Experimental Section

L-Tyrosine, 3-iodo-L-tyrosine, and 4-hydroxyphenylpyruvic acid were used as obtained from the Nutritional Biochemicals Corp. 2-Amino-4-hydroxy-6,7-dimethyltetrahydropteridine ($DMPH_4$) and α-ketoglutaric acid were supplied from Calbiochem. 3-Iodo-4-hydroxyphenylpyruvic acid was obtained from the Cyclo Chemical Corp. Pyridoxal phosphate and 4-hydroxyphenyl-DL-lactic acid were obtained from the Sigma Chemical Corp.; the compound was recrystallized (Et_2O) before use. 3,5-^{3}H-L-Tyrosine and ^{14}C-L-tyrosine, uniformly labeled, were obtained from the New England Nuclear Corp. *p*-Bromo-*o*-hydroxybenzoxyamine, a powerful dopa decarboxylase inhibitor, was supplied generously by Dr. Sidney Udenfriend of N. I. H. Elemental analyses were performed by G. Weiler and F. B. Strauss, Oxford, England.

3-Iodo-4-hydroxyphenylacetic Acid.—The iodination of 4-hydroxyphenylacetic acid was carried out in an ice–salt bath according to the procedure of Nakano and Danowski.[6] The white crystalline material obtained after one recrystallization (hot H_2O) was dried at 79° *in vacuo* over P_2O_5; mp 182–186°. *Anal.* ($C_8H_7IO_3 \cdot H_2O$) C, H, I.

3-Iodo-^{14}C-L-tyrosine.—Uniformly labeled 3-iodo-^{14}C-L-tyrosine was prepared from labeled ^{14}C-L-tyrosine according to the method of Pitt-Rivers,[7] adapted for microsynthesis in the following manner. Uniformly labeled ^{14}C-L-tyrosine (0.5 mCi, 1.08 μM, in 1.0 ml of 1 *N* HCl) was carefully dried under N_2 and the residue dissolved in 320 μl of 1 *N* NH_4OH. The solution was cooled to near freezing, then iodinated with 212 μl of 0.01 *N* I_2 over 30 min. The iodinated mixture was dried under N_2 and redissolved in 200 μl of 1 *N* NH_4OH. The solution was chromatographed in the BuOH–AcOH–H_2O system described below and radioautographed for 10 min. The band corresponding to monoiodotyrosine was cut from the paper and eluted slowly with 20 ml of 2 *N* HCl, and the eluate was dried under N_2. All but 3% of the radioactivity could be recovered in this manner. The residue was taken up in 6 ml of 0.005 *N* NaOH, the amount of monoiodotyrosine was determined from the absorbance measurement at 300 mμ, and the radioactivity of the product was determined from a planchet count of a 20-μl aliquot. The specific activity of the monoiodotyrosine was found to be 0.46 mCi/μmole; the yield of MIT was 79%. The NaOH solution was dried and the residue was dissolved in 2 ml of Krebs–Ringer phosphate buffer without Ca^{2+}; the final concentration was 426 nmoles/ml.

Paper Chromatography.—Ascending paper chromatography was carried out on Whatman No. 1 paper at room temperature using *n*-BuOH–AcOH–H_2O (120:35:50) and *n*-BuOH–dioxane–2 *N* NH_4OH (4:1:2).[8] Approximately 50 μg of MIT and of authentic metabolites were chromatographed with alkalized samples from slice and homogenate experiments. After development the location of the authentic compounds was established with uv light and with the following spray reagents: ninhydrin for amino acids, 2,4-dinitrophenylhydrazine for α-keto acids, and fast blue salt B followed by NaOH for phenols.[8] Bands of concentrated radioactivity identified by radioautography and intervening areas were counted on a low-background planchet counting system.

Tyrosine Hydroxylase.—A tyrosine hydroxylase preparation was obtained using the directions of Nagatsu, *et al.*[10] The enzyme was precipitated from the 105,000*g* supernatant of a bovine medullary tissue homogenate by the slow addition of solid $(NH_4)_2SO_4$ to 40% saturation. The precipitate was centrifuged at 14,000*g*, suspended in a minimum of 0.15 *M* phosphate buffer, and lyophilized under 1 mm for 3 hr; the dry powder was collected and stored in a dessicator over silica gel at 0° until ready for use.

Tyrosine hydrolyase was assayed according to the method of Udenfriend, *et al.*,[9] but $DMPH_4$ was used instead of tetrahydrofolate and Fe^{2+}; L-dititiotyrosine was diluted to yield a solution containing 5×10^{-5} mmole and an activity of 1.0 μCi in the 20-μl aliquot used in the assay.

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(7) R. Pitt-Rivers, *Chem. Ind.* (London), 21 (1956).
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Fluorinated α,α-Dialkylphenethylamines

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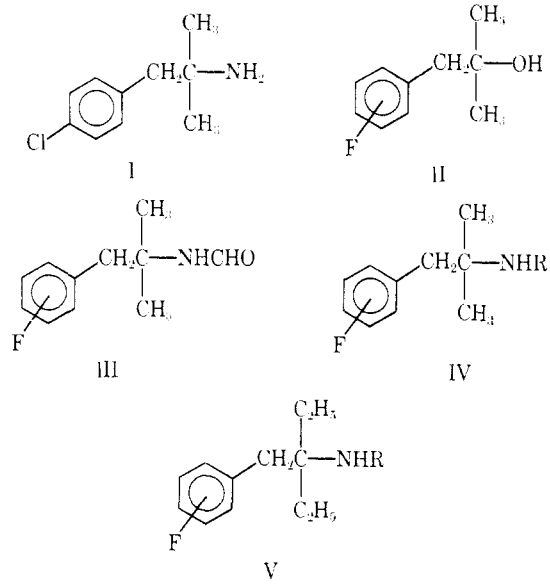
Holms, *et al.*,[1] have shown that the anorexigenic activity of phenethylamines can be dissociated from their effect on the central nervous system; halogenation in the nucleus and α,α-dimethylation produce compounds which are only anorexigenic;[2-4] groups bulkier than methyl in the α position and N substitution also give active anorexigenic compounds.[5] The most important of them is chlorophentermine (α,α-dimethyl-4-chlorophenethylamine, I), and it is known that the chloro compounds are more active than the bromo analogs. It thus seemed of interest to prepare the analogous fluorine compounds.

The condensation of 3- and 4-fluorobenzylmagnesium chloride with acetone gave the tertiary alcohols II, which, when subjected to the Ritter reaction[6,7] with NaCN in the presence of AcOH and H_2SO_4, led to the fluorinated α,α-dimethyl-*N*-formylphenethylamines (III). Their acid hydrolysis afforded the desired compounds IV (R = H),[8] while their reduction ($LiAlH_4$) led to the corresponding N-methyl derivatives (IV, R = CH_3). When diethyl ketone was used instead of acetone, compounds of type V were obtained.



Of the compounds described only N,α,α-trimethyl-4-

(1) T. Holms, I. Hous, R. Kopf, I. Møller-Nielsen, and P. V. Petersen, *Acta Pharmacol. Toxicol.*, **17**, 121 (1960).
(2) K. Opitz, *Arzneimittel-Forsch.*, **10**, 952 (1961).
(3) G. C. Boxill, M. Ben, I. W. Hillyard, and M. R. Warren, *J. Pharmacol. Exptl. Therap.*, **137**, 198 (1962).
(4) J. R. Gylys, J. J. D. Hart, and M. R. Warren, *ibid.*, **137**, 365 (1962).
(5) For a review see G. I. Poos, *Ann. Rept. Med. Chem.*, 51 (1965).
(6) J. Ritter and J. Kalish, *J. Am. Chem. Soc.*, **70**, 4048 (1948).
(7) A. Kaluszyner, S. Blum, and E. D. Bergmann, *J. Org. Chem.*, **28**, 3588 (1963).
(8) In several patents, the synthesis of α,α-dimethyl-4-fluorophenethylamine (IV, R = H) has been carried out by a similar method: Troponwerke Dinklage, Belgian Patent 636,858 (1964); *Chem. Abstr.*, **62**, 3976 (1965); French Patent CAM66 (1965); *Chem. Abstr.*, **62**, 13087 (1965); German Patent 1,199,779 (1965); *Chem. Abstr.*, **63**, 17967 (1965); A. Weber and J. Frossard, French Patent M4288 (1966); *Chem. Abstr.*, **68**, 59250 (1968).

TABLE I
PHARMACOLOGICAL DATA

No.	Compd	Tests performed[a] CNS	Analgetic	Local anesth	Anorexic	Antimicrobial	Significant act. obsd	MED[b]
1	IV, 3-F, R = H	+[c]	+	−[d]	−	+	CNS stimulation	<12.5
							Analgetic	<60
2	IV, 4-F, R = H	+	+	+	−	+	CNS stimulation	15
3	IV, 3-F, R = CH_3	+	+	−	−	+	CNS stimulation	15–30
							Analgetic	15
4	IV, 4-F, R = CH_3	+	+	−	+	+	CNS stimulation	15
							Analgetic	15
							Anorexic	50
5	V, 3-F, R = H	+	+	−	−	+	Antidepressant	15
6	V, 4-F, R = H	+	+	+	+	+	None	
7	V, 3-F, R = CH_3	+	+	+	+	+	Local anesthetic (tissue irritant)	2
8	V, 4-F, R = CH_3	+	+	+	+	+	Analgetic	15

[a] In addition to the testing described, compounds **4**, **6–8** were tested for several other general pharmacological activities and found to be inactive. [b] Minimal effective dose in mg/kg *po*, except for local anesthetic activity (%). [c] Test performed. [d] Not tested for activity indicated.

fluorophenethylamine showed some anorexigenic activity. The other pharmacological observations made on these compounds are summarized in Table I.[9]

Experimental Section

The peaks of the ir spectra were as expected.

N-Formyl-α,α-dimethyl-4-fluorophenethylamine (III).—A Grignard solution was prepared from 0.1 mole of *p*-fluorobenzyl chloride and 2.5 g of Mg in 50 ml of Et_2O, diluted with C_6H_6 (30 ml), and treated with a solution of 7 g of Me_2CO in 30 ml of C_6H_6 at such a rate that the mixture was kept boiling gently. The reaction mixture was refluxed for 30 min and decomposed at 0° with 300 ml of 10% NH_4Cl. The organic layer was washed (H_2O), dried, and concentrated *in vacuo* at below 70°. The oily carbinol (II) so obtained was added to a solution prepared from 0.1 mole of NaCN in 15 ml of glacial AcOH and 25 g of concentrated H_2SO_4 in 15 ml of the same solvent. The addition was carried out so that the temperature rose to 40–50°. The reaction mixture was stirred at 70° for 1 hr and at room temperature for 2 more hr and poured onto 300 g of ice. When the solution was neutralized with solid Na_2CO_3, the product separated as an oil. It was extracted (C_6H_6) and the solution so obtained was washed (H_2O), dried, and concentrated *in vacuo*; bp 134–136° (1 mm), yield 50%. The product solidified on standing and had mp 83° after recrystallization from cyclohexane. *Anal.* ($C_{11}H_{14}FNO$) F.

N-Formyl-α,α-dimethyl-3-fluorophenethylamine (III) was prepared analogously from 3-fluorobenzyl chloride; bp 128–130° (6 mm), 47%. *Anal.* ($C_{11}H_{14}FNO$) F.

N-Formyl-α,α-diethyl-4-fluorophenethylamine (V, R = CHO) was prepared analogously from 4-fluorobenzyl chloride and diethyl ketone; mp 128° (from cyclohexane), yield 55%. *Anal.* ($C_{13}H_{18}FNO$) F, N.

N-Formyl-α,α-diethyl-3-fluorophenethylamine (V, R = CHO) was prepared from 3-fluorobenzyl chloride and diethyl ketone; mp 116° (from cyclohexane), yield 55%. *Anal.* ($C_{13}H_{18}FNO$) F, N.

α,α-Dimethyl-4-fluorophenethylamine (IV, R = H) Hydrochloride.—A mixture of 2 g of the N-formyl compound, 5 ml of concentrated HCl, and 20 ml of EtOH was refluxed for 4 hr and brought to dryness *in vacuo*. The residue was recrystallized from *i*-PrOH–Et_2O; mp 186°, lit.[8] mp 185–186°, yield 1.6 g (80%).

α,α-Dimethyl-3-fluorophenethylamine (IV, R = H) hydrochloride was obtained in 80% yield and melted at 204°, lit.[10] mp 210° (from *i*-PrOH–Et_2O).

α,α-Diethyl-4-fluorophenethylamine (V, R = H) hydrochloride was obtained in 77% yield; mp 177° (from EtOAc–Et_2O). *Anal.* ($C_{12}H_{19}ClFN$) C, H, F.

α,α-Diethyl-3-fluorophenethylamine (V, R = H) hydrochloride was prepared in 80% yield, mp 167–168° (from Me_2CO–Et_2O). *Anal.* ($C_{12}H_{19}ClFN$) F, N.

N,α,α-Trimethyl-4-fluorophenethylamine (IV, R = CH_3) Hydrochloride.—A solution of 2 g of the N-formyl compound (III) in 50 ml of Et_2O was slowly added to a well-stirred, ice-cooled slurry of 0.4 g of LAH in 50 ml of Et_2O. The reaction mixture was stirred for 24 hr at room temperature, cooled in ice, and carefully decomposed with H_2O (*ca.* 5 ml); 5 g of $MgSO_4$ was added and the gelatinous mixture was stirred for 10 min, filtered, and washed several times with Et_2O. Dry HCl was passed into the ethereal solution to precipitate the hydrochloride, which was recrystallized from Me_2CO–Et_2O, yield 70%, mp 135–136°, lit.[10] mp 135°.

N,α,α-Trimethyl-3-fluorophenethylamine (IV, R = CH_3) hydrochloride was obtained analogously in 82% yield, mp 187° (from *i*-PrOH–Et_2O). *Anal.* ($C_{11}H_{17}ClFN$) C, H, N.

α,α-Diethyl-N-methyl-4-fluorophenethylamine (V, R = CH_3) hydrochloride was prepared in 83% yield, mp 179° (from Me_2CO). *Anal.* ($C_{13}H_{21}ClFN$) C, H, F.

α,α-Diethyl-N-methyl-3-fluorophenethylamine (V, R = CH_3) hydrochloride was prepared in 83% yield, mp 156° (from Me_2CO). *Anal.* ($C_{13}H_{21}ClFN$) C, H, F, N.

Pharmacology.—A summary of the pharmacological testing and results is given in Table I. All of the compounds described were tested for general CNS activity in the mouse. The CNS testing includes determination of reflex depressant, behavioral depressant, muscular relaxation, motor stimulation, and antidepressant activities. The test compound treated animals were observed and compared to control animals employing a rigid scoring system. The mice were carefully observed for signs of behavioral depression or stimulation. Their reaction to auditory, painful, and tactile stimuli was also determined. Spontaneous motor activity was compared to that of control animals. The animals were observed for the presence or absence of the righting, pinna, and corneal reflexes. The status of muscle tone was determined by evaluation of the abdominal and limb tone. In addition, grip strength was checked by placing the mice on a vertical pole. Inability to climb the pole or to maintain themselves on the pole indicated a reduction of the parameter.

Testing for antidepressant activity was performed in mice by intravenous injection of reserpine (5 mg/kg) 3 hr after the oral administration of test compound. Reserpine normally induces ptosis and descreased motor activity in these animals. A "reserpine reversal" as indicated by one or more of the observations given (Table I) suggests antidepressant activity: (a) increased motor activity, (b) profuse salivation, (c) reversal of reserpine ptosis.

Analgetic activity was determined employing a modification of the phenylquinone method of Sigmund, *et al.*[11] The total number of writhes for a group of test compound–phenylquinone treated mice was compared to that of a group receiving only phenylquinone. Evaluation was 60 min after test compound administration. A reduction of 30% in the number of writhes was considered significant.

(9) These studies have been carried out by Messrs. Interlab, Dewitt, N. Y. We are grateful to this firm for the permission to publish their pharmacological results.

(10) *Cf.* Science Union et Cie-Société Francaise de Recherches Medicales, Belgian Patent 609,630 (1962); *Chem. Abstr.*, **60**, 1647 (1964).

(11) E. Sigmund, R. Cadmus, and G. Lu, *Proc. Soc. Exptl. Biol. Med.*, **95**, 72 (1957).

Anorexic activity was evaluated in mice which were trained to consume their daily food intake over an 8-hr period. The amount of food intake for a group of mice treated with a test compound was determined and compared to that of a nontreated control group. A reduction of 30% in the test group was considered significant.

Local anesthetic activity was determined by instilling a solution of the test compound into a rabbit eye and determining the presence or absence of the corneal reflex. Absence of the corneal reflex is taken as an indication of local anesthetic activity. The lowest concentration (per cent) preventing the corneal reflex is taken as the minimal effective dose for local anesthetic activity.

Microbiological Testing.—Test compounds were evaluated for antimicrobial activity employing a series of gram-negative and gram-positive organisms. In addition, these compounds were also tested for antitrichomonal and antifungal activity. All of these tests were performed *in vitro*.

Phenothiazines Exhibiting Lesser Extrapyramidal Manifestations

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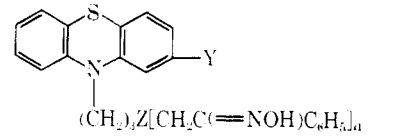
Since alkylaminoalkylphenothiazines are valuable agents in psychiatry, control of their extrapyramidal side effects offers an important challenge. Several intriguing views regarding the relationship of tranquilizing potency and extrapyramidal reactions are reported in the literature. Freyhan,[1] Haase,[2] and Brune, *et al.*,[3] are of the opinion that the ability of a drug to induce a Parkinson-like syndrome bears a direct correlation to its therapeutic efficacy. Cole and Clyde,[4] Brooks,[5] and Hollister,[6] on the other hand, believe that the suppression of parkinsonism does not lead to the impairment of therapeutic efficacy of tranquilizers and that the clinical apperance of extrapyramidal syndromes in no way predicts the therapeutic response of a tranquilizer.

An endeavor to find out the relationship between parkinsonism and antipsychotic activity of a tranquilizer can be made profitably on the basis of the hypothesis of McGeer,[7] *et al.* According to this hypothesis, parkinsonism may be brought about by a disturbance in normal brain equilibrium between serotonin and catecholamines on one hand, and histamine as well as acetylcholine on the other. Since the therapeutic efficacy of the phenothiazine group of tranquilizers bears a close correlation to their antiadrenergic[8] and antiserotonin[9] activity, the two possible ways to inhibit parkinsonism without interfering with antipsychotic activity is to enhance antihistaminic or anti-acetylcholine action. But potent antihistaminics among phenothiazines like promethazine or ethopropazine possess little tranquilizing activity, while perphenazine and trifluoperazine which are weak antihistaminics are potent tranquilizers; therefore the only way to reduce parkinsonism liability of phenothiazine tranquilizers is to increase their antiacetylcholine activity. Since oximes are well known as antidotes for organophosphorus poisoning (anti-DFP), we could speculate that anticholinergic quaternary oximes of phenothiazine drugs might provide a tranquilizer with less liability for Parkinson disease, and that the study of these compounds would lead to certain clues for the relationship between these two actions.

Three well-established phenothiazine tranquilizers, *viz.* chlorpromazine hydrochloride (CPZ), trifluoperazine dihydrochloride (TFP), and perphenazine dihydrochloride (PER), were selected for the present study. These hydrochlorides, on treatment with 40% sodium hydroxide solution, provided their respective free bases, which were treated with phenacyl bromide oxime. This resulted in the precipitation of the quaternary oxime of each drug, *viz.*, phenacyloxime chlorpromazine bromide (I), diphenacyloxime trifluoperazine dibromide (II), and diphenacyloxime perphenazine dibromide (III).



$(CH_2)_3Z[CH_2C(=NOH)C_6H_5]_n$

I. Y = Cl; Z = $N^+(CH_3)_2 \cdot Br^-$; $n = 1$

II. Y = CF$_3$; Z = ^+N⟨⟩$N^+CH_3 \cdot 2Br^-$; $n = 2$

III. Y = Cl; Z = ^+N⟨⟩$N^+(CH_2)_2OH \cdot 2Br^-$; $n = 2$

These compounds were assayed for their liability to induce catatonia in rats, a method which is believed to produce symptoms similar to those of parkinsonism.[10,11] The effects were compared to those of their parent drugs. Compound I at 10 mg/kg produced no catatonia while CPZ at this dose produced 62.5% catatonic reaction; II at 3 mg/kg produced only insignificant (20% catatonia) imbalance of posture, but TFP exhibited 100% catatonia at the same dose level. Similarly III at 3 mg/kg exhibited only 16.66% catatonia while PER at an equivalent dose produced 100% catatonic reactions.

Since these derivatives (I–III) offered a marked reduction in parkinsonism-like reactions over their parent drugs, it became of interest to find out to what extent these derivatives were devoid of tranquilizing property. The tests employed were as follows.

(i) Qualitative assessment of spontaneous motor activity (SMA), ptosis, and the influence of tactile and auditory stimuli on the mobility of mice by following a double blind observational method.[12]—In general, these derivatives affected the SMA to a lesser extent as compared to their parent drugs. III at 3 mg/kg compared well with 1 mg/kg of PER. The case was similar

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(2) H. J. Haase, ref 1, p 329.

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