Effects of Aromatic Aldehydes on O-Methylation of Dopamine and Norepinephrine In Vitro

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Abstract □ An increase in extracted radioactivity was found when the aromatic aldehydes 2-nitrobenzaldehyde or pyridine-4-carboxaldehyde were incubated with catechol-O-methyltransferase in the presence of ¹⁴C-methyl-S-adenosylmethionine and dopamine or norepinephrine. The condensation products of dopamine with the two aldehydes were synthesized. The Schiff bases and tetrahydroisoquinoline derivatives were better substrates for catechol-O-methyltransferase than was dopamine or norepinephrine in terms of either K_m or V_{max}.

Keyphrases □ Aldehydes, aromatic—effects on catechol-O-methyltransferase and O-methylation of dopamine and norepinephrine in vitro, syntheses of Schiff bases and tetrahydroisoquinoline derivatives as substrates □ Catechol-O-methyltransferase—effects of aromatic aldehydes, O-methylation of dopamine and norepinephrine in vitro □ Dopamine—effects of 2-nitrobenzaldehyde and pyridine-4-carboxaldehyde on O-methylation in vitro □ Norepinephrine—effects of 2-nitrobenzaldehyde and pyridine-4-carboxaldehyde on O-methylation in vitro

The biological effects of aldehydes, derived endogenously or from the ingestion of foodstuffs, drugs, or other foreign compounds, are gaining widespread attention. The effects of aldehydes on the disposition of monoamines have been reported (1–3), as has evidence (4) that aldehydes derived from brain amines induced behavioral effects in laboratory animals. Recently, salsolinal and tetrahydropapaveroline, the condensation products of dopamine with acetaldehyde or 3,4-dihydroxyphenylacetaldehyde, respectively, were reported (5) to be better substrates for the enzyme catechol-O-methyltransferase than either dopamine or norepinephrine.

This report describes the effects of some aromatic aldehydes on the O-methylation of norepinephrine and dopamine in vitro.

EXPERIMENTAL[1]

4-(N-3,4-Dihydroxyphenethyliminomethyl)pyridine (II)— A mixture of 1.89 g (10 mmoles) of dopamine hydrochloride, 1.07 g (10 mmoles) of pyridine-4-carboxaldehyde, 3 g of sodium bicarbonate, and 100 ml of water was stirred under nitrogen for 2 hr. The resulting precipitate was filtered and recrystallized from dimethyl sulfoxide–water to give 2.25 g (93%) of a cream-colored solid, mp 239–240° dec. N-(2-Nitrobenzylidene)-3,4-dihydroxyphenethylamine (I) and N-(2-nitrobenzylidene)-3-methoxy-4-hydroxyphenethylamine (III) were prepared by this procedure. All compounds traveled as one spot on silica gel plates developed in n-butanol–acetic acid–water (4:1:1) and chloroform–methanol–ammonia (12:12:1.25). Table I lists their physical constants and analytical data.

1-(2-Nitrophenyl)-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (IV)—A mixture of 0.31 g (1.1 mmoles) of I, 0.1 ml of concentrated hydrochloric acid, and 10 ml of n-propanol was refluxed for 6 hr and the resulting mixture was kept in a refrigerator for 18 hr. The solid was collected by filtration, yielding 0.27 g (75%), mp 226–232° dec. The compound traveled as one spot on silica gel plates developed in n-butanol–acetic acid–water (4:1:1).

1-Pyridyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline Hydrochloride (V)—To a solution of 1 g (5 mmoles) of dopamine hydrochloride in 16 ml of ethanol and 2 ml of methanol was added 0.5 g (5 mmoles) of pyridine-4-carboxaldehyde. The mixture was refluxed with stirring for 8 hr. After standing at room temperature for 24 hr, the solid was filtered and washed twice with a small amount of ethanol, yielding 71%, mp 235–240° dec. The compound traveled as one spot on silica gel plates developed in n-butanol–acetic acid–water (4:1:1). Table I lists the analytical data.

Enzyme Purification and Assay—Catechol-O-methyltransferase from rat liver was prepared according to Axelrod and Tomchick (6). Assay of the enzyme was performed according to Axelrod and Vesell (7) using 78.3 nmoles of either dopamine or L-norepinephrine and 50 nmoles (40 nCi) of ¹⁴C-methyl-S-adenosylmethionine[2] in a total volume of 1 ml. Compounds I, II, III, 2-nitrobenzaldehyde, and pyridine-4-carboxaldehyde[3] were dissolved in dimethyl sulfoxide unless otherwise stated. Compounds IV and V were dissolved in water. When dimethyl sulfoxide was used as a solvent, a corresponding volume (0.1 ml) was added to the controls. The K_m and V_{max} values were determined from a Lineweaver–Burke plot constructed using the method of least squares.

Recovery Studies—3-Methoxytyramine hydrochloride[4] was dissolved in 0.01 N HCl and scanned on a spectrophotometer[5] between 190 and 340 nm; absorbance at 282 and 225 nm was found to be linear in a range of 7.5–150 nmoles in a volume of 3 ml. Twenty nanomoles of 3-methoxytyramine in 0.01 N HCl was added to a solution containing 0.3 ml of 0.05 M sodium phosphate buffer (pH 7.9), 0.1 ml of 0.02 M MgCl₂, 0.1 ml of dimethyl sulfoxide, and 0.42 ml of water for a final volume of 1 ml. A blank contained all but the 3-methoxytyramine. Following the addition of 0.5 ml of 0.5 M sodium borate buffer (pH 10), the mixture was extracted with 6 ml of toluene–isoamyl alcohol (3:2 by volume). Five-milliliter aliquots from three samples were pooled and lyophilized, and the residue was dissolved in 3 ml of 0.01 N HCl and transferred to cells. The absorbance at 282 and 225 nm was averaged from triplicate samples, and nanomoles of 3-methoxytyramine was determined from the standard curve. Eighty-four percent of the added 3-methoxytyramine was recovered.

The recovery of III was performed similarly to that of 3-methoxytyramine, with the following exceptions: the compound was dissolved in methanol rather than 0.01 N HCl and it was quantitated by its absorbance at 280 nm, which was linear between 7.5 and 150 nmoles. For extraction, the compound was dissolved in dimethyl sulfoxide rather than 0.01 N HCl, extracted, and lyophilized, and the residue was dissolved in methanol. The amount of compound extracted was 74%.

Since the O-methylated derivatives of II, IV, and V were not available, their recoveries were determined using the corresponding ¹⁴C-labeled forms synthesized enzymatically. Compound IV (0.4 μmole in water) was used as the substrate in the reaction mixture containing 0.1 ml dimethyl sulfoxide as described under Enzyme Purification and Assay. Following extraction of the product, 4-ml aliquots from two samples were pooled to obtain a reference sample and 4-ml aliquots from another eight samples were pooled
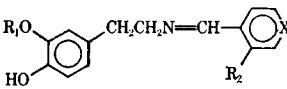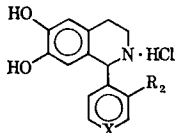
Table I—Physical Constants of Schiff Bases and Tetrahydroisoquinolines

R_1O—⟨ ⟩—CH_2CH_2N=CH—⟨ ⟩—X, HO, R_2

	Compound			Melting Point	Yield, %	Empirical Formula	Analysis, % Calc.	Found
Number	R_1	R_2	X					
I	H	NO₂	C	117–119° dec.[a]	93	$C_{15}H_{14}N_2O_4$	C 62.93 / H 4.93 / N 9.79	63.03 / 5.04 / 9.68
II	H	H	N	239–240° dec.[b]	93	$C_{14}H_{14}N_2O_2$	C 69.40 / H 5.82 / N 11.56	69.36 / 5.70 / 11.50
III	CH₃	NO₂	C	67.5–69°[c]	61	$C_{16}H_{16}N_2O_4$	C 63.99 / H 5.37 / N 9.33	63.97 / 5.44 / 9.43

HO—⟨ ⟩—$N\cdot HCl$, HO, R_2, X

	Compound			Melting Point	Yield, %	Empirical Formula	Analysis, % Calc.	Found
IV	—	NO₂	C	226–232° dec.	75	$C_{15}H_{15}ClN_2O_4$	C 55.82 / H 4.68 / N 8.68	56.08 / 4.65 / 8.61
V	—	H	N	235–240° dec.	71	$C_{14}H_{15}ClN_2O_2$	C 60.32 / H 5.42 / N 10.05	60.60 / 5.37 / 10.05

[a] Recrystallized from absolute ethanol. [b] Recrystallized from dimethyl sulfoxide–water. [c] Recrystallized from ethanol–water.

Table II—Effect of 2-Nitrobenzaldehyde and Pyridine-4-carboxaldehyde on O-Methylation of Dopamine *In Vitro*

	Product Formed[a,b], nmoles			
Experiment Number	Dopamine in Water	Dopamine in Dimethyl Sulfoxide	Dopamine plus 2-Nitrobenz-aldehyde[c]	Dopamine plus Pyridine-4-carboxaldehyde[c]
1	3.34	4.96	6.19 (25%)	—
2	5.29	8.52	8.66 (2%)	—
3	5.30	8.19	10.65 (30%)	—
4	4.48	7.08	10.52 (49%)	—
5	4.56	7.72	7.80 (1%)	9.78 (27%)
6	9.62	10.70	10.90 (2%)	11.94 (12%)
7	3.12	5.12	—	8.28 (62%)
8	5.50	9.57	—	13.43 (40%)
9	5.16	10.51	—	12.28 (17%)
10	5.16	8.26	—	10.11 (22%)
11	10.96	—	—	12.03 (10%)[d]
12	7.46	—	—	8.14 (9%)[d]
13	9.20	—	—	10.23 (11%)[d]
14	9.61	—	—	10.84 (13%)[d]

[a] Each value represents the average of two samples. [b] Values in parentheses represent percent increase from controls. [c] Concentration of aldehydes was 1 mM in dimethyl sulfoxide unless otherwise noted. [d] Pyridine-4-carboxaldehyde was dissolved in water.

for preparative isolation of the product. The reference sample was lyophilized and the residue, dissolved in methanol, was then chromatographed on a silica gel F-243-coated plastic sheet[6] (20 × 20 cm) with a solvent system of n-butanol–triethylamine–ethyl acetate (3:2:2). The R_f value of the product was determined by cutting the sheet in 0.5-cm sections for measurement of radioactivity by liquid scintillation spectrometry.

After establishing the R_f value of the radioactive product (0.79), the corresponding area on a chromatogram of the preparative sample was cut out, minced, and eluted three times with 5 ml of methanol. The eluates were pooled and evaporated under nitrogen, and the residue was dissolved in 20 ml of methanol. After determining the specific activity of this solution, triplicate samples of 0.5 ml were transferred to 10-ml centrifuge tubes and evaporated to dryness; the residue was then dissolved in 0.1 ml of dimethyl sulfoxide before the addition of magnesium chloride, sodium phosphate

buffer (pH 7.9), and water to a final volume of 1 ml. The samples were extracted with 6 ml of toluene–isoamyl alcohol (3:2) after being made basic with 0.5 ml of 0.5 M sodium borate buffer (pH 10). A 4-ml aliquot of the extract was transferred to a counting vial and the ¹⁴C was assayed as already described with the addition of 3 ml of methanol and 15 ml of liquifluor. Recovery of the O-methylated derivative of IV determined by the radioactivity of the extracts was 92.5%.

The method used to determine the recovery of O-methylated derivatives of II and V was similar to that used for IV with the following exceptions: 78.3 nmoles of II (dissolved in dimethyl sulfoxide) and 47.0 nmoles of V (dissolved in water) were substituted as substrates, and the product was chromatographed on cellulose thin-layer plates[6] (20 × 20 cm) with n-butanol–water–triethylamine (25:4:1). The R_f values of the products formed from II and V were 0.89 and 0.91, respectively; the recovery of the products was then established at 87.8 and 87.5%, respectively.

DL-Normetanephrine-³H² (7 nmoles, 20 nCi) was dissolved in water and added to quadruplicate samples containing 0.1 ml of di-

[6] Merck, Darmstadt, Germany.

Table III—Kinetic Constants of Substrates for Catechol-O-methyltransferase

| Substrate	K_m, μM^a	V_{max}, μmoles/hr
I	23.6	27.39
II	15.1	21.62
IV	28.5	38.66
V	25.9	33.44
Norepinephrine	51.1	25.55
Dopamine	32.7	18.51

[a] All assays were carried out in the presence of 0.1 ml of dimethyl sulfoxide.

methyl sulfoxide, sodium phosphate buffer (pH 7.9), magnesium chloride, and water to a final volume of 1 ml. The solution was made basic with 0.5 ml of 0.5 M sodium borate buffer (pH 10) and extracted with 6 ml of toluene–isoamyl alcohol (3:2). The radioactivity in 4-ml aliquots was assayed as already described, and only 26% of the added normetanephrine was found to have been extracted.

RESULTS AND DISCUSSION

The effects of 2-nitrobenzaldehyde and pyridine-4-carboxaldehyde on dopamine O-methylation are shown in Table II. In all experiments, an increase in extracted radioactivity (in terms of nanomoles of product) was found when these aldehydes were added to the reaction. Although dimethyl sulfoxide exerted activation in itself, this effect did not contribute to the action of pyridine-4-carboxaldehyde which, when dissolved in water instead of dimethyl sulfoxide, still produced an increase in ^{14}C (see last four experiments in Table II). One explanation for the increased product is an interaction of the aldehyde with the substrate dopamine. To examine the possibility that a Schiff base was being formed between these aldehydes and dopamine, providing an additional substrate for catechol-O-methyltransferase, or that the Schiff base cyclized via the Pictet–Spengler reaction, producing a tetrahydroisoquinoline, these derivatives were synthesized and examined as possible substrates for catechol-O-methyltransferase.

As seen in Table III, the Schiff bases and tetrahydroisoquinoline derivatives were better substrates for catechol-O-methyltransferase than either dopamine or norepinephrine; II was most active in
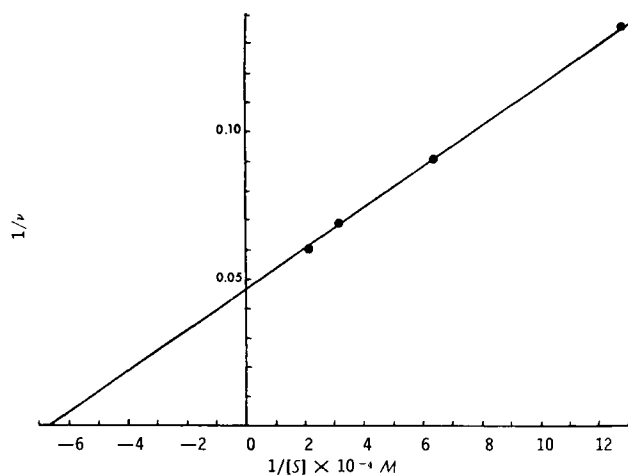
Figure 1—*Determination of kinetic constants of II for catechol-O-methyltransferase* (K_m = *15.1* μM, V_{max} = *21.62 nmoles/hr*).

terms of K_m (Fig. 1) while the tetrahydroisoquinolines had greater V_{max} values. All values were corrected for 100% recovery (see *Recovery Studies*). No attempt was made to distinguish between the *meta*- and *para*-O-methylated isomers. Although it is possible that one optical isomer of either IV or V might be a better substrate for catechol-O-methyltransferase than its counterpart, no attempt was made to resolve the stereoisomers.

Due to the wide variability in activation found when dopamine was used as substrate, it was thought that norepinephrine would yield more consistent results in the presence of the aldehydes. As seen in Table IV, activation with norepinephrine was of a greater magnitude and less variable than when dopamine was used. In addition, dimethyl sulfoxide had little or no effect on the methylation of norepinephrine; this was evident also in runs where pyridine-4-carboxaldehyde was dissolved in water. Concentration variation of the aldehydes (see Experiments 7, 13, and 16, Table IV) gave a linear response.

Preliminary findings using epinephrine or 3,4-dihydroxymandelic acid as a substrate indicate that the aldehydes were ineffec-

Table IV—Effects of 2-Nitrobenzaldehyde and Pyridine-4-carboxaldehyde on O-Methylation of Norepinephrine *In Vitro*

Experiment Number	Product Formed[a,b], nmoles				
	Norepinephrine in Water	Norepinephrine in Dimethyl Sulfoxide	Norepinephrine plus Pyridine-4-carboxaldehyde in Water[c]	Norepinephrine plus Pyridine-4-carboxaldehyde in Dimethyl Sulfoxide[c]	Norepinephrine plus 2-Nitrobenzaldehyde in Dimethyl Sulfoxide[c]
1	1.32	1.97	—	4.07 (107%)	—
2	1.76	2.00	—	4.10 (105%)	—
3	2.05	2.02	—	3.59 (78%)	—
4	1.58	1.99	—	3.81 (91%)	—
5	1.34	1.97	—	3.60 (83%)	—
6	2.33	3.26	—	4.62 (42%)	—
7	3.85	3.76	—	6.45 (72%)	—
				5.20 (38%)[d]	
				3.99 (6%)[e]	
8	2.05	—	3.55 (73%)	—	—
9	1.58	—	2.84 (80%)	—	—
10	2.03	—	3.45 (70%)	—	—
11	3.43	—	4.98 (45%)	—	—
12	3.74	—	6.31 (69%)	—	—
13	3.82	—	5.94 (56%)	—	—
			5.34 (40%)[d]		
			4.09 (7%)[e]		
14	2.31	2.47	—	—	4.65 (88%)
15	2.33	3.26	—	—	4.60 (41%)
16	3.97	3.70	—	—	6.37 (72%)
					5.35 (45%)[d]
					4.15 (12%)[e]

[a] Each value represents the average of two samples. [b] Values in parentheses represent percent increase from controls. [c] Concentration of aldehydes was 1 mM unless otherwise stated. [d] Aldehyde concentration was 0.5 mM. [e] Aldehyde concentration was 0.1 mM.

tive in producing activation, further supporting the hypothesis of Schiff-base formation for which a terminal primary amine group is required. It is hoped that results of these studies may provide further information concerning the metabolism of dopamine and norepinephrine in terms of endogenous aldehyde levels and agents that alter these levels.

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Synthesis and Screening of Potential Antimalarial Agent α-(2-Piperidyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol Hydrochloride

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Abstract □ The synthesis and biological testing of α-(2-piperidyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol hydrochloride and its O-acetyl and N-acetyl derivatives are reported. Direct hydrogenation of the α-pyridyl ketone gave very low yields. A novel procedure was developed which permits formation of piperidyl quinolinemethanols in satisfactory yields.
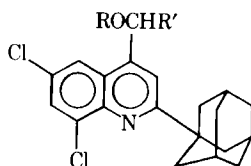
Keyphrases □ α-(2-Piperidyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol hydrochloride (and N-acetyl and O-acetyl derivatives)—synthesized and screened as potential antimalarial agents □ Quinolinemethanols, α-(2-piperidyl) analog and N-acetyl and O-acetyl derivatives—synthesized and screened as potential antimalarial agents □ Antimalarial agents, potential—synthesis and screening of α-(2-piperidyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol hydrochloride and N-acetyl and O-acetyl derivatives

Antimalarial activity has been reported for many derivatives of the quinine analog 4-quinolinemethanol. Mono- or disubstitutions at the 6-, 7-, or 8-position of the quinoline nucleus enhance activity relative to the unsubstituted compound, while substitution at the 2-position appears necessary to retard metabolic oxidation of the quinoline skeleton (1–4).

Unfortunately, the more active 2-aryl-4-quinolinemethanols also have undesirably high phototoxic-

Ia: R = H, R' = CH₂N(C₄H₉)₂
Ib: R = H, R' = 2-piperidyl
Ic: R = H, R' = N-acetyl-2-piperidyl
Id: R = CH₃CO, R' = 2-piperidyl

ity, which might detract from their use as antimalarial agents in humans (3, 5–8). Recently, a number of 4-quinolinemethanols were prepared with nonaryl substitution at the 2-position (9–11). Mixed results indicated further search for appropriate substitution at this position. Use of an adamantyl substituent at position 2 was suggested by the enhanced biological activity of this substituent in comparison to more conventional saturated moieties in other medicinal agents (12–14). Synthesis of several 2-adamantylquinolinemethanols has been reported (15). The promising antimalarial activity of α-(di-n-butylaminomethyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol (Ia) (15) suggested that the α-(2-piperidyl) analog (Ib) should be synthesized and tested.

The synthesis and biological screening of α-(2-piperidyl)-2-(1-adamantyl)-6,8-dichloro-4-quinolinemethanol (Ib) as well as the related N-acetyl (Ic) and O-acetyl (Id) derivatives are reported here. Preparation of (Id) was necessitated when the usual selective catalytic hydrogenation of the pyridyl ketone (4, 16) produced the desired product in very small yields. The modification, a two-step reduction, permits good yields of the α-2-piperidyl-4-quinolinemethanol (Ib).

CHEMISTRY

Compound Ib was prepared as illustrated in Scheme I. Acetyladamantane (II) (17, 18) was obtained in 59% yield, by the method of Tegner (19), from the addition of methyllithium to adamantane-1-carboxylic acid followed by hydrolysis. A by-product of this reaction, 2-(1-adamantyl)propan-2-ol (20), was also isolated in low yield (about 4%). The formation of this product was somewhat surprising, because Tegner (19) suggested that methyllithium should not react further with the dilithium salt of the ketone, even though phenyllithium has been shown to give tertiary alcohols in its reaction with carboxylic acids and their derivatives (21).