20-one (**2**) were prepared by the method of Karmas[11] and Mallory,[12] respectively.

The oral progestational activity of these compounds was determined by the Clauberg test[13] and the endometrial response was scored according to the index of McPhail.[14] The McPhail index for 6α-methyl-17α-acetoxyprogesterone and compounds **5** was found to be +3.0 and +3.2, respectively, at 0.5 mg. These data, though limited in nature, do point out that the oxygen function at C-3 is not an absolute necessity for progestational response. On the other hand the McPhail index of 6α,16α-dimethylprogesterone and the oxime of **6** (2.1 and 0, respectively, at 5.0 mg) points out the importance of the C-20 functional group for protein binding. The polarity of the carbonyl group constitutes a point of contact with the receptor superior to that of the oxime. The oxidation–reduction of the oxygen function at C-3 as a possible factor in the mechanism of action probably can be ruled out.

Experimental Section

All melting points were taken with a Fisher-Johns melting point apparatus and are uncorrected. The uv and ir data were obtained on a Cary Mode 111 and Beckman IR-5 spectrophotometers, respectively. Nmr spectra were determined on a Varian A-60 spectrometer in $CDCl_3$ using TMS as an internal standard. Elemental analyses were performed by Midwest Microlab Inc., Indianapolis, Ind. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

17α-Acetoxy-6-methylpregn-3,5-dien-20-one (3).—Raney Ni (88.0 g) was suspended in 800 ml of Me_2CO and refluxed with stirring under N_2 for 0.5 hr. The mixture was cooled to room temperature and to it was added 8.0 g of **1** in 100 ml of THF. Stirring was continued at 25° for 2.5 hr. The reaction mixture was filtered and the filtrate was evaporated to give an oil. Recrystallization from ether–hexane yielded 4.5 g (70%) of **3**: mp 132–133°; λ_{max}^{EtOH} 241 mμ; λ_{max}^{KBr} 1650, 1720, 1731 cm^{-1}; nmr, multiplets at 5.68 and 6.41 ppm (C-3, C-4). *Anal.* ($C_{24}H_{34}O_3$) C, H.

17α-Acetoxy-6-methylpregn-5-en-20-one (5).—Compound **3** (1.0 g) was dissolved in 15 ml of AcOH and 700 mg of 5% Pd–C was added. The mixture was hydrogenated at room temperature until 1 equiv of H_2 was consumed. It was filtered and the filtrate was evaporated to give an oil. Repeated recrystallization from hexane gave 250 mg (36%) of **5**: mp 166–168°; $\lambda_{max}^{cyclohexane}$ 198 mμ (ε 8900); λ_{max}^{KBr} 1723, 1737 cm^{-1}; nmr, no resonance due to vinyl protons. *Anal.* ($C_{24}H_{36}O_3$) C, H.

6,16α-Dimethylpregn-3,5-dien-20-one (4).—A mixture of 80 g of Raney Ni in 700 ml of Me_2CO was stirred and refluxed for 0.5 hr under N_2. The mixture was cooled to room temperature and to it was added a solution of 6.9 g of **2** in 75 ml of THF. Stirring was continued for 3.5 hr at 25°. The mixture was filtered and the filtrate was evaporated to a dark residue. Chromatographic separation on neutral alumina gave 3.9 g (80%) of **4** on elution with 9:1 hexane–Et_2O. Recrystallization from hexane gave analytically pure sample: mp 125–126°; λ_{max}^{EtOH} 241 mμ; λ_{max}^{KBr} 1650, 1705 cm^{-1}; nmr, 5.30 ppm (vinyl multiplets). *Anal.* ($C_{23}H_{34}O$): C, H.

6,16α-Dimethylpregn-5-en-20-one (6).—Compound **4** (500 mg) was dissolved in 15 ml of AcOH and to it was added 250 mg of 5% Pd–C. The mixture was hydrogenated at room temperature until 1 equiv of H_2 was consumed. The mixture was filtered and the filtrate was evaporated to give an oil which failed to crystallize; λ_{max}^{NaCl} 1705 cm^{-1}; nmr, no resonance due to vinyl protons. Analyzed as oxime, mp 75–76°. *Anal.* ($C_{23}H_{37}NO$) C, H, N.

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(11) G. Karmas, *Tetrahedron Letters*, 1093 (1964).
(12) R. Mallory, personal communications.
(13) C. Clauberg, *Zentr. Gynaekol.*, **54**, 2757 (1930).
(14) M. K. McPhail, *J. Physiol.* (London), **83**, 145 (1935).

2-Hydroxy-2-phenylethylhydrazine Monoamine Oxidase Inhibitors

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2-Hydroxy-2-phenylethylhydrazine (**1**) was synthesized first by Benoit[2] and observed to have typical sympathomimetic activity with a potency 1/500th that of epinephrine.[3] Later, Biel, *et al.*,[4] evaluated **1** for monoamine oxidase (MAO) inhibitory activity and found it to be eight times as potent as iproniazid *in vivo*, but inactive at 10^{-5} *M in vitro*. In this study we desired to determine the effect of position and number of carbethoxy groups on MAO inhibitory activity and to verify the lack of *in vitro* activity of **1**.

The N^1-carbethoxy derivative (**2**) was prepared by treating **1**[2] with ethyl chloroformate. The first approach to the synthesis of the N^2-carbethoxy derivative (**3**) involved the reaction of styrene oxide and ethyl carbazate. This procedure led to a complex mixture of products, a situation not unexpected in light of the mixture obtained by treating styrene oxide with ethyl glycinate.[5]

Compound **3** was synthesized by treating styrene bromohydrin with ethyl carbazate. The N^1,N^2-dicarbethoxy derivative (**4**) was prepared from either **2** or **3** by reaction with ethyl chloroformate. That the positions of the carbethoxy groups are correctly assigned to nitrogens and are not on the alcoholic oxygens is shown by the ir absorption of the carbonyl groups of **2**, **3**, and **4**. The carbonyls of ethyl carbazate and diethyl carbonate are at 1716 and 1750 cm^{-1}, respectively. Compounds **2**, **3**, and **4** are at 1690, 1715, and 1725 and 1705 cm^{-1}, respectively. Attempts to cause N → O migration of the carbethoxy group according to the method of Lyle and Durand[6] failed.

Biological Results.—Mitochondrial monoamine oxidase from beef liver was isolated and purified as described by Ho, *et al.*[7] Incubation was carried out at 37° for 30 min in a solution containing 0.15 mμmole of substrate, tyramine-1-C^{14}, per milliliter, varying amounts of inhibitor, 20 μl of enzyme, phosphate buffer pH 7.4, and water to make a final volume of 1 ml. The product, a mixture of *p*-hydroxyphenylacetaldehyde and *p*-hydroxyphenylacetic acid, was extracted with EtOAc in strongly acidic medium. After removal of solvent, the product was assayed for C^{14} in a liquid scintillation spectrometer and the concentration of the inhibitor at which enzyme activity was 50% inhibited (I_{50}) was determined. The results are shown in Table I.

(1) National Science Foundation undergraduate research participant.
(2) G. Benoit, *Bull. Soc. Chim. France*, **6**, 708, (1939).
(3) G. Benoit and D. Bovet, *Compt. Rend. Soc. Biol.*, **136**, 356 (1942).
(4) J. H. Biel, A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Horita, *J. Am. Chem. Soc.*, **81**, 2805 (1959).
(5) K. Jankowski and C. Berse, *Can. J. Chem.*, **44**, 1513 (1966).
(6) G. G. Lyle and M. L. Durand, *J. Org. Chem.*, **32**, 3295 (1967).
(7) B. T. Ho, W. M. McIsaac, K. E. Walker, and V. Estevez, *J. Pharm. Sci.*, **57**, 269 (1968).

TABLE I
INHIBITION OF MAO BY $C_6H_5CHOHCH_2NHR_2$ (with R^1)

Compd	R^1	R^2	I_{50}, mM
1·tartrate	H	H	0.16
2·HCl	$CO_2C_2H_5$	H	7.8
3·HCl	H	$CO_2C_2H_5$	5.4
4·HCl	$CO_2C_2H_5$	$CO_2C_2H_5$	16.0

Discussion.—The potency order of **1** > **3** > **2** > **4** is consistent with accepted structure–activity relationships for MAO inhibition in phenylethylhydrazine molecules.[8] The presence of a carbethoxy group on either N adversely affects the *in vitro* MAO inhibitory potency. The discrepancy between the *in vitro* activity of **1** observed in this study and the results of Biel, *et al.*,[4] may be due to the use of different substrates.

Experimental Section[9]

2-Hydroxy-2-phenylethylhydrazine (1).—The method of Benoit[2] was employed. The oxalate salt, mp 170° [*Anal.* ($C_{10}H_{14}N_2O_5$) C, H, N], and the tartrate salt, mp 141–143°, were recrystallized from MeOH–Et_2O.

N^1-Carbethoxy(2-hydroxy-2-phenyl)ethylhydrazine (2) was synthesized by treating **1** with ethyl chloroformate as described by Matzner, *et al.*[10] The HCl salt was recrystallized from $CHCl_3$–EtOAc; yield 76%, mp 134–136°. *Anal.* ($C_{11}H_{17}ClN_2O_3$) C, H, N.

N^2-Carbethoxy(2-hydroxy-2-phenyl)ethylhydrazine (3).—To a solution of styrene bromohydrin (2.42 g, 0.012 mole) in 100 ml of EtOH was added a solution of ethyl carbazate (1.4 g, 0.0133 mole) in 50 ml of EtOH. The solution was refluxed overnight, evaporated under reduced pressure, and treated with 20 ml of 10% $NaHCO_3$ solution and 200 ml of EtOH. The precipitate was removed and the solution was evaporated to yield **3** as an oil, yield 70%). The oxalate, mp 112–115° [*Anal.* ($C_{13}H_{18}N_2O_7$) C, H, N], and the HCl salt, mp 108–110°, were recrystallized from MeOH–Et_2O.

N^1,N^2-Dicarbethoxy(2-hydroxyl-2-phenyl)ethylhydrazine (4).—To a cold solution of **2** (4.0 g, 0.018 mole) in 150 ml of Et_2O was added ethyl chloroformate (2.95 g, 0.027 mole) in 100 ml of Et_2O. After stirring for 2 hr the product (**4**) was removed by filtration. The HCl salt, mp 142–143°, yield 65%, was recrystallized from $CHCl_3$–EtOAc. *Anal.* ($C_{14}H_{21}ClN_2O_5$) C, H, N.

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(8) B. M. Bloom, *Ann. N. Y. Acad. Sci.*, 107, 878 (1963).

(9) Melting points were determined on a Hoover apparatus and are uncorrected. Where analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(10) M. Matzner, R. P. Kurkjy, and R. S. Cotter, *Chem. Rev.*, **64**, 645 (1964).

Thiomethyltetrazole Hypoglycemic Agents

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A report[1] of the potentiation of insulin hypoglycemia by a series of phenylsulfinyl- and -sulfonylacetic acid derivatives prompted us to synthesize and test for hypoglycemic effects a group of related congeners in which the carboxyl group was replaced by an acidic, 5-tetrazolyl moiety.[2] It was anticipated that the tetrazole would impart the same degree of acidity[3] to the resulting compounds as the carboxyl group but would not undergo the facile decarboxylation typical for β-sulfinyl- and β-sulfonylacetic acids (Table I).

A series of alkyl-, aralkyl- and arylthioacetonitriles (Table II), prepared by alkylation of the requisite mercaptan with chloroacetonitrile, was converted to the corresponding tetrazoles using the method of Finnegan, *et al.*[4] Oxidation of the resulting thiomethyltetrazoles using H_2O_2 in AcOH provided a route to the desired sulfones.

Titration in 1:1 dioxane–water showed the thiomethyltetrazoles to be weak acids; the sulfonylmethyl congeners, because of the inductive effect of the sulfone group, were somewhat more acidic, differing from the sulfides by more than 1 pK_a unit. Compound **2** gave an anomalous titration curve which was indicative of a dibasic acid with a neutralization equivalent approximately one-half the calculated value. It is probable that the CH_2 group, flanked by the CF_3 and SO_2 moieties, is sufficiently acidic to be titrated along with the tetrazole. The absence of a discernible double break in the titration curve prevented meaningful calculation of pK_{a_1} and pK_{a_2}.

None of the compounds showed any hypoglycemic activity when screened according to previously described procedures.[5]

Experimental Section[6]

5-(2,2,2-Trifluoroethylthiomethyl)tetrazole.—A mixture of 3.1 g (0.02 mole) of 2,2,2-trifluoroethylthioacetonitrile,[7] 2.16 g (0.04 mole) of NH_4Cl, 2.8 g (0.044 mole) of NaN_3, and 0.02 g of LiCl in 15 ml of DMF was heated at steam-bath temperatures overnight. The reaction mixture was cooled, diluted with 30 ml of H_2O, and acidified with concentrated HCl to pH 3–4. The precipitated solid was filtered and dried, 2.5 g.

The thiomethyltetrazoles were prepared by a similar procedure in yields of 60–90%; they are listed with their physical properties in Table I.

5-(Isopropylsulfonylmethyl)tetrazole.—To a solution of 11.8 g (0.075 mole) of 5-(isopropylthiomethyl)tetrazole in 75 ml of glacial AcOH was added 19 g of 30% H_2O_2 dropwise over a period of 15 min. The reaction mixture was stirred at room temperature overnight, and was then heated at steam-bath temperature for 15 min. The solution was cooled, and the product precipitated by the addition of pentane.

The sulfones listed in Table I were all prepared by a similar procedure in 40–60%.

***p*-Chlorophenylthioacetonitrile.**—To a suspension of 59 g (0.42 mole) of *p*-chlorothiophenol and 55 g (0.42 mole) of K_2CO_3 in 275 ml of 1,2-dimethoxyethane was added, dropwise, 32 g (0.42 mole) of chloroacetonitrile. The resulting mixture was stirred for 1 hr, and was then refluxed overnight. Most of the solvent was removed under reduced pressure followed by the addition of

(1) E. Riesz, W. Wolf, and H. Alvarez, *Bull. Soc. Chim. France*, 1057 (1963).

(2) R. M. Herbst in "Essays in Biochemistry", S. Graff, Ed., John Wiley and Sons, Inc., New York, N. Y., 1956, pp 141–155.

(3) J. S. Mihina and R. M. Herbst, *J. Org. Chem.*, 15, 1082 (1950).

(4) W. G. Finnegan, R. A. Henry, and R. Lofquist, *J. Am. Chem. Soc.*, **80**, 3908 (1958).

(5) J. M. McManus, J. W. McFarland, C. F. Gerber, W. M. McLamore, and G. D. Laubach, *J. Med. Chem.*, **8**, 766 (1965).

(6) Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are corrected. The analyses were carried out by the Physical Measurements Laboratory of Chas. Pfizer & Co., Inc. Titrations were done in 1:1 (v/v) dioxane–water using a Metrohm potentiograph Model E436. Where analyses are indicated only by symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

(7) J. M. McManus, *J. Heterocycl. Chem.*, **5**, 137 (1968).