destroyed by adding a small amount of the Raney nickel described earlier[18] with additional boiling for 10 min. The mixture was then filtered through No. 3 Whatman paper and boiled down to a small volume and cooled. After separation, the crystalline product was recrystallized from methanol.

Method E.—Acetylation was carried out in benzene with acetic anhydride, and heating was continued for 10 min. on the steam bath. The product was recrystallized from alcohol.

2,7-(N,N′-Dibenzylidene)fluorenediamine.—To a hot solution of 3.9 g. (0.02 mole) of 2,7-fluorenediamine[16] in 120 ml. of alcohol, 4.6 g. (0.04 mole) of benzaldehyde was added slowly. A yellow precipitate came out immediately, and the mixture was heated on the steam bath for 10 min. and allowed to cool. Filtration and drying gave 7.1 g. (91%), m.p. 245–248°. One crystallization from toluene gave the pure product, m.p. 259–260°, lit.[9] m.p. 249–250°.

2,7-(N,N′-Di-*o*-chlorobenzylidene)fluorenediamine.—The foregoing procedure was used to obtain 97.5% of the crude product, m.p. 209–210°. One recrystallization from benzene (Darco) gave an analytically pure sample with the same melting point.

Anal. Calcd. for $C_{27}H_{18}Cl_2N_2$: C, 73.47; H, 4.11; Cl, 16.07; N, 6.35. Found: C, 73.33; H, 4.13; Cl, 16.10; N, 6.59.

2,5-(N,N′-Di-*o*-chlorobenzylidene)fluorenediamine.—A 99% crude yield (m.p. 148–150°) was obtained using the above procedure with 2,5-fluorenediamine.[16] Recrystallization from benzene (Darco) sharpened the melting point to 149–150°.

Anal. Calcd. as above. Found: Cl, 15.80; N, 6.17.

Triphenylphosphonium 2-nitrofluorenylide was made in the same way as the preceding fluorenylide using 9-bromo-2-nitrofluorene. The bromide was obtained in 87% yield. Upon treatment with NH_4OH, the dark purple ylide came out. The yield was quantitative, m.p. 291–292° dec., with slight softening at 290°. Recrystallization from toluene gave an analytical sample.

Anal. Calcd. for $C_{31}H_{22}NO_2P$: C, 78.97; H, 4.70; N, 2.97; P, 6.57. Found: C, 79.28; H, 4.38; N, 3.33; P, 6.79.

Infrared absorption bands showed C–NO_2, 1510 and 1340 cm.$^{-1}$; P–C_{aryl}, 1440 and 692 cm.$^{-1}$; P–C_{alkyl}, 718 cm.$^{-1}$.

Triphenylphosphonium 2-N,N-Dimethylaminofluorenylide.—An identical procedure starting with 9-bromo-2-N,N-dimethylaminofluorene hydrobromide[19] gave a quantitative yield of the bromide and 86% of the ylide, m.p. 275–280° dec.

Anal. Calcd. for $C_{33}H_{28}NP$: C, 84.41; H, 6.01; N, 2.98; P, 6.60. Found: C, 84.24; H, 5.88; N, 3.31; P, 6.59.

Infrared absorption bands showed C–N (vibrational band, *t*-amine), 1333 cm.$^{-1}$; P–C_{aryl}, 1435 and 693 cm.$^{-1}$; P–C_{alkyl}, 720 cm.$^{-1}$.

9-*p*-Nitrobenzylidene-2-N,N-dimethylaminofluorene.—To a solution of 12 g. of 9-triphenylphosphonium 2-N,N-dimethylaminofluorenylide in 200 ml. of chloroform, 3.85 g. (an equimolar amount) of *p*-nitrobenzaldehyde was added. The mixture was refluxed for 30 min. and then boiled down to near dryness. The gummy mass was triturated with 20 ml. of ethanol until the dark purple product solidified. This was filtered off and dried giving 7.5 g. (86%), m.p. 125–127°. One recrystallization (Darco) from ethanol gave an analytical sample, m.p. 126–127°.

Anal. Calcd. for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.18. Found: C, 77.31; H, 5.41; N, 8.27.

Infrared absorption bands showed C–NO_2, 1510 and 1340 cm.$^{-1}$; C–NR_2, 1333 cm.$^{-1}$.

9-*p*-Nitrobenzylidene-2-nitrofluorene.—Reaction of 6 g. of 9-triphenylphosphonium 2-nitrofluorenylide with 1.93 g. of *p*-nitrobenzaldehyde in 100 ml. of chloroform as described in the preceding procedure gave 4.4 g. (100%) of a *cis-trans* mixture of 9-*p*-nitrobenzylidene-2-nitrofluorene,[20] m.p. 240–290°.

Biological Results.—None of the analogs of 9-*o*-chlorocinnamylidenefluorene, thus far submitted, show any antitumor activity. The latest data available from the Cancer Chemotherapy National Service Center, which sponsored the screening, show that the named compound had some activity against S180, having gone to multiple dose assay at two screening laboratories. This was not confirmed, however, and the compound is considered inactive. No activity was found against Adenocarcinoma 755 or leukemia L1210. Multiple dose assay at two laboratories is also being run with the KB cell culture system.

One of the new ylides described above also showed slight initial activity. 9-Triphenylphosphonium 2-N,N-dimethylaminofluorenylide is undergoing multiple dose assay tests against Cloudman melanoma and there is slight activity against the KB cell culture system. Initial activity against S180 was not confirmed.

(18) Ref. 16, footnote 18.

(19) T. L. Fletcher and M. J. Namkung, *J. Chem. Soc.*, 1400 (1961).

(20) E. Fischer and E. D. Bergmann [*Bull. Res. Council Israel*, **1**, 136 (1951)] reported for the *cis* isomer, m.p. 251–252°; for the *trans* isomer, m.p. 287–288°. We prepared these (m.p. 238–290°) by condensing 2-nitrofluorene with *p*-nitrobenzaldehyde and then separating.

5-Methylpyrazole-3-carboxylic Acid. The Potent Hypoglycemic Metabolite of 3,5-Dimethylpyrazole in the Rat

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Biological evaluation of 3,5-dimethylpyrazole, a potent hypoglycemic agent (50 times tolbutamide) in the glucose-primed, fasted, intact rat suggested that this compound was active by virtue of its conversion to an active metabolite. Demonstration that the urine of treated rats possessed considerable hypoglycemic activity in the absence of detectable 3,5-dimethylpyrazole supported this hypothesis. A potent hypoglycemic metabolite (200 times tolbutamide in the rat) has been isolated from the urine of treated rats and identified as 5-methylpyrazole-3-carboxylic acid. Extensive studies of the urine from rats receiving 3,5-dimethylpyrazole indicate that this metabolite accounts for all of the urinary hypoglycemic activity.

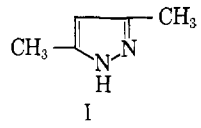
A series of pyrazoles have recently been reported to possess considerable hypoglycemic activity.[1,2] The most active compounds of this series were those containing methyl groups in both the 3- and 5-positions. 3,5-Dimethylpyrazole (I) was the most active of the several pyrazoles tested. Studies, using eviscerate rats, aimed at defining the mechanism of hypoglycemic action of this compound suggested that the gastrointestinal tract and/or the liver was necessary for activity[2]; in this respect its behavior is similar to its close analog, 3,5-dimethylisoxazole.[3]

After oral administration of I to glucose-primed, fasted, intact rats, activity was apparent at 1 hr. and reached a maximum at 2 hr.; the response after intravenous administration, however, was not apparent until

(1) J. B. Wright, W. E. Dulin, and J. H. Markillie, *J. Med. Chem.*, **7**, 102 (1964).

(2) G. C. Gerritsen and W. E. Dulin, *Diabetes*, in press.

(3) W. E. Dulin and G. C. Gerritsen, *Proc. Soc. Exptl. Biol. Med.*, **113**, 683 (1963).



I

2 hr., and was greater at 3 hr. than at 2 hr. This delayed onset of activity following intravenous administration as compared to oral administration was consistent with a hypothesis that the liver was necessary for activity, since a higher concentration of I would be available for metabolism following absorption and transport to the liver *via* the portal vein after oral administration. These observations and the fact that I was inactive in rats previously treated with β-diethylaminoethyl diphenylpropylacetate hydrochloride[4] (II), an agent which inhibits certain microsomal enzyme pathways of drug metabolism *in vivo* and *in vitro*,[5] suggested an active metabolite. Studies were undertaken, therefore, to establish whether the urine of animals treated with I contained an active metabolite and if so, to isolate and identify it.

Results and Discussion

3,5-Dimethylpyrazole could not be detected in the urine of treated rats using either gas chromatography, a modification of the sensitive method of Woods, *et al.*, for amines,[6] or a sensitive thin layer chromatographic procedure. Using the latter method, less than 0.05% of the dose of I (the limit of detection) was excreted intact in the 24-hr. urine collection of rats treated with 25 mg./kg. twice daily. Although the urine did not contain the original hypoglycemic agent, it was hypoglycemically active; 0.5 ml. of urine produced a marked drop in blood sugar (Table I). Since this activity was not associated with 3,5-dimethylpyrazole, the hypothesis of an active metabolite was supported. The lyophilized solid from the urine was biologically active, while the distillate was inactive, thus indicating that the active compound was a solid. Acid (1 *M* HCl) and glucuronidase hydrolysis (pH 5) of the active urine did not yield 3,5-dimethylpyrazole, which indicated that the metabolite was not simply an N-substituted conjugate. The assay for amines of Woods, *et al.*, which employs brom cresol purple indicator, is a general procedure for basic compounds; its failure to detect basic compounds other than those normally present in rat urine showed the metabolite to be either acidic or neutral.

Extraction of the metabolite from the urine at several pH values into various solvents was attempted, and the extracts were tested for hypoglycemic activity. The active metabolite was extracted into ethyl acetate and 1-butanol (but not into chloroform) between pH 1.4 and 4.3, but only with difficulty at pH values of 0.05, or above 4.9 (Table I). This behavior suggested a polar, acidic compound, having a pK_a between 1 and 4, and possessing a protonatable nitrogen. Urine, after it had been extracted with ethyl acetate at pH 1, was inactive. Urine thus extracted was adjusted to pH 5 with acetate buffer, treated with a mixture of sulfatase and β-glucuronidase at 37° for 18 hr., and again tested for hypoglycemic activity. It was still inactive, thus eliminating the possibility of an inactive sulfate or glucuronide conjugate releasing an active compound on enzymatic hydrolysis.

TABLE I

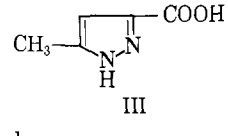
EFFECTS OF URINE AND URINE EXTRACTS[a] FROM 3,5-DIMETHYLPYRAZOLE-TREATED RATS ON BLOOD SUGAR OF INTACT, FASTED (GLUCOSE-PRIMED) RATS[b]

Treatment	Dose of urine or urine equivalent, ml.	Blood sugar[c] % reduction from control
Control urine	0.5–2.0	0
Distilled water	0.5	0
Urine from treated rats	0.5, 1.0, 2.0	*43*,[d] *53*, *44*
Solids from lyophilized urine	1.0	*37*
Distillate from lyophilized urine	1.0	0
Urine after pH 1 ethyl acetate extraction	1.0	0
Ethyl acetate extract of pH 1 urine	1.0	*46*
Ethyl acetate extract of pH 8.9 urine	1.0	1
Ethyl acetate extract of pH 12.6 urine	1.0	−3
Chloroform extract of pH 0.8 urine	1.0	4
Chloroform extract of pH 8.9 urine	1.0	4
Chloroform extract of pH 12.6 urine	1.0	3
Butanol extract of pH 0.05 urine	1.0	7
Butanol extract of pH 1.4 urine	1.0	*42*
Butanol extract of pH 4.3 urine	1.0	*34*
Butanol extract of pH 4.9 urine	1.0	*10*
Butanol extract of pH 5.9 urine	1.0	*12*

[a] The extracts, after evaporation to dryness, and the lyophilized solids were reconstituted with distilled water for blood sugar determinations. [b] Urine was collected for 24 hr. from rats treated twice with 25 mg./kg. of 3,5-dimethylpyrazole during that period. [c] Five or six rats were used for each blood sugar determination. [d] Statistically significant ($P < 0.05$) blood sugar depressions are italicized.

Isolation and purification of this acidic solid compound was accomplished by extraction, ion-exchange chromatography, precipitation, and finally sublimation to yield a highly water-soluble colorless solid, m.p. 239–241°.

Potentiometric microtitration of the sublimed material indicated that it possessed a single, fairly strong carboxyl function (pK_a of 3.8) and an equivalent weight of 132. The infrared spectrum of the metabolite showed C=O, OH, C=C, C=N, and aromatic substitution frequencies and supported a carboxylic acid. Elemental analysis of the isolated material indicated an empirical formula of $C_5H_6N_2O_2$. These data supported the monocarboxylic acid III (equiv. wt., 126), which was subsequently synthesized.[7] The infrared and ultraviolet spectra of the synthesized compound, its chromatographic mobilities in two thin layer and two paper chromatographic systems, and all other analytical data agreed with that of the isolated metabolite and supported the proposed structure.



III

From parallel dose–response curves, the hypoglycemic activity of III was found to be 200 times tolbutamide in the fasted, glucose-primed rat.

(4) Commonly referred to in the literature as SKF-525A.

(5) B. B. Brodie, *J. Pharm. Pharmacol.*, **8**, 1 (1956).

(6) L. A. Woods, J. Cochin, E. J. Fornefeld, F. G. McMahon, and M. H. Seevers, *J. Pharmacol. Exptl. Therap.*, **101**, 188 (1951).

(7) Synthesized by J. H. Markillie of The Upjohn Company, by the method of Knorr and MacDonald.[8]

(8) L. Knorr and J. MacDonald, *Ann.*, **279**, 217 (1894).

The studies involved with the isolation of III indicated that only acidic compound(s) in the urine had hypoglycemic activity since (a) urine which had been extracted at pH 1 with ethyl acetate or butanol no longer possessed hypoglycemic activity, (b) an active compound could not be extracted under neutral or basic conditions, and (c) no basic metabolites could be detected by the dye procedure of Woods, *et al.*,[6] either before or after acid and enzymic hydrolysis of the urine. However, the question as to whether III was in fact the only active metabolite of I still remained.

Exploratory biological studies indicated that III possessed no hypoglycemic activity in rats pretreated with II. The urine from rats treated with I, however, did have activity in rats pretreated with II. The latter experiment indicated either that I was metabolized to (and excreted as) an unknown compound, whose hypoglycemic activity was not inhibited by II, or that the amount of III present in the urine was such that all of its activity was not inhibited by II. In order to select from these two possibilities, it was necessary to determine the amount of III in the urine of rats treated with I.

Rat urine was analyzed for I and III by quantitative thin layer chromatography using the procedures described in Experimental. In a typical experiment, the 24-hr. urine collection from ten rats dosed orally with 50 mg. of I/rat was found to contain 0.085 mg./ml. of III and less than 0.0003 mg./ml. of I. This concentration of III represents 14.8% (by weight) of the total dose of I. Control urine was collected from the same rats prior to treatment, and III was added to give a final concentration of 0.085 mg./ml. Bioassay of these urine samples showed equivalent dose–response curves and the same hypoglycemic potency. Moreover, the activity of each urine sample was depressed to the same extent by II.

In a further experiment, the silica gel above and below the III zone on the thin layer chromatogram of the ethyl acetate extract of acidified urine was removed from the plate and eluted with water. This sample was tested for hypoglycemic activity and found to be inactive, adding further support to the conclusion that all of the activity present in urine from rats treated with I is due to III.

The preliminary experiments with II, instead of showing the presence of another active metabolite, demonstrated the importance of the dose ratio of II and III, *i.e.*, at high enough doses III does have activity in rats pretreated with II. Since the hypoglycemic activity of III is diminished by II, the latter is very likely effective through some pharmacological action other than its well-known effect on microsomal enzyme systems.

3,5-Dimethylpyrazole was inactive as a hypoglycemic agent in the dog, which suggested that metabolism to III might not occur in this species. Accordingly, 24-hr. urine collections from a catheterized mongrel dog, treated with 1.0 g. of I twice daily, was assayed for intact drug by the gas chromatographic method (*cf.* Experimental). No I could be detected; the sensitivity of the method allowed the conclusion that less than 0.2% of the dose was excreted intact.

Thin layer chromatography (system B) of an ethyl acetate extract of the acidified dog urine (pH 1.5) revealed a zone whose R_f corresponded to III. The zone was eluted and identified as III by its infrared spectrum. Thus, the fact that I is inactive as a hypoglycemic agent in the dog is not explained by this animal's failure to metabolize it to III. The latter was subsequently tested in the dog and found to be inactive in lowering blood sugar.

Experimental

Biological.—The determination of hypoglycemic activity was carried out in glucose-primed, fasted, intact rats as previously described.[9] The pure compounds (in 0.25% methyl cellulose vehicle), urine, and urinary extracts were administered orally by stomach tube unless otherwise specified. Extracts were evaporated to dryness and reconstituted to their volume equivalents of urine with water or 0.25% methyl cellulose vehicle. In the metabolism experiments, the rats were fasted overnight; the dose of I was usually 25 mg./kg. twice daily. The dose of β-diethylaminoethyl diphenylpropylacetate hydrochloride was 40 mg./kg., administered intraperitoneally 30 min. prior to drug administration. Water was allowed *ad libitum*. Urine was collected by housing the animals in cages designed to separate the excreta.

Six beagle dogs (three males, three females), weighing about 10 kg. and fasted 15 hr. prior to the experiment were used in the dog studies. The compounds were administered orally in gelatin capsules immediately after a 0-time blood sample was taken from the jugular vein. Blood was also taken at 1, 2, 4, and 6 hr. after treatment and sugars were determined by the Auto-Analyzer. At a dose of 5 mg./kg. orally, I did not alter blood sugar in the dog. It was also inactive at 2.3 or 10 mg./kg. when given intravenously.

Chemical. A. Materials. 3,5-Dimethylpyrazole (I).—The commercial product, Aldrich Chemical Co., was used; m.p. 106–108°, λ_{max} 213 mμ (ε 4700) in ethanol.

Anal. Calcd. for $C_5H_8N_2$: C, 62.47; H, 8.39; N, 29.14. Found: C, 62.03; H, 8.38; N, 28.99.

β-Diethylaminoethyl Diphenylpropylacetate Hydrochloride (II).—This material was a gift from Smith Kline and French Laboratories, Philadelphia, Pa.

5-Methylpyrazole-3-carboxylic Acid (III).—This compound, the active metabolite, was synthesized for comparison with the isolated material by J. H. Markillie of these laboratories, using the method of Knorr and MacDonald,[8] *i.e.*, by condensing 1-carboxy-2,4-pentanedione with hydrazine in KOH solution. The product was crystallized from water; m.p. 241–243°, lit.[8] m.p. 236°, λ_{max} 218 mμ (ε 9150) in ethanol.

Anal. Calcd. for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.67; H, 4.63; N, 22.51.

B. Isolation of III from Urine of Rats Treated with 3,5-Dimethylpyrazole.[10]—The 24-hr. urine collection of 20 rats (350 ml.), each of whom received 50 mg. of I, was adjusted to pH 1.5 with HCl and extracted for 5 hr. with 1 l. of ethyl acetate in a continuous extractor. The ethyl acetate layer was back-extracted twice with minimum volumes of 0.1 *M* phosphate buffer (pH 5.7). The phosphate solution was applied to a 2.5 × 25 cm. Dowex 1-X column in the chloride form, which was then washed thoroughly with water until an ultraviolet spectrum of the eluate showed essentially no absorption above 210 mμ. The column was eluted with 0.2 *M* HCl and the fractions possessing strong ultraviolet absorption were collected (an intense ultraviolet maximum at 216 mμ appeared in this eluate, whereas only nondescript, relatively weak absorption appeared when control urine was used). The eluate (pH 1.5) was extracted several times with equal volumes of ethyl acetate. After evaporation of the ethyl acetate almost to dryness, the biologically active material was precipitated from ethyl acetate–benzene (*ca.* 1:10). Further purification was achieved by sublimation, yielding 32 mg. of colorless solid, m.p. 239–241°.

C. Thin Layer Chromatography.—Silica gel G, 250-μ thickness and impregnated with $ZnSiO_3$ and $(ZnCd)_2S$ phosphors, was used for all thin layer chromatography. The zones were located

(9) W. E. Dulin, H. L. Osler, and F. G. McMahon, *Proc. Soc. Exptl. Biol. Med.*, **109**, 721 (1962).

(10) Urine from both treated and nontreated rats was subjected to this procedure. The isolation procedure was developed and monitored by testing the extracts and residues for hypoglycemic activity.

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).
(3) A. N. Davidson, *Physiol. Rev.*, **38**, 729 (1958).