collagen-synthesizing system. Fluoroproline may offer certain advantages over proline in coding experiments since it is not diluted with endogenous amino acid present in biological preparations.

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Synthesis and Metabolism of 6-Hydroxycatecholamines*

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ABSTRACT: The following 6-hydroxycatecholamines (2,4,5-trihydroxyphenethylamines or -phenethanolamines), potential metabolites of catecholamines, were synthesized (Charts I–III): 3-*O*-methyl-6-hydroxydopamine, 6-hydroxynorepinephrine, 3-*O*-methyl-6-hydroxynorepinephrine, 6-hydroxyepinephrine, and 3-*O*-methyl-6-hydroxyepinephrine. Enzymatic *O*-methylation with catechol *O*-methyltransferase with *S*-adenosylmethionine-^{14}C as donor of $^{14}CH_3$ gave radioactive ^{14}C-labeled 3-*O*-methyl-6-hydroxycatecholamines which were used for metabolic studies in the rat. The corresponding phenylacetic (**20a**) and mandelic acids (**23a**) as well as the phenylglycol (**30a**) were identified as metabolites by comparison with synthetic compounds (Table I, Chart IV). Eleven new potential metabolites were compared and characterized (Table II). The relative substrate activity of some of these 3-*O*-methyl-6-hydroxycatecholamines with monoamine oxidase (Table III) was much lower than that of 3-*O*-methyldopamine or normetanephrine.

Alternate pathways for the metabolism of catecholamines, such as dopamine, norepinephrine, and epinephrine, have received intensive scrutiny in attempts to define normal metabolism and in the hope of detecting metabolic aberrations in congenital and chronic dyscrasias (Daly and Witkop, 1963). One such possible pathway is the demonstrated nuclear hydroxylation of dopamine *in vivo* (Senoh *et al.*, 1959a,c) and *in vitro* (Senoh and Witkop, 1959a,b; Senoh *et al.*, 1959a,c) which leads to 2,4,5-trihydroxyphenethylamine (6-hydroxydop-

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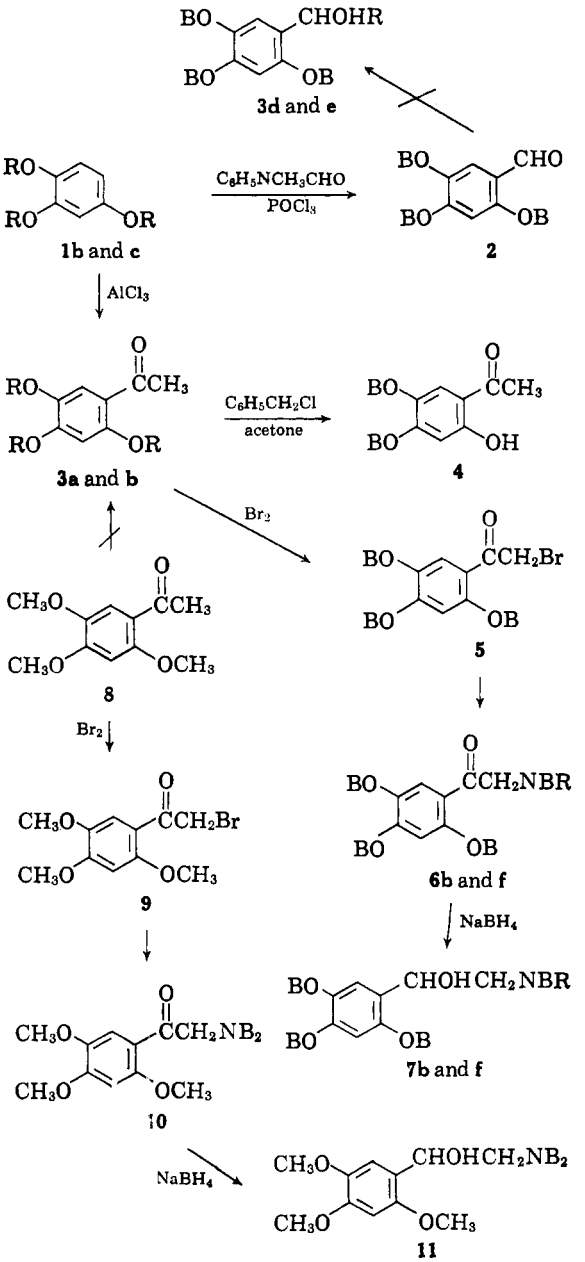
amine) (**35**). Analogous hydroxylations of norepinephrine would yield 6-hydroxynorepinephrines (**36**). As hydroxyhydroquinones these compounds are extremely sensitive and easily autoxidized to *p*-quinoid aminochromes (Senoh and Witkop, 1959b). If such 6-hydroxycatecholamines were formed *in vivo*, they could undergo a variety of transformations, *e.g.*, oxidative cyclization to aminochromes, oxidative deamination by monoamine oxidase (or other amine oxidases), and *O*-methylation of the catechol moiety by catechol *O*-methyltransferase (COMT).[1] The latter reaction has been demonstrated *in vitro* and *in vivo* for 6-hydroxydopamine (Daly *et al.*, 1961) which forms 2,4-dihydroxy-5-methoxyphenethylamine (**16a**). The demonstration that 6-hydroxydopamine causes a profound and prolonged depletion of heart norepinephrine (Porter *et al.*, 1963; Stone *et al.*, 1964; Laverty *et al.*, 1965) *in vivo* by an irreversible alteration of norepinephrine binding sites prompted us to resume and complete our synthetic and metabolic studies of the 6-hydroxycatecholamines.

Synthetic Routes (*Charts I–III*). The synthesis of 6-hydroxynorepinephrine was first attempted starting with 1,2,4-triacetoxybenzene (1,2,4-triphenenyl triacetate, **1c**), which was converted to the tribenzyloxy derivative **1b**, and subsequently to 2,4,5-tribenzyloxybenzaldehyde (**2**). The aldehyde, however, could not be converted to a suitable intermediate **3d** or **e**. An alternate successful synthesis was the formation from **1c** of 2,4,5-trihydroxyacetophenone (**3a**) *via* a Fries rearrangement. Benzylation in acetone led to the 4,5-dibenzyloxy-2-hydroxyacetophenone (**4**), the infrared spectrum of which indicated a hydrogen-bonded carbonyl (1640 cm^{-1}) such as in *o*-hydroxyacetophenone (1647 cm^{-1}) and in 2,4,5-trihydroxyacetophenone (**3a**, 1640 cm^{-1}). At higher temperatures in dimethylformamide complete benzylation to 2,4,5-tribenzyloxyacetophenone (**3b**, C=O, 1667 cm^{-1}) was achieved.

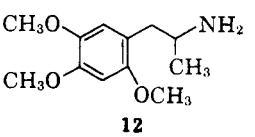
Bromination of the acetophenone **3b** yielded the ω-bromoacetophenone **5** which, on reaction with the appropriate amine, yielded ω-dibenzylamino-2,4,5-tribenzyloxyacetophenone (**6b**) or ω-benzylmethylamino-2,4,5-tribenzyloxyacetophenone (**6f**). The ω-benzylmethylamino derivative **6f** was obtained in good yield *via* the lithium salt of benzylmethylamine. The usual reaction of amine and bromo ketone **5** afforded, in addition to the desired product, varying amounts of an acid, characterized as 2,4,5-tribenzyloxybenzoic acid. These keto compounds were then reduced with sodium borohydride to the benzyl derivatives **7b** and **f** of 6-hydroxynorepinephrine. Attempted conversion of 2,4,5-trimethoxyacetophenone (**8**) to **3a** with hydrochloric acid (sealed tube) was unsuccessful, but the analogous sequence, **8** → **11**, to the amine was successful. The 2,4,5-trimethoxyphenethanolamine (**11**) or its debenzylated product are of interest as psychotomimetics since the related 2,4,5-trimethoxyphenylpropylamine (**12**) is 17 times as active a hallucinogen in humans than mescaline (Shulgin, 1964).

CHART I

SYNTHESIS OF 6-HYDROXYNOREPINEPHRINE



a, R = H
b, R = benzyl
c, R = acetyl
d, R = CN
e, R = CH_2NO_2
f, R = CH_3; B = benzyl



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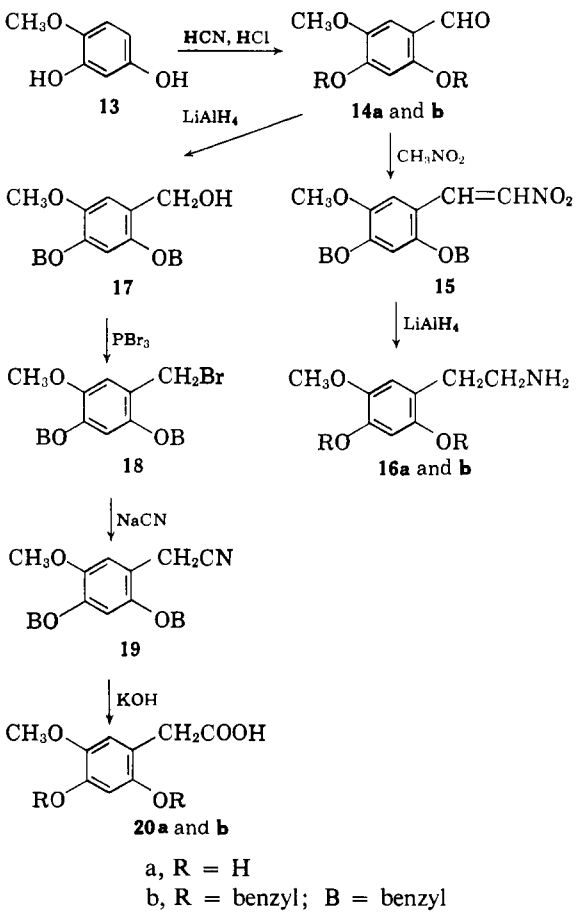
The synthesis of potential *O*-methylated metabolites of 6-hydroxycatecholamines required as a key intermediate 2,4-dihydroxyanisole (**13**), which has been prepared for guaiacol in six steps (Head and Robertson,

[1] Abbreviations used: COMT, catechol *O*-methyltransferase; MAO, monoamine oxidase; nmr, nuclear magnetic resonance.

1931) and from isovanillin *via* a Baeyer–Villiger oxidation (Crosby, 1961). By the use of freshly prepared peracetic acid, which was neutralized with sodium acetate, the yields in the Baeyer–Villiger oxidation became satisfactory. A Gattermann aldehyde synthesis (Head and Robertson, 1931) followed by benzylation afforded 2,4-dibenzyloxy-5-methoxybenzaldehyde (**14b**). 2,4-Dihydroxy-5-methoxyphenethylamine (**16a**) was then prepared from **14b** *via* the nitrostyrene **15**, reduction, and catalytic debenzylation. The corresponding acid, 2,4-dihydroxy-5-methoxyphenylacetic acid (**20a**), was synthesized as shown in Chart II (**14b** → **20a**). In the norepinephrine series (Chart III), 2-amino-1-(2,4-dihydroxy-5-methoxyphenyl)ethanol (**22a**) was prepared by reduction on the mandelonitrile acetate **21** and subsequent debenzylation. Attempted formation of the 2,4-dibenzyloxy-5-methoxymandelic acid (**31b**)

CHART II
SYNTHESIS OF POTENTIAL METABOLITES OF 6-HYDROXYDOPAMINE



a, R = H
b, R = benzyl; B = benzyl

by ethanolysis and hydrolysis of the mandelonitrile acetate **21** led to the formation of α-ethoxy derivatives **23a** and **c**. In another attempt to prepare the mandelic acid **31b** the Hoesch reaction (Spoerri and DuBois, 1949) on 2,4-dihydroxyanisole (**13a**) was studied using mono-, di-, and trichloroacetonitrile. The mono- (**24**) and dichloroacetophenones (**27**) were synthesized, whereas the use of trichloroacetonitrile resulted in the formation of a trichloroacetate of dihydroxyanisole. Neither oxidation of the monochloro ketone **24** in dimethyl sulfoxide at 80° (Kornblum *et al.*, 1957) nor basic hydrolysis of the dichloro ketone **27a** (Aston *et al.*, 1955) resulted in formation of the corresponding glyoxal or mandelic acid. The monochloro ketone **24** with sodium bicarbonate in dimethyl sulfoxide underwent cyclization to 5-methoxy-6-hydroxybenzofuran-3-one (**25**). Treatment of the monochloro ketone **24** with *N*-methylbenzylamine gave β-benzylmethylamino-2,4-dihydroxy-5-methoxyacetophenone (**26**), but this material could not be successfully reduced to the *N*-methylamino alcohol.

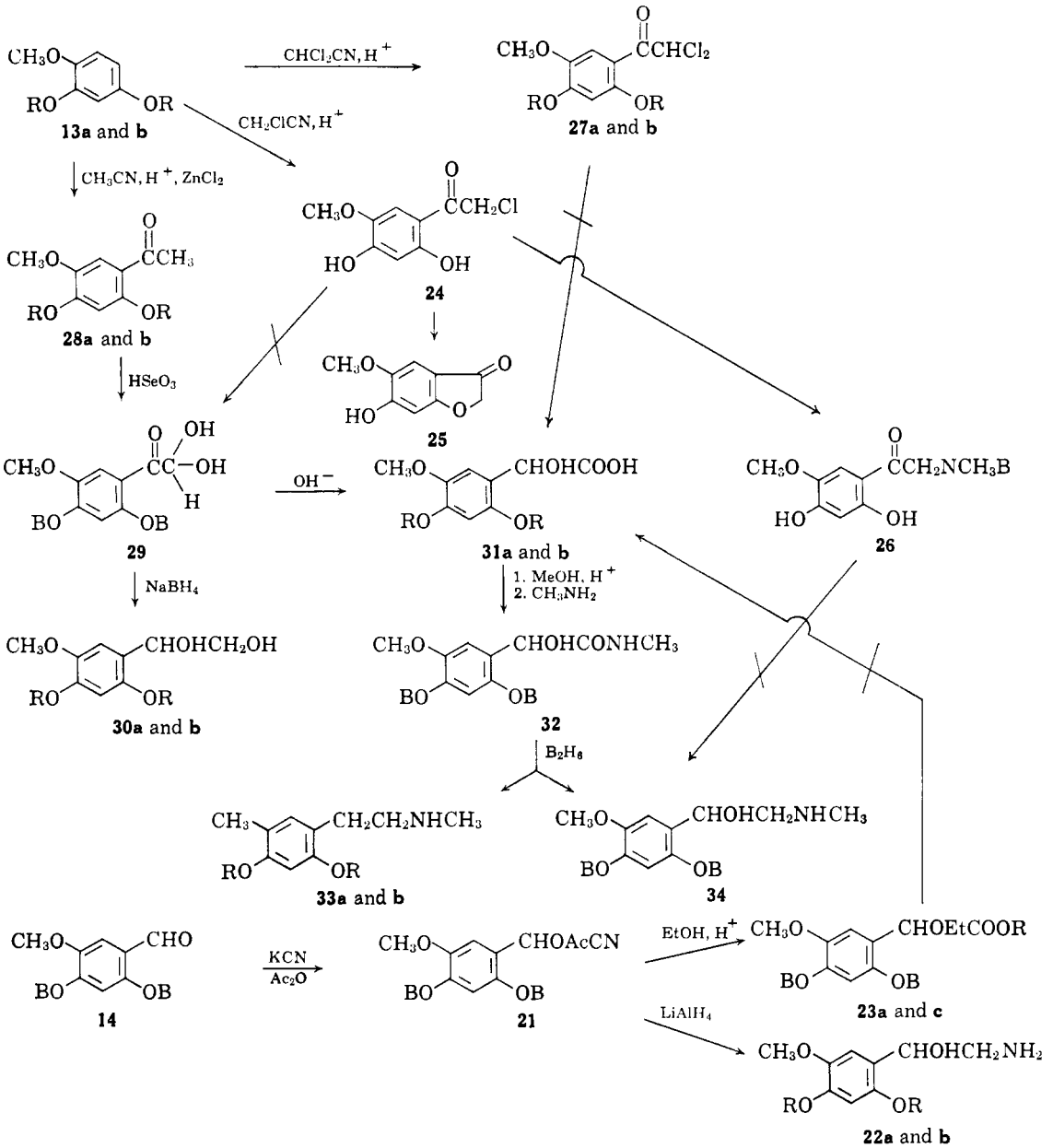
The Hoesch reaction with acetonitrile and 2,4-dihydroxyanisole (**13a**) gave the acetophenone **28a** which was benzylated to **28b** and then oxidized to the glyoxal **29** with selenious acid. Reduction with sodium borohydride afforded the glycol **30b**, while treatment with base yielded the mandelic acid **31b**. The structure of the acid **31b** was confirmed by the nmr spectrum.

In order to obtain the *O*-methylated 6-hydroxyepinephrine **34** the mandelic acid **31b** was converted to the ester and then to the *N*-methylamide **32**. Attempts to reduce this compound with lithium aluminum hydride were unsuccessful. Diborane has recently been used to reduce amides to amines (Brown and Heim, 1964; Papanastassiou and Bruni, 1964; Corsano and Bombardiere, 1964). On reduction of 2,4-dibenzyloxy-5-methoxy-*N*-methylmandelamide (**32b**) with a large excess of diborane, conversion to the phenethylamine (**33b**) was achieved, whereas with smaller amounts of diborane the phenethanolamine (**34**) was isolated.

Certain debenzylated compounds, **7b** and **f** and **34**, because of their inherent lability, were prepared in solution by catalytic (10% Pd–C) reduction with hydrogen in ethanol (3 hr) immediately before chromatography or biochemical studies and no attempt was made to isolate a crystalline product.

Metabolic Studies. Studies on the metabolism of the 6-hydroxycatecholamines, because of their lability, required the use of radioactive isotopes. Since the fate of exogenous catecholamines *in vivo* involves primarily *O*-methylation (Axelrod *et al.*, 1958) the 6-hydroxycatecholamines were enzymatically *O*-methylated by a purified catechol *O*-methyltransferase preparation (Senoh *et al.*, 1959b) in the presence of radioactive *S*-adenosylmethionine-^{14}C as methyl donor. The *O*-methylated amine was then analyzed by paper chromatography to ensure homogeneity, diluted with carrier 3-*O*-methyl-6-hydroxycatecholamine, and administered intraperitoneally to rats. The urine was collected for 24 hr and cooled in ice during collection. One portion was treated with a molluscan glucuronidase and sulfatase preparation, the other assayed directly. Acidic and neutral products were extracted at pH 4 with ethyl acetate. Amines were collected on Dowex 50 (H^+ form); the effluent contained the conjugated metabolites. The amines were then eluted with ammonia. The radioactive metabolites were identified by paper chromatography and radiography in at least two solvent systems; au-

CHART III
SYNTHESIS OF POTENTIAL METABOLITES OF 6-HYDROXYNOREPINEPHRINE



a, R = H
b, R = benzyl
c, R = C_7H_7; B = benzyl

thentic compounds were cochromatographed. The metabolism of 6-hydroxycatecholamines in rats resembles that of dopamine and norepinephrine (Axelrod *et al.*, 1958; Goldstein *et al.*, 1961; Kopin *et al.*, 1961) and leads to amines, acids, and alcohols as metabolic end products. The conjugate fraction, however, appeared much more resistant to enzymatic hydrolysis. Chart IV and Table I summarize the results. R_F values in a variety of solvent systems and color reactions are synoptically presented in Table II.

By the use of a preparation of monoamine oxidase (MAO, rat liver mitochondrial fraction) and *O*-methyl-^{14}C-catecholamine substrates, relative activities (Leeper *et al.*, 1958) for the various 6-hydroxycatecholamines with MAO were determined. Only 3-*O*-methyl-6-hydroxydopamine was moderately active as a substrate, the 3-*O*-methyl-6-hydroxynorepinephrines showing *very low* activity as presented in Table III. *In vitro* studies with dopamine β-oxidase (Creveling *et al.*, 1962) and 3-*O*-methyl-6-hydroxydopamine had indicated that β-hydroxylation to the norepinephrine series does not occur with the 6-hydroxydopamine derivatives.

TABLE I: Metabolic Fate of *O*-Methylated-^{14}C 6-Hydroxycatecholamines in the Rat.[a]

	3-*O*-Methyl-6-hydroxydopamine[b]	3-*O*-Methyl-6-hydroxynorepinephrine	3-*O*-Methyl-6-hydroxyepinephrine
Free Amine	18	20	11
Conjugates[c]	28	26	27
2,4-Dihydroxy-5-methoxyphenylacetic acid	11[d]	..	..
2,4-Dihydroxy-5-methoxymandelic acid	..	6	4
2,4-Dihydroxy-5-methoxyphenyl glycol	..	3	1
Total	57	55	43

[a] Each rat received 1–2 μc of *O*-methylated-^{14}C 6-hydroxycatecholamine (20 μmoles) intraperitoneally. Urine was collected for 24 hr. Results are expressed as percentage of recovered activity. [b] Average of 2 animals. [c] Conjugates consisted mainly of amine conjugates but were quite resistant to hydrolysis with glucuronidase. [d] A minor neutral metabolite (~1%) exhibited the same R_F as a lithium hydride and Pd–C and hydrogen reduction product of 2,4-dibenzyloxy-5-methoxyphenylacetic acid and is assumed to be 2,4-dihydroxy-5-methoxyphenethanol.

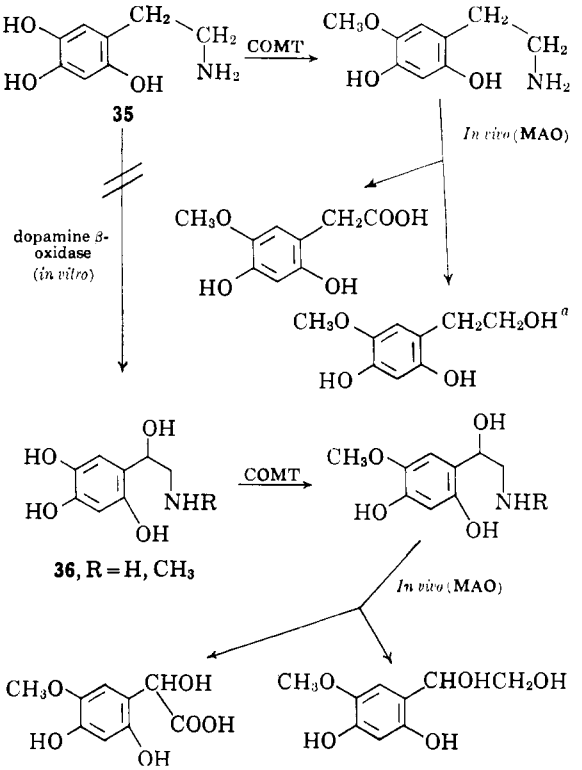
Acetylation, paper chromatography, and a differential fluorometric assay of ethylenediamine condensation products did not detect 6-hydroxydopamine in mice hearts at a level of >0.04 μg/g of tissue (Laverty *et al.*, 1965). Our attempts to develop a more sensitive fluorometric assay for the 6-hydroxycatecholamines in order to investigate their natural occurrence were unsuccessful. None of the 3-*O*-methylated 6-hydroxydopamine could be detected in the urine of patients suffering from phaeochromocytoma (E. LaBrosse, personal communication).

The question of the significance of these 6-hydroxycatecholamines in normal and pathological states remains unanswered but, with the preparation of these amines and their metabolites, has now become more amenable to investigation.

Experimental Section

1,2,4-Tribenzyloxybenzene (**1b**). Anhydrous potassium carbonate (140 g) was added portionwise, with stirring, to a solution of 25.3 g of 1,2,4-triacetoxybenzene (**1c**), 95 g of benzyl chloride, 18 ml of water, and

CHART IV
METABOLISM OF 6-HYDROXYCATECHOLAMINES



[a] Tentative identification as a minor metabolite.

500 ml of acetone. The mixture was refluxed and stirred under nitrogen for 24 hr. After cooling, inorganic salt was removed by filtration and washed with acetone. The combined organic layer was evaporated *in vacuo* and the residue was steam distilled to remove excess benzyl chloride. The remaining oil was dissolved in ether, washed with water, dried (Na_2SO_4), and evaporated. After repeated recrystallization from benzene–hexane, colorless fine needles, mp 81–82°, were obtained; 13.5 g (34%).

Anal. Calcd for $C_{27}H_{24}O_3$: C, 81.79; H, 6.10. Found: C, 81.63; H, 6.14.

2,4,5-Tribenzyloxybenzaldehyde (**2**). When a mixture of 2.43 g of *N*-formylmethylaniline and 2.75 g of phosphoryl chloride was stirred at room temperature for 30 min, it solidified to an orange-yellow mass. A solution of 4.80 g of **1b** in 15 ml of chlorobenzene (Müller, 1954) was added with stirring. The mixture was heated to 60° for 2 hr followed by stirring at room temperature overnight. Ice was added and the product was extracted with ethyl acetate, washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The residual solid was triturated with ether and recrystallized from benzene–hexane to give 3.1 g (61%) of 2,4,5-tribenzyloxybenzaldehyde (**2**), mp 132–133°, ν_{max}^{Nujol} (cm^{-1}) 1663 (C=O).

Anal. Calcd for $C_{28}H_{24}O_4$: C, 79.22; H, 5.70. Found: C, 78.74; H, 5.76.

2,4-Dinitrophenylhydrazone, deep red prisms from ethyl acetate–ethanol; mp 175–177°.

TABLE II: R_F Values and Color Reactions of 6-Hydroxycatecholamines and Metabolites.

Compound	Color Reaction: Gibbs[a]	Ninhydrin	Diazotized[b] *p*-Nitroaniline	Solvent System[c]: 1	2	3	4	5	6	7[d]
6-Hydroxydopamine	Brown	Blue-gray	Reddish	0.17	0.48	0.55	0.23	0.50	0.50	Dec
3-*O*-Methyl-6-hydroxydopamine	Purple-blue	Blue	Yellow-orange	0.39	0.60	0.70	0.47	0.55	0.56	0.35
N-Methyl-3-*O*-methyl-6-hydroxydopamine	Purple-blue	Gray	Yellow-orange	0.57	0.71	0.75	0.59	0.63	0.44	0.33
2,4-Dihydroxy-5-methoxyphenylacetic acid	Purple-gray	...	Yellow-orange	0.56	0.88	0.70	0.80	0.80	0.88	0.12
2,4-Dihydroxy-5-methoxyphenethanol[e]	Purple-gray	...	Yellow-orange	0.67	0.93	0.87	0.88	0.90	0.90	0.53
6-Hydroxynorepinephrine	Brown	Purple	Reddish	0.09	0.42	Dec	0.26	0.43	0.36	Dec
3-*O*-Methyl-6-hydroxynorepinephrine	Purple	Red-purple	Yellow-orange	0.28	0.61	0.71	0.49	0.60	0.56	0.28
6-Hydroxyepinephrine	Brown	Gray-purple	Reddish	0.14	0.45	Dec	0.32	0.31	0.44	Dec
3-*O*-Methyl-6-hydroxyepinephrine	Purple	Gray-purple	Yellow-orange	0.32	0.58	0.70	0.50	0.59	0.50	0.25
2,4-Dihydroxy-5-methoxymandelic acid	Purple	...	Yellow-orange	0.28	0.76	0.58	0.72	0.57	0.90	0.06
2,4-Dihydroxy-5-methylphenyl glycol	Purple	...	Yellow-orange	0.44	0.69	0.78	0.85	0.83	0.85	0.31

[a] Gibbs (1927). [b] Bray *et al.* (1950). [c] Solvent systems (Whatman No. 1 paper): 1, nitroethane–acetic acid–water (90:28:12); 2, methyl ethyl ketone–propionic acid–water (15:5:6); 3, methanol–butanol–benzene–water (2:1:1:1); 4, butanol–acetic acid–water (4:1:1); 5, butanol–pyridine–acetic acid–water (4:2:1:1); 6, tlc (SiO_2), butanol–acetic acid–water (4:1:1); 7, tlc (SiO_2), butanol–concentrated ammonia–ethyl acetate (3:1:1).

TABLE III: Relative Substrate Activity with Rat Liver Monoamine Oxidase.[a]

Substrate	Activity (cpm)
3-*O*-Methyldopamine	3900
Normetanephrine	1800
3-*O*-Methyl-6-hydroxydopamine	1000
3-*O*-Methyl-6-hydroxynorepinephrine	<200
3-*O*-Methyl-6-hydroxyepinephrine	<200

[a] Rat liver mitochondria (see Experimental Section) were incubated with enzymatically *O*-methylated-^{14}C substrates (20,000 cpm/2 μmoles) for 1 hr. Amines were removed *via* a micro Dowex 50 (H^+ form) column and the effluent was counted in a liquid scintillation system. Values are corrected for boiled enzyme blanks.

Anal. Calcd for $C_{34}H_{28}N_4O_7$: C, 67.54; H, 4.67; N, 9.27. Found: C, 67.72; H, 4.89; N, 9.24.

2,4,5-Trihydroxyacetophenone (**3a**). To a suspension of 53 g (0.4 mole) of anhydrous aluminum chloride in 70 ml of chlorobenzene was added slowly a solution of 25 g (0.1 mole) of 1,2,4-triacetoxybenzene and 80 ml of chlorobenzene. The addition was carried out between 30–40° with vigorous stirring over a period of 40 min. The brown mixture was warmed at 80–90° for 30 min with stirring and cooled to 30°. The reaction mixture was poured onto 200 g of ice and 50 ml of concentrated hydrochloric acid with vigorous stirring. The resulting green emulsion was allowed to stand for 1 hr and filtered. The green solid was washed with water, followed by benzene. Recrystallization from 200 ml of 1.0 N hydrochloric acid gave 10 g (60%) of 2,4,5-trihydroxyacetophenone (**3a**), mp 204–206°. Recrystallization from ethanol gave light yellow needles: mp 204–206°; lit. (Bargellin and Avrutin, 1910) mp 200–202°; ν_{max}^{KBr} (cm^{-1}) 3450, 3230 (OH), 1640 (C=O; hydrogen bonded).

Triacetate, colorless needles from carbon tetrachloride; mp 107–108°, lit. (Müller, 1954) mp 110–111°.

2,4,5-Tribenzyloxyacetophenone (**3b**). 2,4,5-Trihydroxyacetophenone (5 g, 0.03 mole), 12.4 g (0.09 mole) of anhydrous potassium carbonate, 12.5 g (0.1 mole) of benzyl chloride, and 180 ml of technical grade dimethylformamide were heated to 100–110° for 2.5 hr with stirring. The brown mixture was cooled and water

was added slowly until a copious amount of brown solid precipitated, at which time the remainder of 200 ml of water was added. The mixture was stirred at 5° for 1 hr and filtered, and the solid was washed well with water and air dried. Recrystallization of the solid from ethanol gave 8.5 g (65%) of 2,4,5-tribenzyloxyacetophenone (**3b**), mp 122–124°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1667 (C=O).

Anal. Calcd for $C_{29}H_{26}O_4$: C, 79.43; H, 5.98. Found: C, 79.19; H, 6.15.

2-Hydroxy-4,5-dibenzyloxyacetophenone (**4**). Benzylation of **3a** (1.7 g) with potassium carbonate in acetone for 24 hr at reflux, followed by filtration and evaporation, afforded 2-hydroxy-4,5-dibenzyloxyacetophenone as colorless needles from methanol; mp 88–90°, 2.1 g (60%), $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1640 (C=O; hydrogen bonded).

Anal. Calcd for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 76.26; H, 6.15.

ω-Bromo-2,4,5-tribenzyloxyacetophenone (**5**). To 2.0 g (0.02 mole) of calcium carbonate, 5 g (0.0114 mole) of 2,4,5-tribenzyloxyacetophenone, and 100 ml of chloroform was added slowly over 30 min with stirring at room temperature a solution of 1.8 g (0.011 mole) of bromine in 20 ml of chloroform. The reaction mixture was stirred for an additional 15 min at room temperature and was poured onto cracked ice and 100 ml of 1.0 N hydrochloric acid. The chloroform layer was separated, washed with water, and dried (Na_2SO_4), and the solvent was removed *in vacuo*. Recrystallization from benzene–petroleum ether (bp 60–70°) afforded 3 g (51%) of ω-bromo-2,4,5-tribenzyloxyacetophenone (**5**), mp 83°. Recrystallization from benzene and petroleum ether with a charcoal treatment gave light yellow crystals, mp 109–111°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1668 (C=O).

Anal. Calcd for $C_{29}H_{24}BrO_4$: C, 67.31; H, 4.87; Br, 15.45. Found: C, 67.57; H, 5.15; Br, 15.46.

ω-Dibenzylamino-2,4,5-tribenzyloxyacetophenone (**6b**). To a solution of 2.1 g of the bromo ketone **5** in 50 ml of benzene was added 1.6 g of dibenzylamine and the mixture was stirred and refluxed for 3 hr. Dibenzylamine hydrobromide was removed by filtration and washed with benzene. The combined benzene solutions were washed with water, dried (Na_2SO_4), and evaporated *in vacuo*. The crystalline residue was dissolved in hot ethanol containing an excess of dry hydrogen chloride. The addition of ether yielded the hydrochloride (2.2 g) as colorless felt of needles, mp 188–190°. The free base was obtained by use of aqueous sodium carbonate and ethyl acetate extraction. After drying and removal of the ethyl acetate, the residue was recrystallized from ethanol to give 2.0 g (79%) of colorless needles: mp 113°; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1682 (C=O); λ_{max}^{EtOH} [mμ (log ε)] 323 (3.89), 267 (4.01), 233 (4.44).

Anal. Calcd for $C_{43}H_{39}NO_4$: C, 81.49; H, 6.20; N, 2.21. Found: C, 81.73; H, 6.45; N, 2.18.

Hydrochloride, colorless fibers from ethanol–ether; mp 189–190°.

Anal. Calcd for $C_{43}H_{39}NO_4 \cdot HCl$: N, 2.09; Cl, 5.28. Found: N, 2.13; Cl, 5.76.

2,4,5-Tribenzyloxy-ω-dibenzylaminomethylbenzyl Alcohol (Pentabenzyl-6-hydroxynorepinephrine) (**7b**). To a solution of 1.7 g of the ketone **6b** in 25 ml of tetrahydrofuran was added a solution of 1.5 g of sodium borohydride in 10 ml of methanol with cooling and stirring over a period of 10 min. The reaction mixture was then stirred for 30 min with cooling and for 3 hr at room temperature. The reduction was followed by measuring the disappearance of the ultraviolet absorption spectrum of the ketone. After warming on a steam bath for 10 min the solution was evaporated *in vacuo*, decomposed with ice–water, and extracted with ethyl acetate. The extract was washed with water, dried (K_2CO_3), and evaporated *in vacuo*. The residue was recrystallized from ethanol to yield colorless needles: mp 134–135°; 1.1 g (65%); $\nu_{max}^{CHCl_3}$ (cm^{-1}) 3500 (broad) (OH), no carbonyl; λ_{max}^{EtOH} [mμ (log ε)] 288 (3.67).

Anal. Calcd for $C_{43}H_{41}NO_4$: C, 81.23; H, 6.50; N, 2.20. Found: C, 81.15; H, 6.26; N, 2.33.

ω-Benzylmethylamino-2,4,5-tribenzyloxyacetophenone (**6f**). A. *Via* THE LITHIUM SALT OF BENZYLMETHYLAMINE. To 5.0 g (9.66 mmoles) of ω-bromo-2,4,5-tribenzyloxyacetophenone and 50 ml of dry benzene was added a benzene solution of lithium benzylmethylamine, prepared in the following manner. To a solution of 1.17 g (9.66 mmoles) of benzylmethylamine and 10 ml of dry benzene was added a solution of 3.41 ml (9.66 mmoles) of commercial *n*-butyllithium in heptane. After addition the mixture of bromo ketone and amine salt was stirred and refluxed for 3 hr. The reaction mixture was cooled and the precipitate was collected. The solid was treated with boiling water, and on cooling an oily solid precipitated which on stirring crystallized. Recrystallization from ethanol yielded 2.65 g (49%) of benzylmethylamino-2,4,5-tribenzyloxyacetophenone, mp 93–94°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1667 (C=O).

Anal. Calcd for $C_{37}H_{35}NO_4$: C, 79.68; H, 6.33. Found: C, 79.93; H, 6.43.

B. REACTION OF ω-BROMO-2,4,5-TRIBENZYLOXYACETOPHENONE (**5**) WITH BENZYLMETHYLAMINE. To 0.94 g (7.7 mmoles) of benzylmethylamine and 25 ml of dry benzene there was added with stirring at reflux over a period of 45 min a solution of 2.0 g (3.9 mmoles) of ω-bromo-2,4,5-tribenzyloxyacetophenone in 25 ml of dry benzene. The stirring and refluxing were continued for 3 hr. After cooling to room temperature the white crystalline benzylmethylamine hydrobromide was collected, 0.70 g (90% of the bromine), mp 166–168° (authentic sample, mp 165–167°). On removal of the solvent *in vacuo*, there remained a red oil which on trituration with a small amount of ethanol left a tan solid. The solid was recrystallized from ethanol as yellow needles, 0.47 g (41%), mp 93–94°, and was identical with the ω-benzylmethylamine-2,4,5-tribenzyloxyacetophenone (**6f**) obtained by A.

The red oil obtained on evaporation of the ethanolic mother liquors was covered with dilute ethanolic sodium hydroxide and allowed to stand for 1 week at room temperature. The basic solution was decanted from the red oil and acidified. The yellow precipitate was collected, washed with water, and air dried. There resulted 0.1 g of an acid, mp 145–150°. Recrystallization from ethanol gave yellow needles, mp 149–151°. In other runs larger quantities of this acid, 2,4,5-tri-

benzyloxybenzoic acid, were isolated; nmr ($CDCl_3$, δ), 5.25 (2-OCH_2), 5.30 (OCH_2), 6.88 (1H), 7.58 (15H), 7.98 (1H), 10.7 (COOH); $\nu_{max}^{HCCl_3}$ (cm^{-1}) 1730 (C=O); λ_{max}^{EtOH} [mμ (log ϵ)] 210 (4.61), $\lambda_{max}^{EtOH\ 0.005\ N\ NaOH}$ 216 (4.52).

Anal. Calcd for $C_{28}H_{31}O_5$: C, 76.34; H, 5.49; O, 18.16. Found: C, 76.47; H, 5.66; O, 17.91.

2,4,5-Tribenzyloxy-ω-benzylmethylaminomethylbenzyl Alcohol (Pentabenzyl-6-hydroxyepinephrine). Reduction of the ketone with borohydride was carried out as above (**6b** → **7b**) to yield colorless crystals, mp 83–84° (65%).

Anal. Calcd for $C_{37}H_{37}NO_4$: C, 79.40; H, 6.66; N, 2.50. Found: C, 79.86; H, 6.84; N, 2.52.

ω-Bromo-2,4,5-trimethoxyacetophenone (**9**). 2,4,5-Trimethoxyacetophenone (**8**) (3.2 g) (Reigrodski and Tambor, 1910) was dissolved in 40 ml of carbon tetrachloride and stirred and refluxed with 2.7 g of *N*-bromosuccinimide for 6 hr. After purification (see above), recrystallization from methanol gave colorless long needles of mp 141–142°, 2.5 g (57%).

Anal. Calcd for $C_{11}H_{13}BrO_4$: C, 45.69; H, 4.53. Found: C, 45.58; H, 4.73.

2,4,5-Trimethoxy-ω-dibenzylaminoacetophenone (**10**). The bromo ketone **9** (1.35 g) was treated with 1.9 g of dibenzylamine in benzene as above. The base was obtained from methanol as slightly yellow prisms, mp 91–92°, 1.6 g.

Anal. Calcd for $C_{25}H_{27}NO_4$: C, 74.05; H, 6.71; N, 3.45. Found: C, 73.77; H, 6.90; N, 3.51.

Hydrobromide, colorless fine needles from ethanol–ethyl acetate–ether; mp 183–184°.

Anal. Calcd for $C_{25}H_{27}NO_4 \cdot HBr$: N, 2.88. Found: N, 3.30.

2,4,5-Trimethoxy-ω-dibenzylaminomethylbenzyl Alcohol (**11**). Reduction of the ketone **10** with sodium borohydride was carried out as described above. The colorless product was converted to the hydrochloride and recrystallized from ethanol–ethyl acetate–ether to yield 0.2 g (66%) of colorless needles, mp 162–165° dec.

Anal. Calcd for $C_{25}H_{29}NO_4 \cdot HCl$: Cl, 7.99. Found: Cl, 8.09.

2,4-Dibenzyloxy-5-methoxybenzaldehyde (**14b**). Benzylation of 2,4-dihydroxy-5-methoxybenzaldehyde (**14a**) (Head and Robertson, 1931) was carried out as described for **3b**. Recrystallization from methylcyclohexane yielded 2,4-dibenzyloxy-5-methoxybenzaldehyde, mp 117–119° (82%).

Anal. Calcd for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 75.77; H, 5.76.

Oxime, recrystallized from EtOH; mp 165–167°.

Anal. Calcd for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.83; N, 3.86. Found: C, 72.59; H, 5.82; N, 3.85.

2,4-Dibenzyloxy-5-methoxy-β-nitrostyrene (**15**). A solution of 2 g (0.0057 mole) of 2,4-dibenzyloxy-5-methoxybenzaldehyde (**14b**) in 20 ml of nitromethane containing 170 mg of ammonium acetate was heated for 5 hr on a steam bath. The mixture was cooled in a Dry Ice–acetone bath until crystallization was complete. The yellow solid after collection and air drying overnight weighed 1.9 g (84.8%), mp 132.5–134°. Recrystallization from methylcyclohexane gave 2,4-dibenzyloxy-5-methoxy-β-nitrostyrene as orange feathery plates, yield 1.7 g (76.0%), mp 133–135°.

Anal. Calcd for $C_{23}H_{21}NO_5$: C, 70.57; H, 5.41; N, 3.58. Found: C, 70.83; H, 5.47; N, 3.48.

2,4-Dibenzyloxy-5-methoxyphenethylamine (**16b**). Lithium aluminum hydride (1.15 g, 0.030 mole) was added cautiously with stirring to 45 ml of tetrahydrofuran. Nitrogen was passed over the solution and 1.5 g (0.0028 mole) of 2,4-dibenzyloxy-5-methoxy-β-nitrostyrene (**15**), dissolved in 18 ml of tetrahydrofuran, was added dropwise. The reaction mixture was refluxed for 1.5 hr after addition. The mixture was cooled, 1.64 g (0.0912 mole) of water in 20 ml of tetrahydrofuran was added dropwise with stirring, the mixture was then refluxed for 5 min and filtered hot, and the colorless tetrahydrofuran solution was evaporated under vacuum. A yellow oil remained which was dissolved in ether. The ether solution was filtered and evaporated. The residual oil solidified upon standing. The crude yield was 1.2 g (86.3%). A small amount of the solid was recrystallized from benzene to give 2,4-dibenzyloxy-5-methoxyphenethylamine (**16b**) as a white powder, mp 143–146°.

Anal. Calcd for $C_{23}H_{25}NO_3 \cdot 1.5H_2O$: C, 70.74; H, 7.22; N, 3.59. Found: C, 70.12; H, 6.87; N, 3.72.

Hydrochloride, recrystallized from EtOH–Et_2O; mp 143–145°.

Anal. Calcd for $C_{23}H_{26}ClNO_3$: C, 69.08; H, 6.56; Cl, 8.86; N, 3.50. Found: C, 68.97; H, 6.78; Cl, 9.11; N, 3.70.

2,4-Dihydroxy-5-methoxyphenethylamine Hydrochloride (**16a**). A solution of 300 mg of 2,4-dibenzyloxy-5-methoxyphenethylamine hydrochloride in 50 ml of ethanol was shaken in the presence of 180 mg of 5% palladium on carbon under 30 psi of hydrogen for 2 hr. The reaction mixture was filtered, and the filtrate was evaporated at 40° under vacuum. A light yellow solid separated which was filtered and dried to yield 128 mg (77.8%), mp 211–214° dec. The solid was recrystallized from ethanol–ether (Norit A) to give 90 mg of 2,4-dihydroxy-5-methoxyphenethylamine hydrochloride, mp 214.5–217° dec.

Anal. Calcd for $C_9H_{14}ClNO_3$: C, 49.21; H, 6.42; Cl, 16.14; N, 6.38. Found: C, 49.70; H, 7.09; Cl, 16.60; N, 5.86.

2,4-Dibenzyloxy-5-methoxybenzyl Alcohol (**17**). To 21.8 g (0.57 mole) of lithium aluminum hydride, suspended in 425 ml of tetrahydrofuran in a nitrogen atmosphere, was added dropwise 25 g (0.072 mole) of 2,4-dibenzyloxy-5-methoxybenzaldehyde dissolved in 250 ml of tetrahydrofuran. The reaction mixture was heated to reflux for 2 hr with stirring and allowed to cool. To the cooled solution was added dropwise 31.1 g (1.728 moles) of water in 150 ml of tetrahydrofuran. The mixture was heated to reflux with stirring and filtered hot, and the inorganic solid was washed twice with hot tetrahydrofuran. After evaporation of the tetrahydrofuran *in vacuo*, a light yellow oil remained. Hexane was added and the flask was refrigerated overnight to yield after collection 19.7 g (78%) of 2,4-

dibenzyloxy-5-methoxybenzyl alcohol (**17**), mp 88–91°. On recrystallization from cyclohexane, fine white needles were obtained, mp 90–91°.

Anal. Calcd for $C_{22}H_{22}O_4$: C, 75.41; H, 6.33. Found: C, 75.55; H, 6.49.

2,4-Dibenzyloxy-5-methoxybenzyl Bromide (**18**). To a cooled solution of 10 g (0.028 mole) of 2,4-dibenzyloxy-5-methoxybenzyl alcohol and 230 ml of dry carbon tetrachloride was added 17 g (0.063 mole) of fresh phosphorus tribromide in 30 ml of dry carbon tetrachloride. The light yellow solution was stoppered under nitrogen and allowed to stand in the dark at room temperature for 3.5 hr. The solution was decanted from the precipitated phosphorus compound and refluxed for 10 min. The carbon tetrachloride solution was cooled with ice and washed with dilute sodium bicarbonate containing ice. The solution was extracted until neutral, dried over sodium sulfate, and evaporated *in vacuo*. The colorless solid residue was recrystallized twice with cyclohexane (charcoal) to yield 8.5 g of impure 2,4-dibenzyloxy-5-methoxybenzyl bromide: mp 104–106°; nmr ($CDCl_3$, δ), 3.32 (OCH_3), 4.55 (CH_2Br), 4.98 (OCH_2), 5.09 (OCH_2), 6.55 (1H), 6.92 (1H), 7.38 (10 aromatic H).

Anal. Calcd for $C_{22}H_{21}BrO_3$: C, 63.93; H, 5.12; Br, 19.34. Found: C, 64.53; H, 5.41; Br, 20.74.

2,4-Dibenzyloxy-5-methoxyphenylacetonitrile (**19**). To a solution of 6.5 g (0.13 mole) of sodium cyanide in 120 ml of dimethyl sulfoxide at 50° was added 5.0 g (0.012 mole) of (unrecrystallized) 2,4-dibenzyloxy-5-methoxybenzyl bromide. The mixture, which turned red as the benzyl bromide was added, was stirred for 2.5 hr and diluted with 600 ml of water. After refrigeration overnight, the solid was collected, washed well with water, and air dried. Recrystallization from cyclohexane gave 3.4 g (77%) of crystalline 2,4-dibenzyloxy-5-methoxyphenylacetonitrile, mp 89–92°. The material was recrystallized from cyclohexane to a constant melting point of 94–95°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 2250 (C≡N).

Anal. Calcd for $C_{23}H_{21}NO_3$: C, 76.86; H, 5.89; N, 3.90. Found: C, 76.61; H, 6.10; N, 3.80.

2,4-Dibenzyloxy-5-methoxyphenylacetic Acid (**20b**). A mixture of 2.1 g (0.0058 mole) of 2,4-dibenzyloxy-5-methoxyphenylacetonitrile, 13 ml of dioxane, 10 ml of methanol, and 4 g (0.071 mole) of potassium hydroxide dissolved in 4 ml of water was refluxed until ammonia ceased to be evolved (24 hr). The mixture was concentrated to half its volume, diluted with water, and acidified with hydrochloric acid. The resulting yellow solid was collected, washed with water, and air dried. The yield of 2,4-dibenzyloxy-5-methoxyphenylacetic acid, mp 153–157°, was 2.0 g (91%). Recrystallization from benzene gave a colorless crystalline solid, mp 162–162.5°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1700 (COOH).

Anal. Calcd for $C_{23}H_{22}O_5$: C, 73.00; H, 5.86. Found: C, 73.16; H, 5.98.

2,4-Dihydroxy-5-methoxyphenylacetic Acid (**20a**). The benzyloxy compound **20b** was reduced in the same way as described for **16a**. Two recrystallizations of the product from ethyl acetate–hexane gave 2,4-dihydroxy-5-methoxyphenylacetic acid (96%), mp 150–151.5°.

Anal. Calcd for $C_9H_{10}O_5$: C, 54.54; H, 5.09. Found: C, 54.31; H, 5.35.

2,4-Dibenzyloxy-5-methoxymandelonitrile Acetate. (**21**). To a stirred solution of 6 g (0.017 mole) of 2,4-dibenzyloxy-5-methoxybenzaldehyde dissolved in 70 ml of dioxane was added 6.6 g (0.10 mole) of potassium cyanide dissolved in 12 ml of water followed by 4.6 ml (0.055 mole) of concentrated hydrochloric acid. The mixture was refluxed with stirring for 1 hr and cooled. After filtration the filtrate was poured into a separatory funnel with 15 ml of benzene. To the organic layer over sodium sulfate was slowly added 500 ml of benzene until all of the water had been forced out of the dioxane. After filtration 21 ml of acetic anhydride and 8 ml of pyridine were added and the mixture was allowed to stand overnight at room temperature. After evaporation *in vacuo* the orange solid was air dried and recrystallized from ethanol yielding 6.27 g (88%) of orange needles, mp 117–120°. After several recrystallizations from isopropyl alcohol, an analytically pure sample of 2,4-dibenzyloxy-5-methoxymandelonitrile acetate, mp 120–122°, was obtained.

Anal. Calcd for $C_{25}H_{23}NO_5$:C , 71.93; H, 5.55; N, 3.36. Found: C, 71.60; H, 5.53; N, 3.33.

2-Amino-1-(2,4-dibenzyloxy-5-methoxyphenyl)ethanol Hydrochloride (**22b**). Reduction of **21** was carried out with lithium aluminum hydride as described for **17**. The oil was dissolved in a minimum of benzene, and ethanolic hydrogen chloride was added, followed by ether. After three recrystallizations (EtOH–Et_2O) colorless crystals of the hydrochloride, mp 181–182° dec, was obtained in 34% yield.

Anal. Calcd for $C_{23}H_{26}ClNO_4$: C, 66.42; H, 6.30; N, 3.37. Found: C, 66.25; H, 6.58; N, 3.29.

2-Amino-1-(2,4-dihydroxy-5-methoxyphenyl)ethanol Hydrochloride (**22a**). The catalytic debenzylation was carried out in methanol as described for **16a**. The product was purified by recrystallization from ethanol–ether. It was extremely hydroscopic and unstable, mp 100° dec.

Anal. Calcd for $C_9H_{14}ClNO_4$: N, 5.97. Found: N, 5.44.

Ethyl α-Ethoxy-2,4-dibenzyloxy-5-methoxyphenylacetate (**23c**). 2,4-Dibenzyloxy-5-methoxymandelonitrile acetate (7 g, 0.17 mole) was dissolved in 200 ml of ethanol containing 20% (by weight) of anhydrous hydrogen chloride. The solution was covered with nitrogen, stoppered, and stirred for 20 hr at room temperature. Water (10 ml) was added and the solution was evaporated to a dark oil. The oil was covered with 100 ml of water, stirred for 45 min, and extracted with ether. The ether was dried and evaporated to an oil, which showed several spots on thin layer chromatography. The oil was chromatographed on an alumina column in chloroform–ethyl acetate (97:3). The main fraction was evaporated to a yellow oil which solidified. Three recrystallizations from hexane (charcoal) gave colorless crystals, mp 77–79°; the nmr and infrared spectra were consistent with structure **23c**.

Anal. Calcd for $C_{27}H_{30}O_6$: C, 71.97; H, 6.71; CCH_3

(Kuhn Roth), 6.65. Found: C, 71.96; H, 6.65; CCH_3, 6.09.

α-Ethoxy-2,4-dibenzyloxy-5-methoxyphenylacetic Acid (**23a**). A sample of **23c** was dissolved in dioxane and stirred with 5% sodium hydroxide for 2.5 hr. The solution was diluted with water and acidified with hydrochloric acid to give a yellow oil. The yellow oil was extracted with ether, and the ether was dried and evaporated to an oil. When attempts to crystallize the oil failed, it was taken up in isopropyl alcohol and cyclohexylamine was added. Recrystallization from isopropyl alcohol gave cyclohexylammonium α-ethoxy-2,4-dibenzyloxy-5-methoxyphenylacetate, mp 162–164°.

Anal. Calcd for $C_{31}H_{39}NO_6$: C, 71.37; H, 7.53; N, 2.68. Found: C, 71.27; H, 7.41; N, 2.56.

ω,ω-Dichloro-2,4-dihydroxy-5-methoxyacetophenone (**27a**). A stirred solution of 6.0 g (0.042 mole) of 2,4-dihydroxyanisole, 4.6 g (0.042 mole) of dichloroacetonitrile, and 70 ml of dry ether was saturated with dry gaseous hydrogen chloride at 5° for 45 min. The reaction mixture was stirred at 5° for an additional hour and filtered. There resulted 9.7 g (80%) of orange crystals, mp 187–190° dec. The crystals were dissolved in water and refluxed in a nitrogen atmosphere for 1 hr and cooled. There resulted 2.4 g (22%) of yellow crystals of ω,ω-dichloro-2,4-dihydroxy-5-methoxyacetophenone, mp 104–107°. Recrystallization from ether–hexane (charcoal) yielded light yellow needles, mp 105–107°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1630 (C=O).

Anal. Calcd for $C_9H_8Cl_2O_4$: C, 43.06; H, 3.21; Cl, 28.29. Found: C, 42.95; H, 3.27; Cl, 28.06.

ω-Chloro-2,4-dihydroxy-5-methoxyacetophenone (**24**). A stirred solution of 8.0 g (0.057 mole) of 2,4-dihydroxyanisole, 4.4 g (0.060 mole) of chloroacetonitrile, and 70 ml of dry ether was saturated with dry hydrogen chloride at 5°. The mixture was stirred at 0° for 0.5 hr. The precipitate was collected and washed with fresh ether to yield 12.18 g (84.5%) of solid, mp 189–192° dec. The salt was treated with 700 ml of boiling water, and, on cooling, there was obtained 4.9 g (60%) of colorless crystalline ω-chloro-2,4-dihydroxy-5-methoxyacetophenone, mp 139–142°. Recrystallization from benzene raised the melting point to 142–145°; $\nu_{max}^{HCCl_3}$ (cm^{-1}) 1630 (C=O).

Anal. Calcd for $C_9H_9ClO_4$: C, 49.90; H, 4.19; Cl, 16.37. Found: C, 49.77; H, 4.08; Cl, 16.31.

6-Hydroxy-5-methoxybenzofuran-3-one (**25**). To a stirred solution of 1 g (0.012 mole) of sodium bicarbonate and 15 ml of dimethyl sulfoxide was added under nitrogen at 150° a solution of 0.40 g (0.0195 mole) of ω-chloro-2,4-dihydroxy-5-methoxyacetophenone (**24**) and 10 ml of dimethyl sulfoxide. The mixture was stirred at 150° for 5 min, cooled, and poured into 50 ml of water. The aqueous solution was acidified with hydrochloric acid, and extracted with ether, and the ether extract was dried (Na_2SO_4) and evaporated *in vacuo* to yield 0.1 g (30%) of off-white needles, mp 171–173°. Recrystallization from chloroform–cyclohexane gave 6-hydroxy-5-methoxybenzofuran-3-one as colorless needles: mp 172–174°; $\nu_{max}^{CHCl_3}$ 3520 (OH), 1690 (C=O).

Anal. Calcd for $C_9H_8O_4$: C, 60.00; H, 4.47. Found: C, 59.89; H, 4.42.

ω-N-Benzyl-N-methylamino-2,4-dihydroxy-5-methoxyacetophenone Hydrochloride (**26**). A solution of 1.0 g (0.0041 mole) of ω-chloro-2,4-dihydroxy-5-methoxyacetophenone (**24**), 1.1 g (0.0092 mole) of *N*-methylbenzylamine, and 7 ml of ethanol was refluxed for 0.5 hr with stirring. The ethanol was concentrated *in vacuo* and ether was added to precipitate 0.86 g of solid. The ethereal mother liquors were evaporated to give a dark red oil, which was treated with ether and water. The ether extracts were dried and evaporated to yield 0.8 g of a glass, which was taken up in fresh ether. On addition of a few drops of 20% hydrogen chloride in ethanol there was obtained 0.4 g (25.6%) of yellow solid. After two recrystallizations from ethanol–ether, light yellow crystals of ω-benzyl-*N*-methylamino-2,4-dihydroxy-5-methoxyacetophenone hydrochloride, mp 240–241° dec, were obtained; ν_{max}^{HBr} 1630 (C=O).

Anal. Calcd for $C_{17}H_{20}ClNO_4$: C, 60.44; H, 5.97; Cl, 10.49; N, 4.15. Found: C, 60.54; H, 6.13; Cl, 10.56; N, 4.19.

2,4-Dibenzyloxyanisole (**13b**). Benzylation of 2,4-dihydroxyanisole (**13a**) was carried out as described for **3b**. Recrystallization of the product from cyclohexane gave tan platelets of 2,4-dibenzyloxyanisole (88%), mp 88–90°.

Anal. Calcd for $C_{21}H_{20}O_3$: C, 78.73; H, 6.29. Found: C, 78.84; H, 6.25.

ω,ω-Dichloro-2,4-dibenzyloxy-5-methoxyacetophenone (**27b**). A solution of 1.6 g (0.005 mole) of 2,4-dibenzyloxyanisole, 0.55 g (0.005 mole) of dichloroacetonitrile, and 75 ml of anhydrous ether was saturated with dry hydrogen chloride at 5°. Since no precipitate occurred after 1 hr of stirring between 5 and 20°, 0.8 g (0.005 mole) of anhydrous ferric chloride was added to the mixture. On stirring for 1.5 hr there precipitated 0.13 g of an orange solid, which was collected and boiled with water to give on cooling 70 mg of a yellow solid, mp 90–92°. Recrystallization from cyclohexane gave ω,ω-dichloro-2,4-dibenzyloxy-5-methoxyacetophenone as colorless needles: mp 92.5–93°; $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1670, 1650 (C=O).

Anal. Calcd for $C_{23}H_{20}Cl_2O_4$: C, 64.04; H, 4.68; O, 14.84. Found: C, 63.93; H, 4.65; O, 15.43.

2,4-Dihydroxy-5-methoxyacetophenone (**28a**). A stirred solution of 20 g (0.140 mole) of 2,4-dihydroxyanisole, 12 g (0.30 mole) of anhydrous acetonitrile, 3.2 g (0.024 mole) of anhydrous zinc chloride, and 180 ml of dry ether was saturated with hydrogen chloride at 5° for 100 min. After storage in the refrigerator overnight the ether was decanted, and the salt refluxed with 250 ml of water for 15 min. The mixture was cooled, and the solid was collected, washed with water, and air dried. The product was obtained as yellow crystals, mp 174–176°, 11.3 g (43.5%), and was recrystallized from ethyl acetate–hexane to give yellow platelets, mp 174–176°, ν_{max}^{KBr} (cm^{-1}) 1625 (C=O).

Anal. Calcd for $C_9H_{10}O_4$: C, 59.34; H, 5.53. Found: C, 59.55; H, 5.52.

2,4-Dibenzyloxy-5-methoxyacetophenone (**28b**). Ben-

zylation was carried out as described for **3b**. Recrystallization of the product from cyclohexane gave bright yellow needles (95%), mp 100–102°.

Anal. Calcd for $C_{23}H_{22}O_4$: C, 76.22; H, 6.12. Found: C, 76.15; H, 6.17.

2,4-Dibenzyloxy-5-methoxyphenylglyoxal Hydrate (**29**). To a solution of 8 g (0.062 mole) of 93% selenious acid dissolved in 400 ml of dioxane at 45° was added 18.7 g (0.052 mole) of 2,4-dibenzyloxy-5-methoxyacetophenone. The solution was stirred and refluxed for 24 hr. The selenium was removed by filtration, and the filtrate was evaporated to dryness *in vacuo*. The resulting oil was dissolved in hot dioxane, water was added to turbidity, and the solution was refrigerated overnight. The yield of 2,4-dibenzyloxy-5-methoxyphenylglyoxal, mp 110–113°, was 20.0 g (99%). Recrystallization from dioxane–water gave yellow crystals, mp 114–116°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1650 (C=O).

Anal. Calcd for $C_{23}H_{20}O_5 \cdot H_2O$: C, 70.04; H, 5.62. Found: C, 70.25; H, 5.88.

2-(2,4-Dibenzyloxy-5-methoxyphenyl)quinoxaline. A small amount of **29** in methanol was treated with a warm methanolic solution of *o*-phenylenediamine. Recrystallization of the product from methanol gave the product in the form of light yellow crystals, mp 138–140°.

Anal. Calcd for $C_{29}H_{24}N_2O_3$: C, 77.66; H, 5.39; N, 6.25. Found: C, 77.65; H, 5.44; N, 6.30.

2,4-Dibenzyloxy-5-methoxyphenylglycol (**30b**). To a stirred solution of 4 g (0.01 mole) of 2,4-dibenzyloxy-5-methoxyphenylglyoxal hydrate in 150 ml of methanol was added portionwise over 4 hr 8 g (0.21 mole) of sodium borohydride. The methanol was evaporated, the resulting red solid was treated with water, and the solution was brought to pH 7–8 with hydrochloric acid. The aqueous mixture was extracted with ether, and the extracts were dried over magnesium sulfate and evaporated to dryness. The yield of colorless **30b**, mp 85–88°, was 3.0 g (80%). Colorless needles, mp 91–93°, were obtained from ethyl acetate–hexane.

Anal. Calcd for $C_{23}H_{24}O_3$: C, 72.61; H, 6.36. Found: C, 72.57; H, 6.59.

2,4-Dihydroxy-5-methoxyphenylglycol (**30a**). The catalytic debenzylation was carried out as described for **16a**. Recrystallization from ethyl acetate–hexane (Norit A) gave light pink crystals (88%), mp 144–145°.

Anal. Calcd. for $C_9H_{12}O_5$: C, 53.78; H, 6.42. Found: C, 53.76; H, 6.12.

2,4-Dibenzyloxy-5-methoxymandelic Acid (**31b**). A solution of 4.0 g (0.01 mole) of 2,4-dibenzyloxy-5-methoxyphenylglyoxal hydrate in 120 ml of ethanol and 60 ml of water containing 15 g (0.26 mole) of potassium hydroxide was stirred at room temperature for 2.5 hr. The solution was partially evaporated, diluted with water, extracted with ether, and acidified with hydrochloric acid. The acid was extracted with ether, and the extracts were washed with fresh water, dried over sodium sulfate, and evaporated to give 3.0 g (75%) of yellow crystals, mp 104–107°. Recrystallization from carbon tetrachloride gave 2,4-dibenzyloxy-5-methoxymandelic acid as colorless crystals: mp 106–107°; nmr ($CDCl_3$, δ), 3.83 (OCH_3), 4.95 (OCH_2), 5.08 (OCH_2), 5.30 (CH–O), 6.54 (1H), 6.93 (1H), 7.31 (10H).

Anal. Calcd for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62. Found: C, 69.74; H, 5.75.

In another preparation of the above acid, the product isolated had mp 126–128° after recrystallization from cyclohexane. Infrared spectra of solutions of the two allotropic crystalline forms were identical.

Anal. Calcd for $C_{23}H_{22}O_6$: C, 70.04; H, 5.62. Found: C, 69.96; H, 5.65.

Cyclohexylammonium 2,4-Dibenzyloxy-5-methoxymandelate. 2,4-Dibenzyloxy-5-methoxymandelic acid (100 mg) was added to a hot solution of 25 mg of cyclohexylamine in 10 ml of isopropyl alcohol. On cooling overnight colorless crystals separated which after recrystallization from isopropyl alcohol had mp 149–151°.

Anal. Calcd for $C_{29}H_{35}NO_6$: C, 70.57; H, 7.15; N, 2.84. Found: C, 70.74; H, 7.28; N, 2.88.

2,4-Dihydroxy-5-methoxymandelic Acid (1,4-Diazabicyclo[2.2.2]octane Salt) (**31a**). Catalytic reduction was carried out as described for **16a**. A solution of 1,4-diazabicyclo[2.2.2]octane in ethanol was added to the reduction medium after removal of catalyst. After evaporation, two recrystallizations from ethanol–hexane gave brown crystals (74%), mp 164–165° dec.

Anal. Calcd for $C_{15}H_{22}N_2O_2$: C, 55.21; H, 6.80; N, 8.58. Found: C, 54.97; H, 6.72; N, 8.31.

Methyl 2,4-Dibenzyloxy-5-methoxymandelate. To a solution of 1.1 g (0.0028 mole) of 2,4-dibenzyloxy-5-methoxymandelic acid in 100 ml of methanol was added 50 mg of *p*-toluenesulfonic acid. The solution was allowed to stand for 2 days, and the methanol was evaporated. The residual oil was taken up in ether, the ether solution was washed with aqueous sodium bicarbonate, followed by water, and dried over anhydrous sodium sulfate. The ether was evaporated, and the resulting oil upon standing crystallized to an off-white solid (1.0 g, 90%), mp 63–66°. Recrystallization from cyclohexane gave methyl 2,4-dibenzyloxy-5-methoxymandelate in colorless crystals, mp 55–56°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1705 (C=O).

Anal. Calcd for $C_{24}H_{24}O_6$: C, 70.58; H, 5.92. Found: C, 70.53; H, 6.11.

2,4-Dibenzyloxy-5-methoxy-N-methylmandelamide (**32**). A solution of 2.5 g (0.0061 mole) of methyl 2,4-dibenzyloxy-5-methoxymandelate in 40 ml of methanol containing 130 ml of liquid methylamine was allowed to stand for 4 hr. The reaction mixture was evaporated to give an off-white solid. Hexane was added, and the solid was collected, washed with hexane, and air dried to yield 2.3 g (92%) of product, mp 151–153°. Recrystallization from benzene gave fine colorless needles of **32**, mp 152–154°, $\nu_{max}^{CHCl_3}$ (cm^{-1}) 1660 (C=O).

Anal. Calcd for $C_{24}H_{25}NO_5$: C, 70.75; H, 6.18; N, 3.44. Found: C, 70.87; H, 6.28; N, 3.45.

N-Methyl-2,4-dibenzyloxy-5-methoxyphenethylamine Hydrochloride. To 50 ml (about 0.05 mole) of 1.0 M borane in tetrahydrofuran at 5° was added over 20 min a solution of 3.3 g (0.008 mole) of **32** in 65 ml of

tetrahydrofuran. The solution was stirred and refluxed in a nitrogen atmosphere for 4 hr. To the clear solution was then added dropwise 15 ml of methanol and the reaction mixture refluxed for 2 hr. Evaporation of the reaction mixture gave a gray oil which was refluxed in aqueous ethanolic sodium hydroxide for 11 hr. The mixture was then concentrated and extracted with ether. Upon addition of ethanolic hydrogen chloride solution and refrigeration there was obtained 3 g of a colorless powder, mp 134–137°. Recrystallization from ethanol–ether gave 2.7 g (81%) of colorless crystals, mp 145.5–147°, $\nu_{max}^{CHCl_3}$ no OH absorption.

Anal. Calcd for $C_{24}H_{27}NO_3 \cdot HCl$: C, 69.64; H, 6.82; N, 3.38; Cl, 8.56. Found: C, 69.62; H, 6.93; N, 3.15; Cl, 8.75.

N-Methyl-2,4-dihydroxy-5-methoxyphenethylamine Hydrochloride (**33a**). Reductive debenzylation was carried out as described for **16a**. Recrystallization from ethanol–ether gave a colorless crystalline powder, mp 145–148°.

Anal. Calcd for $C_{10}H_{15}NO_3 \cdot HCl$: C, 51.40; H, 6.90; Cl, 15.16; N, 6.00. Found: C, 51.32; H, 7.00; Cl, 15.38; N, 6.16.

2-N-Methylamino-1-(2,4-dibenzyloxy-5-methoxyphenyl)ethanol Hydrochloride. To 7 ml (0.007 mole) of 1.0 M borane in tetrahydrofuran at 5° was added over 15 min a solution of 3.3 g (0.008 mole) of **32** in 65 ml of tetrahydrofuran. The solution was stirred in a nitrogen atmosphere at room temperature for 2 hr and then refluxed in a nitrogen atmosphere for 4 hr. About 8 ml of 1.0 M diborane in tetrahydrofuran was added at 3° and the solution was allowed to stand overnight. The solution was again cooled to 3° and 5 ml more of 1.0 M diborane in tetrahydrofuran was added. After stirring for 0.5 hr in the cold, the solution was refluxed for about 0.75 hr. To the clear solution was then added dropwise 15 ml of methanol. Evaporation of the reaction mixture gave a gray oil which was refluxed in aqueous ethanolic sodium hydroxide for a total of 23 hr. The mixture was concentrated and extracted with ether. After drying over anhydrous magnesium sulfate, the ether was evaporated to an ivory-colored solid. The solid was washed with ether to yield 0.9 g (26%) of a colorless powder, mp 124–127°. A small amount of the material was dissolved in a minimum of hot ethanol, ethanolic hydrogen chloride was added dropwise until an acidic solution was obtained, and ether was added to turbidity. On cooling, the product, mp 123–124°, was obtained. The material was recrystallized again from ethanol–ether; mp 125–127°.

Anal. Calcd for $C_{24}H_{27}NO_4 \cdot HCl$: C, 67.05; H, 6.56; Cl, 8.24; N, 3.26. Found: C, 67.07; H, 6.69; Cl, 8.30; N, 3.27.

Enzymatic O-Methylation-^{14}C with S-Adenosylmethionine-^{14}C. Enzymatic *O*-methylation of 6-hydroxydopamine and 6-hydroxynorepinephrine was carried out with 2.0 ml of enzyme preparation containing catechol *O*-methyltransferase, 1 ml of 0.5 M potassium phosphate buffer, pH 7.9, 0.05 ml of 0.5 M magnesium chloride, 5 μmoles of substrate, and 5 μc (0.25 μmole) of *S*-adenosylmethionine-^{14}C. The mixtures were incubated at 37° under N_2 for 30 min, and 6 μmoles of cold *S*-adenosylmethionine was added. After an additional hour, the reaction was stopped by the addition of 20 volumes of ethanol. After centrifugation, evaporation *in vacuo*, resolution in 5 ml of ethanol, centrifugation, and re-evaporation, the residue was dissolved in 2 ml of isotonic saline (0.9%) and extracted two times with ethyl acetate. Cochromatography of the saline solutions indicated one radioactive peak for each of the substrates corresponding to the respective 3-*O*-methylated 6-hydroxycatecholamines. These solutions were diluted with carrier and employed for *in vivo* metabolic studies.

In Vivo Metabolism of 3-O-Methylated-^{14}C 6-Hydroxycatecholamines. Isotonic saline solutions from enzymatic *O*-methylation (above) containing 1–2 μc (20 μmoles) of 3-*O*-methyl-^{14}C-6-hydroxydopamine or 3-*O*-methyl-^{14}C-6-hydroxynorepinephrine were injected intraperitoneally into 300-g Sprague–Dawley male rats. Urines were collected in ice for 24 hr. An aliquot (5 ml) of the urine was adjusted to pH 6 with 0.5 M sodium acetate buffer and 0.2 ml (20,000 units of β-glucuronidase and 70,000 units of sulfatase) of Glusulase (Endo Products, Inc., New York, N. Y.) was added. After enzymatic hydrolysis at 37° for 18 hr the material was treated as described below.

Urine was adjusted to pH 4 and extracted with ethyl acetate (four times) to remove acids and neutral metabolites. The amines and conjugates were then isolated by column chromatography (Kakimoto and Armstrong, 1962). Paper chromatography was used to identify the radioactive compounds and counting was done in a liquid scintillation system. Enzymatic hydrolysis of conjugates was only 20–30% complete at the end of 18 hr. Most of the product formed was parent amine. The results are presented in Table I.

Enzymatic Monoamine Oxidase Studies. Enzymatically labeled *O*-methyl-^{14}C-amines (3-*O*-methyldopamine, normetanephrine, 3-*O*-methyl-6-hydroxydopamine, 3-*O*-methyl-6-hydroxynorepinephrine) were prepared as described above but on a smaller scale. A preparation of rat liver mitochondria containing monoamine oxidase (0.2 ml) (Hogeboom *et al.*, 1948), 0.1 ml of 0.5 M phosphate buffer, pH 7.4, and 2 μmoles (20,000 cpm) of *O*-methylated-^{14}C catecholamine was incubated for 1 hr. The mixture was passed through a micro Dowex 50 column (H^+ form) washed with 0.5 ml of water and the eluent radioactivity was determined in a scintillation system. Table III presents the data obtained after correction for boiled enzyme blanks.

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