TABLE V

LOG P VALUES FOR MISCELLANEOUS COMPOUNDS

Compd.	Log P
$C_6H_5C{\equiv}CH$	2.53 ± 0.01
$CH_3COC_2H_5$	0.32 ± 0.01
$CH_3CO(CH_2)_3CH_3$	1.38 ± 0.01
$CH_3CO(CH_2)_2CH{=}CH_2$	1.02 ± 0.01
$CH_3COCH_2CH_2$-◁	1.50 ± 0.01
Thiophene	1.81 ± 0.01
Indole	1.14 ± 0.01
Pyridine	0.65 ± 0.01
Quinoline	2.03 ± 0.01
$C_6H_5B(OH)_2$	1.58 ± 0.01
$CH_3COCH_2CH_2COOCH_3$	−0.23 ± 0.02
$CH_3CO(CH_2)_4COOCH_3$	0.55 ± 0.03
$CH_3(CH_2)_3SCN$	2.03 ± 0.02

that the logarithm of the partition of a compound in one set of solvents (P_1) is linearly related to the logarithm of the partition coefficient in a second similar set of solvents (P_2).

Since R_M and ΔR_M are so readily obtained *via* tankless chromatography, they should prove to be valuable supplements to log P and π in the extrathermodynamic substituent constant analysis of structure–activity relationships.

Experimental

The partition coefficients were determined according to our previously reported[4] procedure. Most of the compounds whose log P values are reported in Tables I and V were purified for partitioning by preparative vapor phase chromatography. Several of the compounds employed in this work have not been reported previously.

1-Fluoro-3-phenylpropane.—A mixture of 1-chloro-3-phenylpropane (35 g.), dry powdered potassium fluoride (21 g.), and 120 ml. of ethylene glycol was heated at 150–160° for 12 hr. with vigorous stirring. The mixture was then cooled, diluted with water, and extracted with ether. Evaporation of the ether and fractionation of the residue yielded 11 g. of product boiling from 173–195°. This material was purified for partitioning by means of an Aerograph autoprep using a silicon column; b.p. 183.5° (730.5 mm.), $n^{23}D$ 1.4870.

Anal. Calcd. for $C_9H_{11}F$: C, 78.26; H, 7.97. Found: C, 78.01; H, 8.12.

2-Amino-4-phenylpentanoic acid was prepared by the malonic ester method.[11] The melting point of our product after recrystallization from water was 245–246° dec.; von Braun and Kruber[11] reported 203–206°.

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.42; H, 7.76. Found: C, 68.47; H, 7.83.

4-Cyclopropyl-2-butanone.—Cyclopropylmethyl bromide was condensed with ethyl acetoacetate in the usual way.[12] The resulting product was hydrolyzed with 5% KOH (yield 33%). The crude material was purified by vapor phase chromatography; b.p. 155° (732 mm.), $n^{25}D$ 1.4260.

Anal. Calcd. for $C_7H_{12}O$: C, 74.94; H, 10.78. Found: C, 74.89; H, 10.74.

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The Formation of O-Methylated Catechols by Microsomal Hydroxylation of Phenols and Subsequent Enzymatic Catechol O-Methylation. Substrate Specificity

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A microsomal hydroxylating system which converts phenols to catechols and requires niacinamide adenine dinucleotide phosphate and glucose-6-phosphate has been assayed for a variety of phenols using the enzyme catechol-O-methyltransferase and radioactive S-adenosylmethionine-methyl-C^{14}. This system specifically methylates catechols converting them to radioactive methoxyphenols which can be extracted and assayed. Among the phenols which are converted to catechols are N-acetylserotonin, hydroxyindoles, tyramine, octopamine, hordenine, metanephrine, morphine, phenazocine, levorphanol, and estradiol. 2,4,6-Trichlorophenol formed an O-methylated product. Products from a variety of substrates were identified by cochromatography with authentic compounds.

Liver microsomes have been shown to hydroxylate a variety of aromatic compounds to phenols.[1-3] Recently, Axelrod[4] has demonstrated that phenolic amines are further hydroxylated by microsomal preparations to yield catechol amines such as (nor)epinephrine and dopamine. The conversion of tyramine to norepinephrine has been demonstrated *in vivo*,[5] a finding that focuses attention on the group of microsomal hydroxylases which convert phenols to catechols. Among the compounds containing phenolic groups and thus potential substrates for the formation of catechols are various physiologically active hydroxyindoles and phenolic phenethylamines, and a large number of important drugs (morphine, levorphanol, etc.). In order to carry out a survey of substrates, a convenient and widely applicable assay was needed. Catechol-O-methyltransferase[6] is an enzyme occurring in the soluble supernatant fraction of homogenized liver, which readily

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O-methylates a large variety of catechols[6-8] but does not carry out methylation of simple phenols.[6,7] By use of this enzyme and S-adenosylmethionine-methyl-C^{14}, a specific and simple biochemical assay of catechol formation was available. By organic extraction of radioactive O-methylated products and determination of radioactivity in a liquid scintillation counter, hydroxylation of a variety of phenolic substances was studied.

Preparation of Enzyme.—Rabbits were killed by a blow on the head. The livers were immediately removed, chilled on crushed ice, and homogenized with 3 vol. of ice-cold isotonic KCl. The homogenate was centrifuged at 10,000 *g* for 30 min. The supernatant, consisting of microsomes and a soluble fraction, was decanted, dialyzed, and used for subsequent enzymic assays.

Enzymatic Assay.—Enzyme activity was measured by incubating the "liver supernatant" containing the microsomes and the soluble fraction, obtained from 25 mg. of liver, with 0.5 μmole of substrate, 0.2 ml. of 0.5 *M* phosphate buffer (pH 7.9), 10 μmoles of $MgCl_2$, 0.6 μmole of NADP (niacinamide adenine dinucleotide phosphate), 1.5 μmoles of glucose-6-phosphate, and 3.7 mμmoles of S-adenosylmethionine-methyl-C^{14} (10,000 c.p.m.) in a final volume of 0.8 ml. After 1-hr. incubation at 37°, the reaction was stopped by the addition of 0.5 ml. of 0.5 *M* borate (pH 10.0). The radioactive metabolites were extracted into 5 ml. of toluene–isoamyl alcohol (3:2). A 4-ml. aliquot was transferred to a vial containing 1 ml. of ethanol and 10 ml. of phosphor. The radioactivity was measured in a liquid scintillation counter. The extractions were nearly quantitative for most compounds studied, so that the activities obtained were reported without correction for small losses during partitions. Acidic substances were extracted from acid or neutral pH in accordance with their dissociation constants. A control incubation, in which substrate was omitted, was run concurrently. Activities were then corrected for this blank which represents S-adenosylmethionine-methyl-C^{14} that is extracted and methylated products that are formed from endogenous material.

Materials.—Substrates were obtained from commercial sources. S-Adenosylmethionine-methyl-C^{14} was prepared enzymatically with methionine-methyl-C^{14}.[9] Certain compounds such as tyrosol,[10] N-acetyltyramine,[11] and *p*-hydroxyphenylglycol (m.p. 143.5–145°, C and H analysis correct) were prepared by standard synthetic methods.

O-Methylation of Enzymatically Formed Catechols.—Since catechol amines are unstable and difficult to measure, advantage was taken of the unique biochemical properties of these compounds. It has been shown[6,7] that only catechols are O-methylated by catechol-O-methyltransferase with S-adenosylmethionine serving as the methyl donor. Thus when monophenols are incubated with an enzyme preparation from liver together with S-adenosylmethionine-methyl-C^{14}, any catechol formed enzymatically will be methylated immediately by catechol-O-methyltransferase in liver, and thus rendered radioactive. This radioactive O-methylated derivative can be extracted readily into an organic solvent and measured. The catechol-O-methyl transferase in these experiments is present in such a large excess that it is quite unlikely that it represents the rate-limiting step for any of the reactions studied, so that the data may be presumed to reflect the rate of conversion of phenols to catechols. However, it is possible that some of the new catechols formed are very poor substrates for catechol-O-methyltransferase, and in this case the results would reflect a combination of rates.

Tyramine has been shown to form dopamine through the action of an enzyme in liver microsomes that requires NADPH.[4] When tyramine was incubated with rabbit liver microsomes and the soluble supernatant fraction together with S-adenosylmethionine-methyl-C^{14}, a radioactive product was formed which was extracted into toluene–isoamyl alcohol. The radioactive metabolite was subjected to paper chromatography using two solvent systems. After chromatography there was a single radioactive peak which had the R_f values of authentic 3-methoxytyramine in both solvent systems.[4] These observations suggest a sensitive and specific means for studying the enzymatic formation of catechols from various monophenols of diverse chemical structures. Typical quantitative results obtained with *p*-tyramine as the substrate are presented in Table I. The results reported in subsequent tables have been derived from similar data.

TABLE I

ENZYMATIC FORMATION OF O-METHYLATED CATECHOL FROM TYRAMINE[a]

Substrate	C.p.m.	Activity, mμmoles/g. of liver
Blank	212	
Tyramine	1340	15.8

[a] Incubation and assay as described in the text.

Enzymatic Formation of Methoxyhydroxyindoles.—A variety of phenolic indoles were incubated with the fortified rabbit liver preparation (Table II). All simple monohydroxyindoles formed methoxyhydroxyindoles regardless of the position of the original hydroxy group. The 5-hydroxyindole gave, however, greater conversion values. Conversely 4-hydroxyindole was the least active substrate. In compounds containing ethylamine side chains, N-acetylation or N-methylation was observed to increase activity. Thus N-acetylserotonin and N-methylhydroxytryptamines served as substrates while primary amines such as serotonin and 6-hydroxytryptamine were not acted upon by the enzyme. Compounds related to tryptamine, the β-carbolines, were readily hydroxylated and O-methylated.

Enzymatic Formation of O-Methylated Catechol Amines from Monophenolic Amines.—Almost all monophenolic amines examined (Table II) formed O-methylated catechols during incubation. As in the indole series, N-methylated and N-acetylated derivatives were readily hydroxylated. Acids and amino acids did not serve as substrates. Both *p*- and *m*-octopamine formed normetanephrine (ref. 4 and Table III), the O-methylation product of norepinephrine.

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TABLE II

ENZYMATIC FORMATION OF METHOXYHYDROXYINDOLES AND RELATED COMPOUNDS[a]

Substrate	Activity, mμmoles/g. of liver
Serotonin	0.0
N-Acetylserotonin	12.6
N-Methylserotonin	3.5
Bufotenin	0.7
6-Hydroxytryptamine	0.0
6-Hydroxy-N,N-dimethyltryptamine	0.8
N-Acetyl-4-hydroxytryptamine	0.0
5-Hydroxytryptophan	0.0
4-Hydroxyindole	3.6
5-Hydroxyindole	19.6
6-Hydroxyindole	12.5
7-Hydroxyindole	13.3
5-Hydroxy-6-methoxyindole	6.3
6-Hydroxy-5-methoxyindole	15.4
Harmalol	35.1
Dihydroharmalol	18.2
6-Hydroxy-1-methyltetrahydro-2-carboline	6.3
N-Acetyl-6-hydroxy-1-methyltetrahydro-2-carboline	10.7

[a] Incubation and assay as described in the text.

TABLE III

ENZYMATIC FORMATION OF O-METHYLATED CATECHOL AMINES AND RELATED COMPOUNDS[a]

Substrate	Activity, mμmoles/g. of liver
p-Tyramine	16.4
m-Tyramine	10.5
N-Methyltyramine	26.6
Hordenine	50.2
N-Acetyltyramine	52.3
Tyrosol	36.4
dl-Tyrosine	0.0
p-Hydroxyphenylacetic acid	0.0
p-Hydroxymandelic acid	0.0
3-Methoxytyrosol	10.5
3-Methoxytyramine	0.0
p-Hydroxyamphetamine	38.5
p-Octopamine	0.5
m-Octopamine	0.2
p-Hydroxy-α-(methylaminomethyl)benzyl alcohol (Synephrine)	8.4
m-Hydroxy-α-(methylaminomethyl)benzyl alcohol (phenylephrine)	16.8
p-Hydroxyephedrine	14.0
p-Hydroxyphenylglycol	8.3
Normetanephrine	0.0
Metanephrine	1.4
N-Methylmetanephrine	2.4

[a] Incubation and assay as described in the text.

Enzymatic Formation of O-Methylated Catechols from Phenolic Drugs and Alkaloids.—Of the large number of medicinals that contain phenolic grouping, a small number (7) were tested. All showed activity in spite of quite diverse structures, as exemplified by morphine, phentolamine, and estradiol (Table IV).

Enzymatic Formation of Methoxyphenols.—In order to attempt to elucidate substrate requirements of the enzyme, a large series of simple mono-, di-, and trisubstituted phenols were tested (Tables V and VI). Many showed very high activity while others, either for stereochemical or other reasons, showed negligible activity. These results will be discussed below.

TABLE IV

ENZYMATIC HYDROXYLATION AND O-METHYLATION OF PHENOLIC ALKALOIDS AND DRUGS[a]

Substrate	Activity, mμmoles/g. of liver
Morphine	1.2
Nalorphine	7.1
Levorphanol	8.3
Phenazocine	28.5
Phentolamine	35.6
Diethylstilbestrol	20.5
Estradiol	37.2

[a] Incubation and assay as described in the text.

TABLE V

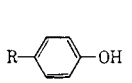
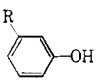
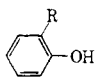
ENZYMATIC FORMATION OF METHOXYPHENOLS[a]

Substrate	Activity, mμmoles/g. of liver
Phenol	28.6
α-Naphthol	14.1
β-Naphthol	12.6
2-Hydroxyquinoline	0.0
4-Hydroxyquinoline	5.8
8-Hydroxyquinoline	16.0
2,3-Dimethylphenol	38.5
3,5-Dimethylphenol	49.0
2,4-Dimethylphenol	75.9
3,4-Dimethylphenol	74.8
2,6-Dimethylphenol	0.0
2,5-Dichlorophenol	37.6
2,4-Dichlorophenol	65.2
2,4,6-Trichlorophenol	15.6
2,4,6-Triiodophenol	2.1
2,6-Dibromophenol	2.4

[a] Incubation and assay as described in the text.

TABLE VI

ENZYMATIC HYDROXYLATION AND O-METHYLATION OF MONOSUBSTITUTED PHENOLS[a]

	Activity, mμmoles/g. of liver		
R			
CHO	39.3	32.5	4.2
NO_2	61.7	71.5	22.6
Cl	54.5	73.0	43.6
CH_3	43.4	69.0	20.0
OCH_3	42.0	60.2	2.8
$N(C_2H_5)_2$	...	28.1	...
$NHCOCH_3$	20.7	53.0	1.2
OH	8.1	11.2	...
$COCH_3$	70.2	65.1	28.5
$CHOHCH_3$	47.6	...	...
NH_2	1.6	17.8	5.1
$C(CH_3)_3$	60.8	28.6	0.6
C_6H_5	42.0	...	...

[a] Incubation and assay as described in the text.

Enzymatic Hydroxylation and O-Methylation of 2,4,6-Trichlorophenol.—The trisubstituted phenol, 2,4,-6-trichlorophenol, formed an O-methylated product (Table VII) while its structure would seem to preclude such a result, both *ortho* positions being occupied by chlorine atoms. Since the formation of O-methylated product required NADP and glucose-6-phosphate, it would seem to involve initial hydroxylation, followed by O-methylation of the catechol formed. Activity was lowered when magnesium ion, essential to catechol-O-methyltransferase,[6] was absent and the system was

TABLE VII

ENZYMATIC HYDROXYLATION AND O-METHYLATION OF 2,4,6-TRICHLOROPHENOL[a]

Substrate	Complete system	Minus NADP and glucose-6-phosphate	Minus Mg^{+2}	Plus 10^{-3} tropolone
2,4,6-Trichlorophenol	15.4	0.0	7.2	1.2

[a] Conditions and assay as described in the text.

inhibited by tropolone, a known catechol-O-methyltransferase inhibitor.[12] The results suggest *ortho* hydroxylation with concommitant loss of chlorine followed by O-methylation.

Identification of Metabolites Formed by Enzymatic Hydroxylation and O-Methylation. The conversion of *p*- or *m*-hydroxy-α-(methylaminomethyl)benzyl alcohol (Synephrine) to metanephrine, *p*- or *m*-tyramine to 3-methoxytryamine, and *m*-octopamine to normetanephrine have been previously reported.[4] The following reactions have now been authenticated by identification of the radioactive product by cochromatography with authentic compound: hordenine to N,N-dimethyl-3-methoxytyramine, tyrosol to 3-methoxy-4-hydroxyphenylethanol, *p*-hydroxyphenylglycol to 3-methoxy-4-hydroxyphenylglycol, *p*-octopamine to normetanephrine, N-acetylserotonin to mainly 6-methoxy-N-acetylserotonin, 5-hydroxyindole to a mixture of 5-hydroxy-6-methoxyindole (70%) and 6-hydroxy-5-methoxyindole (30%), and estradiol to 2-methoxyestradiol. In all cases the radioactive peak or peaks coincided with the colored spot given by the standard.

Discussion

The enzymatic formation of catechols from phenols with microsomal preparations first described by Axelrod,[4] has been shown to be a general reaction. The properties of the system and evidence that more than one enzyme is involved will be reported in a subsequent paper.[13] A convenient assay based on the specific methylation of catechols by catechol-O-methyltransferase[6] and using S-adenosylmethionine-methyl-C^{14} has allowed the study of a wide variety of substrates.

Dihydroxyindoles are formed from many monohydroxyindoles. 5-Hydroxyindole, for example, is hydroxylated to 5,6-dihydroxyindole which undergoes O-methylation to 70% 5-hydroxy-6-methoxyindole and 30% 6-hydroxy-5-methoxyindole as assayed by thin layer chromatography.[14] In the same way N-acetylserotonin forms a 5,6-dihydroxyindole and methylation yields chiefly the 6-O-methyl ether, an isomer of the main metabolite of melatonin,[15] a gonadal hormone from pineal glands.[16] 6-Hydroxylation of indoles is a common metabolic fate[8] for this class of compounds, and 5,6-dihydroxyindoles may have interesting physiological properties. 5,6-Dihydroxytryptamine has been reported as a neuro transmitter in crustacea.[17] This system also carries out hydroxylation in the 4-, 5-, and 7-positions as in the formation of 4,5-dihydroxy-6-methoxyindole from 5-hydroxy-6-methoxyindole, 4,5-dihydroxyindole from 4-hydroxyindole, and 6,7-dihydroxy-5-methoxyindole from 6-hydroxy-5-methoxyindole. The above tri-O-substituted indoles are of interest because of structural similarities to mescaline and to indoles which could arise from mescaline metabolites[18,19] on cyclization.[20]

In β-carbolines, which have been suggested as metabolites of tryptamine derivatives,[21] hydroxylation occurs readily. Harmalol, the de-O-methyl homolog of the potent MAO inhibitor harmaline,[22] is quite active as a substrate.

Hydroxylation in the indole series occurs more readily with less polar substrates such as N-acetyl and N-methyl derivatives. Primary amines such as serotonin and amino acids such as 5-hydroxytryptophan are not hydroxylated.

Studies on phenolic phenethylamines and derivatives demonstrate further the potential alternate pathway to catechol amines *via* such compounds as tyramine and octopamine.[5] The conversion of 3-methoxytyrosol, metanephrine, and N-methylmetanephrine to 3,4,5-trioxyphenethyl derivatives is the first absolute demonstration of a confluence of mescaline and catechol amine metabolism.

As in the indole alkylamines, N-acetylation or N-methylation of a primary amine increases enzyme activity. Other structural changes which decrease polarity also increase activity, as in the twofold increase engendered by the addition of an α-methyl group in the pairs of compounds, *p*-tyramine to amphetamine and synephrine to *p*-hydroxyephedrine. Lessened activity is observed upon the addition of a β-hydroxy group which would be expected from the greater polarity thus conferred upon the molecule. This is seen with tyrosol and the corresponding glycol, and with *p*- and *m*-tyramine as compared to the almost inactive β-hydroxy compounds, the octopamines. Acids and amino acids were found inactive as substrates.

Among the alkaloids and drugs tested, morphine and related compounds such as nalorphine, levorphanol, and phenazocine were hydroxylated. As shown with other series of compounds, activity is inversely related to the polarity so that nalorphine is more active 17-fold) than morphine, and the potent analgesic phenazocine in which most of the polar groups are absent is very active. The sympatholytic phentolamine is quite readily hydroxylated. Indeed the high activities shown by many of the compounds tested suggests that formation of catechols *via* phenol hydroxylation may be significant *in vivo*.

Estriol has been previously demonstrated to form a 2-methoxy derivative *in vitro*,[23] and estradiol is converted to 2-methoxyestriol *in vivo*,[24] probably *via* the 2,3-dihydroxy compound.[25] In this paper both estradiol and diethylstilbestrol have been shown to form O-

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methylated derivatives, which in the case of estradiol was identified as 2-methoxyestradiol by paper chromatography.

Simple phenols, naphthols, and hydroxyquinolines undergo hydroxylation to catechols. This reaction has also been demonstrated previously *in vivo* for various simple phenols and hydroxyqinolines.[26] In our studies both 4- and 8-hydroxyquinoline are hydroxylated while the 2-hydroxy isomer which exists mainly in the 2-keto form does not react. Various dimethylphenols are active except for 2,6-dimethylphenol in which catechol formation would require replacement of a methyl group by hydroxyl. Similarly various dichlorophenols were active, and even 2,4,6-trichlorophenol was converted to a methylated product although both *ortho* positions are blocked by chloro groups. Since NADP and glucose-6-phosphate are required, direct methylation of the phenol without an oxidative step is unlikely. Also catechol-O-methyltransferase involvement is indicated since the formation of the O-methylated derivative requires magnesium ions and is inhibited by tropolone.[12] Direct O-methylation by the enzyme which O-methylates 2,6-diiodophenols, described by Tomita, *et al.*,[27] does not seem likely since their enzyme does not require magnesium ions. It seems more likely that a replacement of a chloro group by a hydroxyl group, similar to that described by Kaufman[28] for the conversion of *p*-chloro- or *p*-fluorophenylalanine to tyrosine, has occurred. Certain other 2,6-dihalophenols also show low activity in our microsomal system.

Little correlation of activity with structure was possible for the various monosubstituted phenols (Table VI) studied. Since catechol-O-methyltransferase is present in a large excess and is little influenced by substituents[6] so that O-methylation is observed even in the presence of very bulky *ortho* substituents such as *t*-butyl,[29] most of the effects observed are probably related to hydroxylation. Both *para*- and *meta*-substituted compounds were quite active with *ortho* substitution, often severely reducing activity especially with very bulky groups such as *t*-butyl or with groups that can interact by hydrogen bonding with the phenol such as in salicylaldehyde. Compounds sensitive to oxidation such as aminophenols showed low activity, perhaps due to competitive oxidations.

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Excretion, Distribution, and Metabolism of Doxapram Hydrochloride

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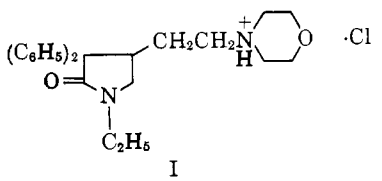
Doxapram hydrochloride, following intravenous injection into the dog, was rapidly metabolized. No unchanged drug was found in any biological material analyzed. The metabolites formed were distributed throughout the animal body with higher levels occurring in the fat, liver, pancreas, and adrenal glands than in other tissues. Blood levels of the metabolites decreased rapidly during the first hour followed by a much more gradual decline. Large concentrations of the metabolites occurred in the bile. The metabolites were excreted rapidly in the urine during the first 24 hr. Small amounts continued to be excreted up to 120 hr. after injection of doxapram hydrochloride. The primary path of metabolism was through opening of the morpholine ring. A large number of metabolites were formed, two of which were identified as 1-ethyl-4-(2-hydroxyethylaminoethyl)-3,3-diphenyl-2-pyrrolidinone and 1-ethyl-4-(2-aminoethyl)-3,3-diphenyl-2-pyrrolidinone.

Doxapram hydrochloride[1] is the nonproprietary name for 1-ethyl-4-(2-morpholinoethyl)-3,3-diphenyl-2-pyrrolidinone hydrochloride (I). This compound is



I

an agent that produces marked respiratory stimulation and pressor effects in animals[2a,b] and in humans.[2c,d] When doxapram is administered to animals under barbiturate-induced sleep, it produces arousal effects.[2a,c]

In this paper we present the results that have been obtained in studies designed to elucidate the metabolism, distribution, and excretion of doxapram. To carry out such studies it has been necessary to develop analytical methods suitable for the determination of the drug in the various biological materials. The methods are ultimately based on the oxidation of doxapram and doxapram-like materials to benzophenone which is subsequently quantitated from its ultraviolet absorption. The methods described, therefore, will determine any material which contains the diphenylmethyl moiety and which appears in the material finally oxidized.

Correlation of the results obtained from the "oxidation method" with those found using C^{14}-labeled doxapram indicate that all of the metabolites formed may be determined by the choice of the appropriate procedure for the "oxidation method."

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(2) (a) J. W. Ward and B. V. Franko, *Federation Proc.*, **21**, 325 (1962); (b) W. H. Funderburk and R. S. Alphin, *ibid.*, **21**, 324 (1962); (c) A. J. Wasserman and D. W. Richardson, *Clin. Pharmacol. Therap.*, **4**, 321 (1963); (d) H. G. Canter, *Am. Rev. Respirat. Diseases*, **87**, 830 (1963).

Experimental

Analytical Methods.—Initially, the ultraviolet spectrum of doxapram was considered as a method for quantitation. The