Dynamic Aspects of Enzymatic O-Methylation and -Demethylation of Catechols in Vitro and in Vivo

JOHN W. DALY, JULIUS AXELROD, AND BERNHARD WITKOP

From the National Institute of Arthritis and Metabolic Diseases and the National Institute of Mental Health, National Institutes of Health, United States Public Health Service, Bethesda, Maryland

(Received for publication, October 5, 1959)

The importance of O-methylation of catecholamines and other catechols as a metabolic pathway has been amply shown (1-4). The isolation of "Substanz Y," a p-O-methylcatechol derivative from beef adrenal glands (5), and the occurrence of p-O-methylcatechol derivatives in urine (6) are suggestive of p-O-methylation in vivo. Previous studies (7) have shown that in vitro the enzyme O-methyltransferase effects both para and meta O-methylation. The following study demonstrates that (nor)epinephrine is converted to p-O-methyl-(nor)epinephrine in vitro and that 3,4-dihydroxyacetophenone, arterenone, and adrenalone undergo p-O-methylation in the intact rat. The para and meta O-methylated derivatives of 3,4-dihydroxyacetophenone (acetovanillone and acetoisovanillone) have been shown to undergo a novel interconversion in vivo.

MATERIALS AND METHODS

3,4-Dihydroxyacetophenone, acetovanillone, acetoisovanillone, 3,4-dihydroxyphenylmethylcarbinol, 3-hydroxy-4-methoxyphenylmethylcarbinol, and 4-hydroxy-3-methoxyphenylmethylcarbinol have been described in a previous paper (7). The isomeric monomethyl ethers of dopamine, epinephrine, and norepinephrine were synthesized at the Sterling-Winthrop Research Institute and made available to us through the courtesy of Dr. Sydney Archer. In analogy to (nor)metanephrine the name (nor)paranephrine is suggested for p-O-methyl-(nor)epinephrine.

Synthesis of p-O-Methyladrenalone—To a vigorously stirred mixture of 7 g of adrenalone and 4 g of sodium bicarbonate in 60 ml of water and 60 ml of ether at 0° a solution of 7.0 g of carbobenzyloxy chloride in 50 ml of ether was added dropwise. Stirring was continued for 24 hours at 0°. Filtration removed 3.0 g of unchanged adrenalone. The solution was then shaken three times with ethyl acetate. The extract was dried and the solvent was removed under reduced pressure. The residue was recrystallized from ethyl acetate-ether-petroleum ether to yield 2.6 g of N-carbobenzyloxyadrenalone, m.p. 180-183°.

C_{11}H_{19}NO_3
Calculated: C 64.75, H 5.43, N 4.44
Found: C 64.77, H 5.48, N 4.51

A solution of 0.45 g of N-carbobenzyloxyadrenalone and 0.4 g of methyl iodide in 30 ml of methanol was refluxed with 1.6 ml of 1 N sodium hydroxide. After 5 hours the mixture was concentrated, the residue taken up in 150 ml of ethyl acetate, and the solution shaken four times with equal volumes of 0.05 N sodium hydroxide to remove any unchanged starting material. The product was then extracted into 1 N sodium hydroxide and precipitated by acidification. After recrystallization from ethyl acetate-ether-petroleum ether 110 mg of p-O-methyl-N-carbobenzyloxyadrenalone, m.p. 121-122°, were obtained.

C_{11}H_{19}NO_3
Calculated: C 65.64, H 5.82, N 4.25
Found: C 65.63, H 5.83, N 4.37

To 0.1 g of p-O-methyl-N-carbobenzyloxyadrenalone were added 3 ml of 32% hydrobromic acid in acetic acid. The solution was concentrated to dryness at room temperature under reduced pressure. The residue was recrystallized (Norit) from methanol-ethyl acetate to give crude p-O-methyladrenalone hydrobromide. The hydrobromide was dissolved in a small volume of water and the calculated amount of sodium carbonate was added. After standing overnight in the cold room the free base was collected and converted to the hydrochloride with a solution of hydrochloric acid in ethanol. Addition of ethyl acetate caused the precipitation of 60 mg of p-O-methyladrenalone-HCl, m.p. 245-246°.

C_{11}H_{19}NO_3\cdot HCl
Calculated: C 51.84, H 6.09, N 6.05, Cl 15.31
Found: C 51.82, H 6.14, N 5.99, Cl 15.47

Synthesis of p-O-Methylarterenone—Carbobenzyloxylation of arterenone was carried out in the same way as described for adrenalone. From 10.2 g of arterenone HCl 4.8 g of N-carbobenzyloxyarterenone, m.p. 153-155°, were obtained; 2.2 g of unchanged arterenone were recovered.

C_{11}H_{19}NO_3
Calculated: C 63.83, H 5.08, N 4.65
Found: C 63.83, H 5.08, N 4.65

The methylation of N-carbobenzyloxyarterenone was carried out as described above for adrenalone. From 0.6 g was obtained 0.5 g of p-O-methyl-N-carbobenzyloxyarterenone, m.p. 142-143°.

C_{11}H_{19}NO_3
Calculated: C 64.75, H 5.43
Found: C 64.86, H 5.43
The decarboxamoyloxylotation of p-O methyl N-carbomethoxyarterenone and the conversion of the resulting hydrobromide to the hydrochloride were carried out as described above. The hydrochloride of p-O-methylarterenone had m.p. 254-256°.  

\[ \text{C}_{19}\text{H}_{18}\text{NO}_5 \cdot \text{HCl} \]

Calculated: C 49.65, H 5.56, N 6.44, Cl 16.29

Found: C 49.69, H 5.57, N 6.39, Cl 16.39

**Synthesis of m-O-Methyladrenalene**—Three grams of 4-acetoxo-3-methoxyacetophenone (8) in 50 ml of glacial acetic acid were cooled and stirred while 2.3 g of bromine was added in 2 ml of acetic acid. The reaction was stirred for 1 hour and the temperature then raised briefly to 70°. After concentration to 3 ml of acetic acid, the reaction was stirred for 1 hour and the temperature then raised briefly to 70°. After concentration to dryness under reduced pressure. Fractional recrystallization from methanol-ethyl acetate afforded 1156

\[ \text{C}_{19}\text{H}_{13}\text{BrO}_4 \]

Calculated: C 46.01, H 3.86, Br 27.84

Found: C 45.70, H 3.70, Br 27.89

A solution of 1 g of 4-acetoxo-3-methoxy-ω-bromoacetophenone in 50 ml of ethyl acetate was added slowly to 50 ml of ethyl acetate through which was passed a stream of methylamine for 10 minutes. The reaction mixture was allowed to stand for 30 minutes and then concentrated to dryness under reduced pressure. The residue was dissolved in 20 ml of 3 N hydrochloric acid, refluxed for 3 hours on the steam bath and then concentrated to dryness under reduced pressure. Fractional recrystallization from methanol-ethyl acetate afforded 100 mg of m-O-methyladrenalone hydrochloride, m.p. 250-254°.  

\[ \text{C}_{19}\text{H}_{13}\text{BrO}_4 \]

Calculated: C 51.84, H 6.09, N 6.10, Cl 15.31

Found: C 51.67, H 6.12, N 6.10, Cl 15.65

**Synthesis of m-O-Methylarterenone**—The compound was prepared as described above for m-O-methyladrenalone with ammonia instead of methylamine. From 1 g of 4-acetoxo-3-methoxy-ω-bromoacetophenone 0.2 g of m-O-methylarterenone hydrochloride was obtained, m.p. 77-79°.  

\[ \text{C}_{19}\text{H}_{13}\text{NO}_5 \cdot \text{HCl} \]

Calculated: C 49.47, H 5.80, N 6.38, Cl 16.50

**Enzymatic O-Methylation of Catecholamines**—Enzymatic O-methylation of epinephrine, norepinephrine, adrenalone, and arterenone was carried out by incubating the hydrochloride of the catecholamine (10 μmoles), dissolved in 1 ml of water, for 1.5 hours at 37° with 4 ml of the soluble supernatant fraction of rat liver, 2.5 ml of 0.5 m phosphate buffer, pH 7.9, 4 μmoles of S-adenosylmethionine, and 0.1 ml of 2.0 m magnesium chloride (9).  

Enzymatic Demethylation of O-Methylated Catechols—The demethylation of O-methylate catechols was carried out as described by Axelrod (10). The formaldehyde formed in the oxidative demethylation was assayed with the Nash reagent (11). The results are presented in Table II.
Enzymatic p- and m-O-Methylation of Arterenone in vivo—The methylation of arterenone in the rat and the subsequent assay was carried out as described above for adrenalone. Chromatography of the purified products indicated the presence of a mixture of O-methylated arterenones. Treatment with sodium borohydride and periodic acid yielded vanillin and isovanillin (RF, color reaction, ultraviolet spectrum) in relative amounts of 18% of p-O-methyl and 82% of m-O-methyl isomer in the purified extract. The direct treatment of the urine with sodium borohydride and periodic acid yielded a mixture of 92% vanillin and 8% isovanillin. The O-methylated derivatives represented about 20% of the administered adrenalone hydrochloride.

Methylation of Epinephrine in vivo—Similar studies as above with rats were conducted with 40 mg per kg (in four divided doses) of L(+)-epinephrine (13, 14), the physiologically less active antipode of the natural hormone, with or without administration of iproniazid. No conversion to isovanillin could be demonstrated.

Administration of paraneprine or metanephrine intraperitoneally—To rats with or without administration of iproniazid, followed by extraction of the amine fraction from the urine and vanillin isoovanillin assay, showed no detectable interconversion of the isomeric ethers, the recovery of which was essentially the same (20 to 25%) within the limits of error.

The nonoccurrence of p-O-methylation of L(+)-epinephrine was also demonstrated in mice.

p- and m-O-Methylation of 3,4-Dihydroxyacetophenone in vivo—Two groups of adult male rats were used. One of these received 120 mg per kg of 3,4-dihydroxyacetophenone in divided doses over a period of 4 hours whereas the other group served as control throughout the experiment. The urine from each was collected for 20 hours, adjusted to pH 6 with acetic acid buffer, and...
incubated overnight with 10,000 units of bacterial 3-glucuronidase. The solutions were brought to an acid strength of 1 N in hydrochloric acid, heated for 90 minutes in a boiling water bath, cooled, and adjusted to ~pH 2. Aliquots were extracted five times with benzene, a procedure that extracts acetovanillone and acetoisovanillone but not 3,4-dihydroxyacetophenone (7). After concentration to dryness under reduced pressure, and so-
times with benzene, a procedure that extracts acetovanillone
NaOH. The concentrated ethanol extract was also purified by
cooled, and adjusted to -pH 2. Aliquots were extracted five
paper chromatography with butanol-concentrated ammonia (4 : 1).

The areas corresponding to acetovanillone and acetoisovanillone
were eluted with 0.1
NaOH and the amounts of these com-
paranephrine, norparanephrine, adrenalone, and arterenone confirm
earlier observations (7) that 3,4-dihydroxyacetophenone, 3,4-
dihydroxyphenylmethylcarbinol, and dopamine undergo p-O-
metabolism, to the extent of 40 to 56% when an unsaturated
side chain is present and to a lesser extent, 10 to 15% when a
saturated side chain is present.

The extension of these studies to intact animals led to the
finding that lesser amounts of the para isomer were formed, 25% in
vivo as opposed to 40% in vitro for 3,4-dihydroxyacetophenone,
and only 10% in vivo, as compared with 40% in vitro for adren-
only and arterenone. Administration of either 3-methoxy-4-hy-
droxy or 4-hydroxy-3-methoxyacetophenone then revealed a
novel interconversion of meta and para O-methyl ethers, the para
isomer being interconverted to a greater extent. Investigation
of all of the isomeric pairs of monomethyl ethers by means of a
crude enzyme preparation of O-demethylase (10) indicated that
the para O-methylated catechol ethers as a rule were demethylated
more readily (Table II) than the meta isomers.

Studies in vivo with L-(+)-epinephrine with or without mono-
amine oxidase inhibitors showed no detectable paraneprine,
whereas paraneprine was formed in vitro to the extent of 9%.
The possibility of a rapid conversion of paraneprine to meta-
nephrine seems to be precluded, since paraneprine, after intra-
peritoneal administration, was recovered with no detectable in-
crease in the excretion of metanephrine. Paraneprine might
conceivably be much more rapidly metabolized in some other way
and thus escape detection; however, the recoveries of admin-
istered metanephrine and paraneprine do not differ significantly.

**Table II**

Enzymatic O-demethylation of isomeric catechol monomethyl ethers

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Rat liver</th>
<th>Guinea pig liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-Hydroxy-3-methoxyphenylmethylcarbinol</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>3-Hydroxy-4-methoxyphenylmethylcarbinol</td>
<td>1.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Acetovanillone</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Acetoisovanillone</td>
<td>1.7</td>
<td>1.8</td>
</tr>
<tr>
<td>m-O-Methylamphetamine-HCl</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>p-O-Methylamphetamine-HCl</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Metanephrine-HCl</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Paraneprine-HCl</td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Normetanephrine-HCl</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Norparaneprine-HCl</td>
<td>0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>m-O-Methylarterenone-HCl</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>p-O-Methylarterenone-HCl</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>m-O-Methyladrenalone-HCl</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>p-O-Methyladrenalone-HCl</td>
<td>1.1</td>
<td>1.5</td>
</tr>
</tbody>
</table>

**Discussion**

The results of the studies in vitro on the methylation of epi-
nephrine, novenepliphine, adrenalone, and arterenone confirm
earlier observations (7) that 3,4-dihydroxyacetophenone, 3,4-
dihydroxyphenylmethylcarbinol, and dopamine undergo p-O-
metabolism, to the extent of 40 to 56% when an unsaturated
side chain is present and to a lesser extent, 10 to 15% when a
saturated side chain is present.

vanillone; on administration of acetoisovanillone the recovery
was 80% and of this 5 to 6% had been converted to the meta
isomer, acetovanillone. No 3,4-dihydroxyacetophenone was
detected.

### FIG. 1

![Diagram](http://www.jbc.org/)

**Enzymatic O-Methylation and -Demethylation of Catechols**

Vol. 235, No. 4
The interconversion of \( m \) and \( p \) O-methyl catechol ethers in the intact rat may be the result of demethylation to the catechol by the TPNH-dependent microsomal enzyme (10) followed by remethylation. Since the catechol is excreted largely as the m-methoxy derivative when given in vivo, the relatively greater conversion of \( p \)- to \( m \)-O-methyl ether may express the relative rates of methylation \((k_d > k_a)\) in the two positions. Further analysis of the factors determining the fates of these compounds requires determination of the number of enzymes capable of methylating and demethylating the catechol derivatives under study, the specificity and affinities of these enzymes, their distribution in the organism, the distribution of the substrates, and other factors.

\( p \)-O-Methylation of catechol derivatives in vivo introduces new metabolic aspects and will have to be studied further before any general conclusions may be reached.

**SUMMARY**

1. Methods for the synthesis of \( p \)-O-methyladrenalone, \( p \)-O-methylarterenone, \( m \)-O-methyladrenalone, and \( m \)-O-methylarterenone are described.

2. The formation of \( p \)-O-methylated compounds in vitro with \( O \)-methyl transferase has been demonstrated for epinephrine, nor-epinephrine, arterenone, and adrenalone.

3. The methylation in vivo of 3,4-dihydroxyacetophenone, arterenone, and adrenalone occurs at the \( para \) as well as \( meta \) positions.

4. An interconversion of the isomeric \( m \)- and \( p \)-methyl ethers of 3,4-dihydroxyacetophenone was observed in rats, the \( para \) isomer undergoing this interconversion to a greater extent.

5. Enzymatic demethylation studies in vitro showed that in most cases the \( para \) O-methyl ethers are demethylated more rapidly than the \( meta \) isomers.

6. No formation of paranephrine from epinephrine could be detected in vivo nor was any interconversion of paranephrine to metanephrine observed in vivo.

**REFERENCES**

Dynamic Aspects of Enzymatic O-Methylation and -Demethylation of Catechols

in Vitro and in Vivo

John W. Daly, Julius Axelrod and Bernhard Witkop


Access the most updated version of this article at
http://www.jbc.org/content/235/4/1155.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/235/4/1155.citation.full.html#ref-list-1