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Cardioselective β-Adrenergic Blocking Agents. 1. 1-[(3,4-Dimethoxyphenethyl)amino]-3-aryloxy-2-propanols

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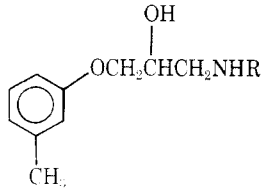
A series of 1-amino-3-aryloxy-2-propanols has been synthesized and examined for cardioselective β-blockade. The introduction of the (3,4-dimethoxyphenethyl)amino group lead to the most cardioselective agents. Structure–activity relationships are discussed. Of the compounds tested 1-[(3,4-dimethoxyphenethyl)amino]-3-(*m*-tolyloxy)-2-propanol was selected for clinical trial because of optimal potency and selectivity.

The first class of compounds which were shown to possess a significant degree of specificity for β-adrenergic receptors in the myocardium was the 3-amino-2-hydroxypropoxy-substituted anilides[1] of which practolol is the best known example. It appears that the *p*-acylamino substituent is responsible for the cardioselectivity in this series and this has been substantiated by other workers.[2]

Recently it has been shown that it is possible to increase the cardioselectivity in a series of 1-aryloxy-3-[(aryloxyalkyl)amino]-2-propanols[3] by the proper choice of the amino substituent. The present report is concerned with the effect of various amino substituents on the β-blocking activity and the cardioselectivity in a series of 1-amino-3-(*m*-tolyloxy)-2-propanols (I). Previous investigators[4] had

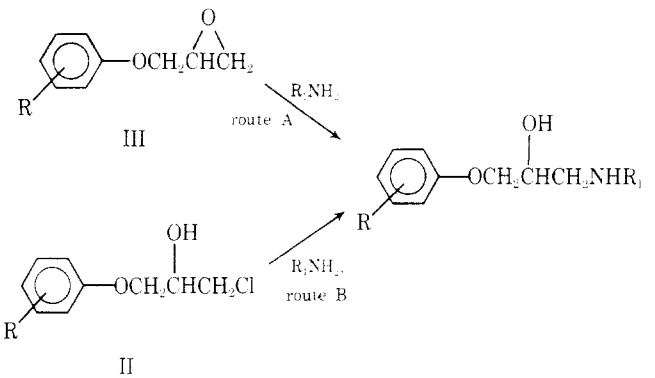


I

shown that compound I (where R = isopropyl or *tert*-butyl) possessed potent β-blocking activity, but no selectivity of action was demonstrated. However, early in our investigations it became apparent that certain amino substituents did have an effect on cardioselectivity in this series and that this selectivity was optimum for the (3,4-dimethoxyphenethyl)amino group. In addition, it was shown that the incorporation of this amino substituent into a series of 1-amino-3-(substituted phenoxy)-2-propanols enhanced cardioselectivity in all cases. Furthermore, incorporation of this same amino substituent into several known β-blocking agents produced the same effect.

Chemistry. The compounds were prepared by methods previously described[4] where the 1-chloro-3-(substituted phenoxy)-2-propanol (II) or 1,2-epoxy-3-(substituted phenoxy)propane (III) was treated with the appropriate amine.

The various substituted phenethylamines were prepared by lithium aluminum hydride reduction of the corresponding substituted phenylacetonitrile, or when this intermediate was not commercially available, the β-nitrostyrenes were prepared as described by Gairaud and Loppin[5] and then reduced with lithium aluminum hydride.[6]



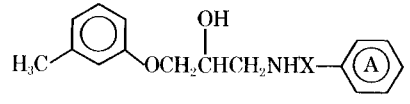
Pharmacology. An *in vitro* guinea pig model was developed to identify β-blocking agents with cardioselective action.[7] Isoproterenol dose–response curves were determined on isolated atria and tracheal chains. Atrial response was represented by an increase in heart rate, while the tracheal response was represented by a decrease in resting tone. Values were calculated and plotted as the per cent of maximum response *vs.* concentration of isoproterenol in the incubation media. The antagonist concentration generally worked with was 10^{-6} *M*. The dose–response curves following β-blockade were shifted to the right of control values proportional to the degree of isoproterenol inhibition. Thus, cardioselective agents demonstrated a greater shift of the atrial *vs.* tracheal curves.

The K_B values, or apparent dissociation constants of the antagonists, were calculated as described by Furchgott.[8] Thus

$$K_B = [B]/(\text{dose ratio} - 1)$$

where [B] is the concentration of the antagonist, and the dose ratio is the ratio of equipotent concentrations of the agonist with/without test compound. The accuracy of the K_B values so determined was contingent upon the presence of competitive inhibition, which was shown to be the case with compound I because of the parallel nature of the

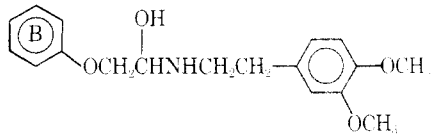
Table I



Compd	–X–	Substituents in ring A	Mp or bp (mm), °C	Crystn solvent[a]	Empirical formula	Analyses	Method of prepn	Apparent K_B Atrium	Apparent K_B Trachea	Ratio of K_B (T/A)
1	$-CH_2CH_2-$	3,4-$(OCH_3)_2$	135–137	A	$C_{20}H_{27}NO_4 \cdot HCl$	C, H, N	A	1.4×10^{-8}	3.6×10^{-7}	26
2	$-CH_2CH_2-$	3-OCH_3-4-OC_2H_5	147–148	A	$C_{21}H_{29}NO_4 \cdot HCl$	C, H, N	A	2.5×10^{-8}	1.4×10^{-7}	5.6
3	$-CH_2CH_2-$	3-OCH_3-4-OH	156–157	A	$C_{19}H_{25}NO_4 \cdot HCl$	C, H, N	A	3.4×10^{-8}	1.5×10^{-7}	4.4
4	$-CH_2CH_2-$	3-OCH_3-4-$OCH_2C_6H_5$	87–88	B	$C_{26}H_{31}NO_4$	C, H, N	A	1.1×10^{-6}	9.1×10^{-6}	8.0
5	$-CH_2CH_2-$	4-OCH_3-3-$OCH_2C_6H_5$	97–98	B–F	$C_{26}H_{31}NO_4$	C, H, N	A	Inactive		
6	$-CH_2CH_2-$	3,4-(–OCH_2O–)	72–73	B–C	$C_{19}H_{23}NO_4$	C, H, N	A	1.6×10^{-7}	6.4×10^{-7}	4.0
7	$-CH_2CH_2-$	2,5-$(OCH_3)_2$	71–72	B–C	$C_{20}H_{27}NO_4$	C, H, N	A	7.1×10^{-7}	3.6×10^{-7}	0.5
8	$-CH_2CH_2-$	2,4-$(OCH_3)_2$	73–74	B–C	$C_{20}H_{27}NO_4$	C, H, N	A	Inactive		
9	$-CH_2CH_2-$	2,3-$(OCH_3)_2$	69–71	B	$C_{20}H_{27}NO_4$	C, H, N	A	2.2×10^{-7}	1.7×10^{-7}	0.8
10	$-CH_2CH_2-$	3,4,5-$(OCH_3)_3$	137–139	A	$C_{21}H_{29}NO_3 \cdot HCl \cdot 0.5H_2O$	C, H, N	A	1.9×10^{-7}	7.1×10^{-7}	3.8
11	$-CH_2CH_2-$	4-OCH_3	212–213 (0.25)		$C_{19}H_{25}NO_3$	C, H, N	A	1.1×10^{-7}	1.7×10^{-7}	1.5
12	$-CH_2CH_2-$	3-OCH_3	116–117	A	$C_{19}H_{25}NO_3 \cdot HCl$	C, H, N	A	7.6×10^{-8}	8.3×10^{-8}	1.1
13	$-CH_2CH_2-$	2-OCH_3	59–60	B–C	$C_{19}H_{25}NO_3$	C, H, N	A	4.5×10^{-7}	4.8×10^{-7}	1.1
14	$-CH_2-$	3,4-$(OCH_3)_2$	163–164	A	$C_{19}H_{25}NO_4 \cdot HCl$	C, H, N	A	1.0×10^{-7}	4.5×10^{-7}	4.5
15	$-CH(CH_3)CH_2-$	3,4-$(OCH_3)_2$	74–76	D	$C_{21}H_{29}NO_4 \cdot C_2H_2O_4$	C, H, N	A	8.5×10^{-8}	9.2×10^{-8}	1.1
16	$-CH_2-$	None	75–76	B	$C_{17}H_{21}NO_2$	C, H, N	A	1.1×10^{-6}	1.2×10^{-6}	1.1
17	$-CH_2CH_2-$	None	164–165	A	$C_{18}H_{23}NO_2 \cdot HCl$	C, H, N	A	6.2×10^{-8}	7.1×10^{-7}	11.0
18	$-CH_2CH_2CH_2-$	None	151–152	A	$C_{19}H_{25}NO_2 \cdot HCl$	C, H, N	A	7.9×10^{-8}	2.5×10^{-7}	3.2
19	$-CH_2CH(OH)-$	None	164–165	E	$C_{18}H_{23}NO_3 \cdot C_2H_2O_4$	C, H, N	A	1.3×10^{-7}	1.1×10^{-6}	8.0
20	$-CH(CH_3)CH(OH)-$	None	119–120	A–B	$C_{19}H_{25}NO_3$	C, H, N	A	4.5×10^{-7}	8.3×10^{-7}	1.8
21	$-CH_2CH_2-$	3,4-$(CH_3)_2$	139–141	A	$C_{20}H_{27}NO_2 \cdot HCl$	C, H, N	A	5.2×10^{-7}	4.3×10^{-7}	0.8
22	$-CH_2CH_2-$	4-Cl	89–90	B	$C_{18}H_{22}ClNO_2$	C, H, N	A	2.2×10^{-7}	1.8×10^{-6}	4.5
23	$-CH_2CH_2-$	3,4-$(Cl)_2$	81–82	B	$C_{18}H_{21}Cl_2NO_2$	C, H, N	A	1.3×10^{-6}	4.3×10^{-6}	3.3
I, R = $CH(CH_3)_2$[b]			119–120		$C_{13}H_{21}NO_2 \cdot HCl$	C, H, N		3.5×10^{-8}	4.9×10^{-8}	1.4

[a] A, MeCN; B, *i*-Pr_2O; C, *n*-hexane; D, MeOH; E, MeC(=O)Me; F, *i*-PrOH. [b] See ref 4.

Table II



Compd	Substituents in ring B	Mp, °C	Crystn solvent[a]	Empirical formula	Analyses	Method of prepn	Apparent K_B Atrium	Apparent K_B Trachea	Ratio of K_B (T/A)
1	3-CH_3	135–137	A	$C_{20}H_{27}NO_4 \cdot HCl$	C, H, N	A	1.4×10^{-8}	3.6×10^{-7}	26
24	2-CH_3	139–141	C	$C_{20}H_{27}NO_4 \cdot HCl$	C, H	A	4.2×10^{-8}	8.7×10^{-8}	21
25	4-CH_3	84–85	B	$C_{20}H_{27}NO_4$	C, H, N	A	8.6×10^{-8}	6.8×10^{-7}	8.0
26	H	98.5–99.5	A–B	$C_{19}H_{27}NO_4$	C, H, N	B	6.5×10^{-9}	1.4×10^{-7}	22
27	3-CH_2CH_3	129–130	A	$C_{21}H_{29}NO_4 \cdot HCl$	C, H, N	A	1.1×10^{-8}	1.0×10^{-7}	9.0
28	3-$C(CH_3)_3$	68–70	B	$C_{23}H_{33}NO_4$	C, H, N	A	1.6×10^{-7}	6.3×10^{-6}	39
29	2-Cl	81–82	B	$C_{19}H_{24}ClNO_4$	C, H, N	A	5.7×10^{-8}	1.4×10^{-7}	25
30	3-Cl	82.5–83.5	B	$C_{19}H_{24}ClNO_4$	C, H, N	A	1.8×10^{-8}	1.9×10^{-7}	11
31	4-Cl	100–101	A–B	$C_{19}H_{24}ClNO_4$	C, H, N	A	6.3×10^{-8}	4.5×10^{-7}	7.0
32	2-OCH_3	149–151	D	$C_{20}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	A	4.2×10^{-8}	1.5×10^{-7}	3.6
33	3-OCH_3	148–150	D	$C_{20}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	A	1.5×10^{-8}	1.5×10^{-7}	10
34	4-OCH_3	137–138	D	$C_{20}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	A	2.2×10^{-7}	2.5×10^{-6}	11
35	2-OCH_2CH_3	113–114	E	$C_{21}H_{29}NO_4 \cdot HCl$	C, H, N	A	7.1×10^{-8}	6.3×10^{-8}	8.9
36	2-CH_2OH	125–127	C	$C_{20}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	A	4.8×10^{-8}	5.9×10^{-7}	12.0
37	2-CH_2NH_2	99–100	C	$C_{20}H_{28}N_2O_4$	C, H, N	A	2.4×10^{-7}	6.3×10^{-6}	26.0
38	2-$C(=O)CH_3$	145–150	C	$C_{21}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	A	2.8×10^{-8}	2.3×10^{-7}	8.2
39	3-$C(=O)CH_3$	136–138	C	$C_{21}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	B	7.6×10^{-8}	3.8×10^{-6}	50
40	4-$C(=O)CH_3$	175–176	F	$C_{21}H_{27}NO_5 \cdot C_2H_2O_4$	C, H, N	B	4.8×10^{-7}	4.2×10^{-5}	88
41	2-CN	125–127	C	$C_{21}H_{24}N_2O_4 \cdot C_2H_2O_4$	C, H, N	A	1.2×10^{-8}	4.2×10^{-7}	35
42	3-CF_3	137–138	A	$C_{20}H_{24}F_3NO_4 \cdot HCl$	C, H, N	A	2.5×10^{-7}	1.1×10^{-6}	4.4
43	3-$NHC(=O)CH_3$	179–180	D–E	$C_{21}H_{28}N_2O_5 \cdot C_2H_2O_4$	C, H, N	A	2.9×10^{-7}	8.3×10^{-6}	29
44	4-C_6H_5	199–200	D	$C_{25}H_{29}NO_4 \cdot HCl$	C, H, N	B	2.4×10^{-7}	1.1×10^{-5}	46
45	3,4-$CH_2CH_2CH_2$–	167–168	C	$C_{22}H_{29}NO_4 \cdot HCl$	C, H, N	A	3.4×10^{-8}	1.1×10^{-6}	32
46	2,3-$(CH_3)_2$	156–157	A	$C_{21}H_{29}NO_4 \cdot HCl$	C, H, N	A	5.1×10^{-8}	1.3×10^{-7}	2.5
47	3,4-$(CH_3)_2$	92–93	B–C	$C_{21}H_{29}NO_4$	C, H, N	A	4.1×10^{-8}	1.4×10^{-7}	3.4
48	3,5-$(CH_3)_2$	113–114	B–C	$C_{21}H_{29}NO_4$	C, H, N	A	3.7×10^{-8}	1.4×10^{-7}	3.8
49	3-CH_3-5-$C(CH_3)_3$	88–89	B	$C_{24}H_{35}NO_4$	C, H, N	A	2.3×10^{-9}	2.5×10^{-8}	11
50	3-CH_3-4-SO_2CH_3	198–199	F	$C_{21}H_{29}NO_6S \cdot 0.5C_2H_2O_4$	C, H, N	A	4.4×10^{-7}	2.3×10^{-6}	52

[a] A, MeCN; B, i-Pr_2O; C, i-PrOH; D, MeOH; E, MeC(=O)Me; F, EtOH.

dose–response curves when tested at 10^{-4}, 10^{-5}, and 10^{-6} M. The ratio of the K_B values calculated for paired atrial and tracheal preparations was used as an index to express cardioselectivity. Control population dose–response curves were determined on 21 paired tissues and these values were used as controls for calculation purposes.

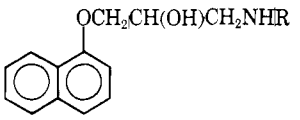
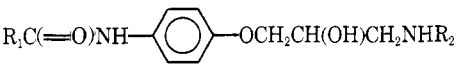
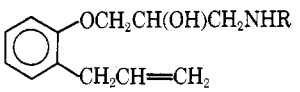
Structure–Activity Relationships. The effect of varying the amino substituent on the cardioselectivity in a series of 1-amino-3-(*m*-tolyloxy)-2-propanols is summarized in Table I. An aralkylamino group was required in order to achieve any significant degree of selectivity, and phenethylamino (**1**, **17**) was superior to either the corresponding benzylamino (**14**, **16**) or (phenylpropyl)amino group (**18**). However, the (α-methylphenethyl)amino group (**15**) caused loss of selectivity.

The nature and position of the substituents on the phenyl ring had a profound effect on the cardioselectivity with the 3,4-dimethoxyphenyl group (**1**) showing the maximum effect. Replacement of the 4-methoxy with a hydroxy (**3**), ethoxy (**2**), or benzyloxy substituent (**4**) decreased the selectivity. Similar results were obtained by changing the positions of the methoxy substituents on the phenyl ring (**7–9**) or by replacing them with methyl (**21**) or chloro groups (**23**). None of the monosubstituted phenyl compounds (**11–13**, **22**) showed any significant selectivity of action.

After the preferred structure of the amino group for maximum cardioselectivity had been established, the effect of varying the substituents on the phenoxy ring B in a series of 1-(3,4-dimethoxyphenethyl)amino-3-(substituted phenoxy)-2-propanols was examined and the results are summarized in Table II. In general, the effect of substituents on the β-adrenergic blocking activity closely follows the results of Crowther and coworkers[4] with the 1-isopropylamino-3-(substituted phenoxy)-2-propanol series. Thus, compounds with substituents in the 2 and 3 positions were more active than those substituted in the 4 position. However, the cardioselectivity seemed to be more dependent upon the nature of the substituent. For nonpolar groups like alkyl (**1**, **24**, **25**, **27**, **28**) or halogen (**29–31**) substitution in the 2 and 3 positions gave the greatest selectivity of action. For more polar groups like methoxy (**32–35**) or acetyl (**38–40**), substitution in the 3 and 4 positions afforded the greatest selectivity. Substitution in the 4 position is preferred for the acetylamino group (**43**, **54**).[1] When there were two substituents on the phenoxy ring (**46–50**) the selectivity was decreased.

Since the (3,4-dimethoxyphenethyl)amino moiety appeared to be unique in conferring cardioselective β-blocking activity in this series, it was incorporated into several known β-blockers and the results are summarized in Table III. In these cases the cardioselectivity was improved, although the degree of enhancement was not predictable. The propranolol analog **52** showed the least selec-

Table III

Compd	Structure	Mp, °C	Crystn solvent[a]	Empirical formula	Analyses	Method of prepn	Apparent K_B Atrium	Apparent K_B Trachea	Ratio of K_B (T/A)
									
51	R = $CH(CH_3)_2$[b]						1.3×10^{-8}	0.8×10^{-8}	0.6
52	R = CH_2CH_2-C_6H_3-*m,p*-$(OCH_3)_2$	145–146	C	$C_{23}H_{27}NO_4$·HCl	C, H, N	A	1.1×10^{-8}	4.8×10^{-8}	4.5
									
53	R_1 = CH_3; R_2 = $CH(CH_3)_2$[c]						1.4×10^{-6}	2.5×10^{-5}	18
54	R_1 = CH_3; R_2 = CH_2CH_2-C_6H_3-*m,p*-$(OCH_3)_2$	127.5–128.5	A–D	$C_{21}H_{28}N_2O_5$	C, H, N	B	2.8×10^{-7}	1.1×10^{-4}	393
55	R_1 = $C_6H_5CH_2$; R_2 = $CH(CH_3)_2$[d]						1.9×10^{-7}	5.5×10^{-6}	29
56	R_1 = $C_6H_5CH_2$; R_2 = CH_2CH_2-C_6H_3-*m,p*-$(OCH_3)_2$	123–125	C	$C_{27}H_{32}N_2O_5$	C, H, N	A	3.3×10^{-7}	6.2×10^{-5}	188
									
57	R = $CH(CH_3)_2$[e]	58–59					8.6×10^{-9}	1.1×10^{-8}	1.3
58	R = CH_2CH_2-C_6H_3-*m,p*-$(OCH_3)_2$	66–67.5	B	$C_{22}H_{29}NO_4$	C, H, N	A	7.6×10^{-9}	9.2×10^{-8}	12

[a] A, MeCN; B, *i*-Pr_2O; C, EtOAc; D, Et_2O. [b] Propranolol, ref 9. [c] Practolol, ref 1. [d] See ref 1. [e] Alprenolol, ref 10.

tivity while the practolol analog **54** was by far the most cardioselective.

A limited number of compounds from this series were chosen for further investigation and 1-[(3,4-dimethoxyphenethyl)amino]-3-(*m*-tolyloxy)-2-propanol (**1**) was selected as the most promising candidate based on potency, selectivity of action, and a minimum of side effects. This compound is currently being studied in man.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus in capillary tubes and are uncorrected. Elemental analyses, indicated by symbols of the elements, were within ±0.3% of the theoretical values.

General Methods. Route A. The required 1-chloro-3-aryloxy-2-propanols were prepared by heating the corresponding phenol and an excess of epichlorohydrin plus a catalytic amount of piperidine hydrochloride at 95° for 6–10 hr.[11] The excess epichlorohydrin was removed by distillation under reduced pressure and the residue was dissolved in chloroform and the resulting solution stirred for 1 hr with an excess of concentrated hydrochloric acid. The chloroform layer was then separated, washed with water, and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the residue distilled *in vacuo*. The resulting product and 2 equiv of amine were heated on the steam bath for 18 hr. The residue was stirred with ethyl acetate and the mixture was filtered to remove the insoluble amine hydrochloride. The filtrate was washed with water, dried over anhydrous magnesium sulfate, and then concentrated under reduced pressure to yield the free base of the product. If a solid was obtained on standing, it was purified by recrystallization. If a solid was not obtained, the free base was dissolved in 2-propanol and the solution was treated with a slight excess of a solution of hydrogen chloride in 2-propanol to yield the monohydrochloride salt of the product which was purified by recrystallization. In a number of cases the oxalate salts were prepared because they were more readily obtained in a crystalline state.

Route B. The 1,2-epoxy-3-aryloxpropanes were prepared in the usual manner[1] and purified by distillation under reduced pressure. The epoxy compound was heated with an equivalent amount of the amine for 1–2 hr on the steam bath to yield the product which was then purified as above.

1-[(3,4-Dimethoxyphenethyl)amino]-3-(*m*-tolyloxy)-2-propanol Monohydrochloride (1) (Route A). A mixture of 40.1 g (0.2 mol) of 1-chloro-3-(*m*-tolyloxy)-2-propanol[4] and 72.4 g (0.4 mol) of 3,4-dimethoxyphenethylamine was heated on a steam bath for 18 hr and then cooled. The residue was stirred with 125 ml of ethyl acetate and the resulting mixture was filtered to remove 3,4-dimethoxyphenethylamine hydrochloride. The filtrate was washed with water, dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was dissolved in 2-propanol and a slight excess of a solution of hydrogen chloride in 2-propanol was added. The resulting precipitate was collected by filtration, washed with ether, and recrystallized from acetonitrile to yield 44.4 g of product (58%), mp 137–138°. *Anal.* ($C_{20}H_{28}ClNO_2$) C, H, N.

4′-[3-[(3,4-Dimethoxyphenyl)amino]-2-hydroxypropoxy]acetophenone Monooxalate (40) (Route B). A mixture of 9.6 g (0.05 mol) of 4′-(2,3-epoxypropoxy)acetophenone and 9.05 g (0.05 mol) of 3,4-dimethoxyphenethylamine was heated on the steam bath for 4 hr. The resulting gum was dissolved in methanol and a solution of 6.3 g (0.05 mol) of oxalic acid in methanol was added and the mixture was heated to the boil and then allowed to cool. The resulting solution was concentrated under reduced pressure until a solid began to precipitate (about 50 ml) and then cooled and 50 ml of 2-propanol was added. The resulting solid was removed by filtration and dried in a vacuum oven yielding 16.0 g of product, mp 158–163°. This product was purified by recrystallization from ethanol yielding 7.1 g (29%), mp 175–176°. *Anal.* ($C_{23}H_{29}NO_9$) C, H, N.

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Catechol O-Methyltransferase. 6. Affinity Labeling with N-Haloacetyl-3,5-dimethoxy-4-hydroxyphenylalkylamines

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Several N-acyl-3,5-dimethoxy-4-hydroxyphenylalkylamines have been synthesized and evaluated for their ability to inactivate catechol O-methyltransferase (COMT). N-Iodoacetyl-3,5-dimethoxy-4-hydroxyphenylethylamine was found to rapidly and irreversibly inactivate this enzyme. The corresponding N-bromoacetyl derivative also produced inactivation of COMT but at a slower rate than the N-iodoacetyl derivative. The N-acetyl and N-fumaryl derivatives were completely inactive. The inactivation of COMT by these reagents appears to proceed by a unimolecular reaction within a dissociable complex rather than by a nonspecific bimolecular reaction. The proximity of the amino acid residue being modified relative to the site which binds the aromatic portion of these inhibitors was determined using N-iodoacetylphenylaklylamines of varying chain length. The number of methylene carbons separating the aromatic ring and the iodoacetamide moiety in these inhibitors did not greatly influence the binding to COMT nor did it affect how rapidly the enzyme was inactivated. From these observations it was concluded that the amino acid moiety being modified by this class of affinity labeling reagents must be relatively close to or part of the site which binds the aromatic region of these inhibitors.

As part of our continuing studies of the enzyme catechol O-methyltransferase (COMT, E.C. 2.1.1.6), we have attempted to develop affinity labeling reagents which could be used to elucidate the relationship between the chemical structure and enzymatic function of this enzyme. Nikodejevic, *et al.*,[1] have previously observed that 3,5-dimethoxy-4-hydroxyphenylethylamine has affinity for the active site of COMT and is a reversible dead-end inhibitor of this enzyme. Therefore, one of our approaches to affinity labeling this enzyme has involved the preparation of various chemically reactive derivatives of 3,5-dimethoxy-4-hydroxyphenylalkylamines. These derivatives should have an affinity for COMT and, in addition, they should be capable of reacting to form a covalent bond with any nucleophiles present at or near the binding site, thereby producing modified amino acids readily amenable to isolation and identification. Since iodoacetamides are capable of alkylating histidine, cysteine, methionine, and lysine residues of proteins,[2,3] we initially prepared N-iodoacetyl-3,5-dimethoxy-4-hydroxyphenylethylamine (4) and found that it rapidly inactivates COMT. Our preliminary studies[4] suggested that this analog indeed serves as an affinity labeling reagent for COMT.

In an effort to further explore the mechanism and specificity of this interaction, we have synthesized a series of N-acyl-3,5-dimethoxy-4-hydroxyphenylalkylamines (Chart I) and studied their interaction with COMT. The objectives of this study were (1) to determine the reactivity of the amino acid residue being modified, and (2) to determine the proximity of this residue to the binding site. The present paper reports the results of this investigation.

Experimental Section

Melting points were obtained on a calibrated Thomas-Hoover Uni-melt and were corrected. Microanalyses were conducted on a F & M Model 185 C, H, N analyzer. The University of Kansas.

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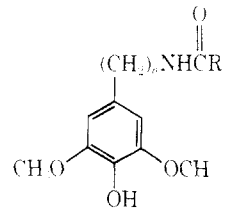
Chart I. Derivatives of 3,5-Dimethoxy-4-hydroxyphenylalkylamines Synthesized as Potential Affinity Labeling Reagents for COMT



Compd	n	R
1	2	$-CH_3$
2	2	$-CH{=}CHCO_2Et$ (trans)
3	2	$-CH_2Br$
4	2	$-CH_2I$
5	3	$-CH_2I$
6	1	$-CH_2I$

Lawrence, Kan., and the Microanalytical Laboratory, National Institutes of Health, Bethesda, Md. Unless otherwise stated, the ir, nmr, and uv data were consistent with the assigned structures. Ir data were recorded on a Beckman IR-33 spectrophotometer, nmr data on a Varian Associates Model T-60 spectrophotometer (TMS), and uv data on a Cary Model 14 spectrophotometer. Scintillation counting was done on a Beckman LS-150 scintillation counter. Tlc were run on Analtech silica gel GF (250 μ). Spots were detected by visual examination under uv light and/or ninhydrin for compounds containing amino moieties.

Materials. SAM-$^{14}CH_3$ (New England Nuclear, 55.0 mCi/mmol) was diluted to a concentration of 10 μCi/ml and stored at −20°F. SAM iodide (Sigma) was stored as a 0.01 *M* aqueous stock solution. Phosphate buffers were prepared as 0.5 *M* stock solutions.

COMT Isolation and Assay. COMT was purified from rat liver (male, Sprague–Dawley, 180–200 g) according to the methods previously described.[4,5] The enzyme was purified through the calcium phosphate gel step resulting in a preparation which contained 1.34 mg of protein per milliliter with a specific activity of 47.8 nmol of product/mg of protein/min with 3,4-dihydroxyben-