Soc.*, **62**, 161 (1940). [g] New compound. [h] J. R. Merchant and A. J. Mountvala, *J. Org. Chem.*, **23**, 1774 (1958). [i] M. P. J. Jansen, *Rec. trav. chim.*, **50**, 291 (1931).

methoxylated phenethylamines have either been synthesized by us[4] or previously reported (see references in Table I).

Rabbit liver was chosen as the source of amine oxidase for these studies because Slotta[5] had shown that the rabbit could metabolize mescaline in large quantities, and Bernheim[6] had demonstrated that rabbit liver contains an enzyme system capable of rapidly deaminating mescaline. Further, Bernheim found that mescaline deamination, unlike that of tyramine, is cyanide inhibited. As reported by Zeller, *et al.*,[7] we found that the deamination of mescaline, but not tyramine, was inhibited by semicarbazide. The ring-methoxylated amines were therefore incubated with soluble amine oxidase from rabbit liver[8] with and without added semicarbazide in order to determine if they were deaminated by Bernheim's "mescaline oxidase" or by "tyramine oxidase." For this purpose, the soluble amine oxidase preparation described by Cotzias served admirably, since he demonstrated conclusively that all of the amine oxidase activity in rabbit liver remained in the supernatant after the solubilizing treatment and centrifugation, even at 181,000*g*.[8]

Synthesis of all the ring-methoxylated phenethylamines except that of the 2,6-dimethoxy derivative has been described previously. Melting points of the hydrochloride salts and the literature references to these phenethylamines are listed in Table I.

2,6-Dimethoxyphenethylamine was obtained from 2,6-dimethoxybenzoic acid[9] *via* reduction with lithium aluminum hydride to the corresponding benzyl alcohol and conversion to 2,6-dimethoxybenzyl chloride with thionyl chloride; treatment with potassium cyanide gave 2,6-dimethoxyphenylacetonitrile, which was catalytically hydrogenated over Raney nickel to the desired 2,6-dimethoxyphenethylamine.

In an alternative method of synthesis of 2,6-dimethoxyphenethylamine *via* 2,6-dimethoxyphenylacetamide, we were unsuccessful in obtaining the desired ω-diazo ketone from the action of diazomethane on 2,6-dimethoxybenzoyl chloride. A similar failure of the Arndt-Eistert reaction is reported for 2,4,6-trimethylbenzoyl chloride.[10]

Ring-methoxylated phenethylamines are divided into three groups by the manner in which they are deaminated by soluble amine oxidase from rabbit liver. Compounds substituted in the 2,6-positions and all those with more than three methoxyl groups are not deaminated at a significant rate. All of the remaining ring-methoxylated phenethylamines are deaminated at appreciable rates, with some variation in rate with structure. Mescaline, however, is unique among the ring-methoxylated phenethylamines in that its deamination is completely inhibited by 0.005 *M* semicarbazide. Semicarbazide does not inhibit the deamination of any of those ring-methoxylated phenethylamines which do undergo deamination, and in this respect these amines behave in the same manner as tyramine or unsubstituted phenethylamine when they are presented as substrates for amine oxidase systems. Results of these experiments are summarized in Table I.

It was also of interest to determine if those amines which are themselves not deaminated might act as inhibitors for either "tyramine oxidase" or "mescaline oxidase." Accordingly, the deamination of tyramine and mescaline in the presence of the six ring-methoxylated phenethylamines which are not deaminated was examined. In no case was the deamination of either tyramine or mescaline altered to any significant extent. It can be concluded from these results that the ring-methoxylated phenethylamines which are not deaminated do not compete with other amines for the receptor sites of the amine oxidase enzymes, since they do not block the deamination of tyramine or mescaline.

Experimental[11]

2,6-Dimethoxyphenylacetonitrile.—A slurry of 54.6 g. of 2,6-dimethoxybenzoic acid in 600 ml. of dry benzene was added to a stirred mixture of lithium aluminum hydride and 500 ml. of anhydrous ether. The mixture was stirred and heated under reflux for 4 hr., cooled in an ice bath, and treated cautiously with 90 ml. of water and then with 850 ml. of 10% H_2SO_4. The organic layer was separated, washed with water, dilute aqueous Na_2CO_3, and again with water. The resulting solution of 2,6-dimethoxybenzyl alcohol in ether–benzene was dried ($MgSO_4$), filtered, and treated with 3.6 ml. of pyridine followed by dropwise

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(5) K. H. Slotta and J. Müller, *Z. physiol. Chem.*, **238**, 141 (1936).

(6) F. Bernheim and M. L. C. Bernheim, *J. Biol. Chem.*, **123**, 317 (1938).

(7) E. A. Zeller, J. Barsky, E. R. Berman, M. S. Clerkas, and J. R. Fouts, *J. Pharmacol. Exptl. Therap.*, **124**, 282 (1958).

(8) G. C. Cotzias, I. Serlin, and J. J. Greenough, *Science*, **120**, 144 (1954).

(9) Available from Aldrich Chemical Co., Milwaukee, Wis.

(10) W. E. Bachmann and W. S. Struve, *Org. Reactions*, **1**, 46 (1942).

(11) Melting points were taken in capillary tubes with a partial immersion thermometer. Calibration of the apparatus against standard compounds showed no need for correction.

addition of 49 ml. of thionyl chloride under ice cooling. After stirring at room temperature for 1 hr., the reaction mixture was poured into ice–water, and the organic layer was separated and washed with water, 10% aqueous Na_2CO_3, water, and finally with saturated aqueous NaCl. After drying ($MgSO_4$), the solution was evaporated under reduced pressure to give a solid residue of 2,6-dimethoxybenzyl chloride, which was used in the next reaction step without further treatment.

The crude solid benzyl chloride was dissolved in 1 l. of acetone, treated with a solution of 39 g. of KCN in 300 ml. of water, and stirred at room temperature for 20 hr. Evaporation of the solvents left an oily residue which was extracted with ether; this solution was washed with water and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The resulting crystalline residue, after recrystallization from hot ethanol, gave 21.7 g. (41%) of **2,6-dimethoxyphenylacetonitrile,** m.p. 94–95°.

Anal. Calcd. for $C_{10}H_{11}NO_2$: C, 67.8; H, 6.2. Found: C, 68.2; H, 6.4.

2,6-Dimethoxyphenethylamine.—A solution of 2,6-dimethoxyphenylacetonitrile (21.7 g.) in 130 ml. of methanol containing 19 g. of ammonia and 10 ml. of Raney nickel catalyst was placed in a mechanically stirred autoclave and flushed with hydrogen. The reaction vessel was charged to a hydrogen pressure of 74 kg./cm.^{2} and heated to 100°, and stirring was initiated; hydrogen absorption was complete in about 1.5 hr. at 100–120°. The reaction mixture was filtered from the catalyst, and the methanol was removed under reduced pressure to give an oily residue of the crude amine. Upon distillation, there was obtained 17.8 g. (80%) of **2,6-dimethoxyphenethylamine,** b.p. 165–171° (28 mm.), m.p. 56–59°. Treatment of an ether solution of the free base with 8.1 ml. of concentrated HCl gave 16 g. of the hydrochloride salt, m.p. 214–215°; recrystallization from ethanol did not change the melting point.

Anal. Calcd. for $C_{10}H_{16}ClNO_2$: C, 55.2; H, 7.4; Cl, 16.3. Found: C, 55.2; H, 7.3; Cl, 16.4.

Preparation of Soluble Amine Oxidase.—Fresh liver, removed from rabbits killed by neck fracture, was weighed, sliced, mixed with twice its volume of ice-cold 0.67 *M* sodium phosphate buffer, pH 7.4, and homogenized in a Waring Blendor for 4 min. To this homogenate, cooled in an ice–water bath, was added 5% of isooctylphenoxypolyethoxyethanol and 1 drop of Dow-Corning Medical Antifoam compound. The homogenate was then centrifuged at 5° and 5000*g* for 3 min., and the supernatant was decanted from sedimented cell debris and stored overnight in a cold room at 4°. This supernatant was then centrifuged at about 25,000*g* for 1 hr. at 5°, decanted from a small amount of additional sediment, and stored at 4° until used. Since the ammonia level can reach undesirably high values on storage, dialysis against phosphate buffer was used as a means of lowering the ammonia concentration in these preparations. The amine oxidase activity of these soluble preparations did not decrease significantly after storage at 4° for periods as long as 6 weeks.

Oxidative Deamination of Substituted Phenethylamines.—The amines (as the hydrochlorides) were incubated with the solubilized homogenate at a concentration of 0.005 *M*. Incubation was carried out for 1 hr. in an air atmosphere at 37° in a Dubnoff shaking incubator.

Flask A contained 1 ml. of the soluble amine oxidase preparation, 0.2 ml. of substrate (0.05 *M*), and 0.8 ml. of 0.67 *M* phosphate buffer, pH 7.4. Flask B contained 1 ml. of the amine oxidase preparation, 0.2 ml. of 0.05 *M* semicarbazide, 0.2 ml. of substrate (0.05 *M*), and 0.6 ml. of buffer. Flask C was identical with flask A except that the substrate was added after the incubation, and flask D was identical with flask B with the same exception. Thus, flasks C and D served as controls for the chromogenicity of the amine itself and the semicarbazide in the colorimetric analysis employed. The final volume of all flasks was 2.0 ml.

The incubation reaction was stopped by adding 8 ml. of 5% trichloroacetic acid in 0.1 *N* HCl, and the filtrate was analyzed directly or stored frozen in capped vials at −20°. Sets of flasks containing tyramine and mescaline were incubated each day to ensure the activity of the tissue preparation.

The incubates were analyzed for ammonia by the automated sodium phenate procedure of Logsdon,[12] except that commercial hypochlorite (Chlorox) was used instead of the more difficultly prepared reagent from calcium hypochlorite. Sodium nitroprusside was used to increase the sensitivity of the colorimetric reaction.[13] Ammonium chloride standards containing 180, 360, 900, 1800, 3600, and 9000 γ of NH_3/100 ml. of solution were analyzed before and after each day's unknowns. Altogether, several thousand ammonia analyses have been conducted by this procedure.

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