is performed at pH 9 and 25 °C and requires only minutes to accomplish, significant P–N bond cleavage does not occur during the assay.<sup>11</sup>

**Registry No.** 1, 74406-94-3; 2-DCHA, 97280-40-5; 3, 82180-41-4; 3-2K, 97280-41-6; 4, 97280-42-7; 5, 97280-42-7; 6, 97293-86-2; 6·2Na,

(22) P. Bohlen, S. Stein, W. Dairman, and S. Udenfriend, Arch. Biochem. Biophys., 155, 213 (1973). 97293-85-1; 7, 97280-44-9; 8, 14295-48-8; 9, 97280-45-0; 10, 97280-46-1; 11, 97280-47-2; 12, 97280-48-3; 13, 86053-89-6; 13-2Na, 82184-87-0; 14, 97280-49-4; 15, 97280-51-8; 15-2K, 97280-50-7; 16, 76166-63-7; L-alanyl-L-proline benzyl ester hydrochloride, 41591-35-9; diphenyl phosphorochloridate, 2524-64-3; L-alanyl-L-proline *tert*-butyl ester hydrochloride, 41591-35-9; bis(4-nitrophenyl) phosphorochloridate, 6546-97-0; (2-phenylethyl)-phosphonic acid, 4672-30-4; phenyl phosphorodichloridate,, 770-12-7; angiotensin converting enzyme, 9015-82-1.

## Selective Thromboxane Synthetase Inhibitors. 1. 1-[(Aryloxy)alkyl]-1H-imidazoles

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1-(2-Phenoxyethyl)-1*H*-imidazole was found to be an inhibitor of thromboxane  $(TxA_2)$  synthetase, but it also inhibited the adrenal cytochrome P-450 enzyme steroid 11 $\beta$ -hydroxylase. The preparation of a series of analogues is described, and activity against  $TxA_2$  synthetase, PGI<sub>2</sub> synthetase, cyclooxygenase, and steroid 11 $\beta$ -hydroxylase is discussed. Potency against  $TxA_2$  synthetase was increased by introduction of a carboxyl group at a suitable distance from the imidazole ring. A distance of 8.1–8.8 Å between N-1 of the imidazole and the carboxyl carbon was found to be optimal. Introduction of a carboxyl group also had the effect of reducing activity against steroid 11 $\beta$ -hydroxylase. The most potent and selective compound was found to be 4-[2-(1*H*-imidazol-1-yl)ethoxy]benzoic acid (14).

During the last decade, two new pathways of prostaglandin endoperoxide metabolism have been elucidated. First, Samuelsson et al.<sup>1,2</sup> reported that an enzyme in blood platelets, thromboxane synthetase, converts the endoperoxide  $PGH_2$  into thromboxane  $A_2$  (TxA<sub>2</sub>). TxA<sub>2</sub> was found to be a potent vasoconstrictor and platelet aggregating agent and was thought to be involved in normal hemostasis.<sup>3</sup> In 1976, Vane et al.<sup>4-9</sup> reported the discovery of prostacyclin  $(PGI_2)$ , which is produced from  $PGH_2$  by an enzyme located in the endothelial lining of blood vessels.  $PGI_2$  is a potent vasodilator and antiaggregatory agent. It was postulated that, under normal conditions, there is a balance between the opposing effects of  $TxA_2$  and  $PGI_2$ , but in certain pathological situations the proaggregatory and vasoconstrictor actions of TxA2 could predominate.4-9 Thus, a compound that inhibits selectively the formation of TxA<sub>2</sub> may be useful in the treatment or prophylaxis of cardiovascular diseases where vasospasm or thrombosis plays a role. A potential advantage of such a compound would be that accumulated PGH<sub>2</sub> produced by platelet cyclooxygenase may be utilized by vascular PGI<sub>2</sub> synthetase, thereby increasing PGI<sub>2</sub> levels.<sup>10,11</sup> By contrast,

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Scheme I



although agents that inhibit cyclooxygenase would also prevent  $TxA_2$  formation, there would normally be no possibility for increased  $PGI_2$  production since no  $PGH_2$  would be produced.

Imidazole has been reported to be a weak inhibitor of  $TxA_2$  synthetase.<sup>12,13</sup> We<sup>14</sup> and other workers<sup>15,16</sup> found that the introduction of a lipophilic 1-substituent increased inhibitory activity, but substitution elsewhere in the imidazole ring was detrimental. Imidazole inhibitors of cytochrome P-450 enzymes such as those present in liver and adrenals follow the same structure-activity trend,<sup>17-22</sup>

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Scheme II



which led to the suggestion that  $TxA_2$  synthetase may be a heme enzyme.<sup>14,15</sup> There is now good experimental evidence that  $TxA_2$  synthetase is in fact a cytochrome P-450 enzyme,<sup>23</sup> and imidazole inhibitors presumably act by coordination of the basic nitrogen at a vacant axial ligand site of a heme unit.

We have reported previously<sup>14</sup> that 3-(1*H*-imidazol-1ylmethyl)-2-(1-methylethyl)-1*H*-indole (UK-34,787) (31) was a potent inhibitor of TxA<sub>2</sub> synthetase without having a significant effect on PGI<sub>2</sub> synthetase or cyclooxygenase. However, further progression of this compound was precluded by the fact that it also inhibited other cytochrome P-450 enzymes. Thus, it was found to inhibit rat adrenal mitochondrial steroid 11 $\beta$ -hydroxylase, and liver enzyme inhibition was indicated by the fact that it prolonged pentobarbitone sleeping time in rats at doses required to cause complete inhibition of TxA<sub>2</sub> synthesis.<sup>24</sup>

1-(2-Phenoxyethyl)-1*H*-imidazole (1) was also found to be a more potent inhibitor of  $TxA_2$  synthetase than imidazole, but it strongly inhibited steroid  $11\beta$ -hydroxylase at  $10^{-5}$  M (Table I). We decided to investigate whether introduction of polar substituents into the phenyl ring would decrease affinity for liver and adrenal cytochrome P-450, while maintaining activity against  $TxA_2$  synthetase.

**Chemistry.** Several compounds were prepared by treatment of imidazole with NaH in DMF followed by an (aryloxy)alkyl chloride or bromide (Scheme I). This method was used for compounds 1–10, 25, and 26. In the reaction of (4-nitrophenoxy)ethyl chloride a 26% yield of 1-(4-nitrophenyl)-1*H*-imidazole (33) was also formed by nucleophilic displacement of the ether side chain. Novel (aryloxy)ethyl chlorides were prepared by treatment of a phenol with NaH in DMF followed by [(toluenesulfonyl)oxy]ethyl chloride except where  $R = CH_2CONH_2$ (34) in which case the reaction was carried out in 2-butanone in the presence of K<sub>2</sub>CO<sub>3</sub>. The latter conditions were also used to prepare 39 from ethyl 4-hydroxybenzoate and 1,3-dibromopropane.

Other compounds were prepared by modification of substituents. Hydrolysis of the esters 2, 4, and 8 under acidic conditions gave the acids 11, 12, and 17, respectively.

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Basic hydrolysis of the esters 6 and 9 gave the acids 16 and 15. The acid 13 was prepared by basic hydrolysis of the carbonitrile 10.

The amide 5 was used as a starting material for the preparation of several other analogues (Scheme II). Thus, acid hydrolysis produced the acid 14, which was then treated with  $SOCl_2$  and either methylamine or dimethylamine to give the amides 18 and 19. Dehydration with acetic anhydride gave the carbonitrile 21, which gave the tetrazole 22 on reaction with NaN<sub>3</sub> and NH<sub>4</sub>Cl. Treatment of 5 with acetyl- or benzoylimidazole gave low yields of the acylamides 23 and 24.

The amide 20 was prepared by ammonolysis of the ester 8. Reduction of the nitro analogue 25 with iron and HCl gave the amine 27, which produced the urea 28 on treatment with KCNO. Chlorosulfonation of 1 followed by treatment with  $NH_3$  gave the sulfonamide 29.

### **Results and Discussion**

1-(2-Phenoxyethyl)-1*H*-imidazole (1) is a more potent inhibitor of  $TxA_2$  synthetase than imidazole (30). However, a marked increase in potency occurs on introduction of a 4-carboxyl group into the phenyl ring (14). A carboxyl group at the 3-position (13) gives no improvement in potency over 1, and at the 2-position it is detrimental (11). Introduction of linking groups such as  $CH_2$  (16) or  $OCH_2$ (17) between the ring and the 4-carboxyl group or extension of the linkage between the rings (15) gives compounds with greater potency than 1 but slightly less than that of 14. The acetic acid 12 is less potent than 1, again showing the adverse effect of an acidic substituent at the 2-position. The amide 5 and tetrazole 22 show a substantial increase in potency over that of 1, but all other substituents examined either have no significant effect or are detrimental.

Thus, most of the substituents that have a beneficial effect on potency are acidic, and the results suggest that there is an optimum distance between the imidazole ring and the carboxyl group. In the case of compound 14 the distance seems to be optimal as a reduction in potency occurs on increasing and, more markedly, on reducing the distance. Other workers have also noted the importance of the distance between the imidazole and carboxyl group for high activity against  $TxA_2$  synthetase,<sup>16,25,26</sup> and an optimal distance of 8.5–9.0 Å between N-1 of the imidazole and the carboxyl carbon was suggested on the basis of examination of Dreiding models of a series of inhibitors.<sup>26</sup>

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								IC	so, M	
	R	u	mp, °C	yield, %	recrystn solvent <sup>a</sup>	formula <sup>b</sup>	TxA <sub>2</sub> synthetase	PGI <sub>2</sub> synthetase	cycloox ygenase	$11\beta$ -hydroxylas
H		21	86-87 c	62	CHCl <sub>3</sub> /PE		$6.6  imes 10^{-7}$	>10-4	$6.7 \times 10^{-5}$	72% at 10
ы с	-CU <sub>2</sub> C <sub>2</sub> H,	21 0	89-90.5	69	i-PrOH	$\mathbf{C}_{14}\mathbf{H}_{16}\mathbf{N}_2\mathbf{O}_3\cdot\mathbf{C}_4\mathbf{H}_4\mathbf{O}_4^{d}$	$4.4 \times 10^{-6}$	>10-4		
4 6	-CH CO C H	9 C	14/-140 190 5.190 5	30	2-Dutanone	$C_{12}H_{13}N_{3}O_{3}$	$2.2 \times 10^{-5}$			
14	-CONH.	1 6	148-149	40 71	FTUR/ELUAC	Cishie N2O3.HCI	$2.0 \times 10^{-8}$	4-0 F .		
4	CH.CO.C.H.	4 0	101 5-103	17 F			$0.6 \times 10^{-6}$	>10-4	>10-4	$1.0 \times 10^{-3}$
4	-CH, CONH.	1 01	147-149	71	2-hiitanone		2.9 × 10-6	- 01<		
4-	-och,co,č,H,	1 01	97-98	55	EtOAc	C H N O C H O e, f	LOA 10 <sup>-7</sup>			
4-	-co <sub>2</sub> Ċ <sub>2</sub> H <sup>5</sup> <sup>2</sup>	n	112-114	83	EtOH/Et.,O	C.H.N.O.HCI	$3.0 \times 10^{-6}$			
÷.	CN	2	126 - 128	56	<i>i</i> -PrOH/EtOAc	C, H, N, O, HCI	$3.5 \times 10^{-6}$			
ŝ	-CO <sub>2</sub> H	7	138-139.5	58	i-PrOH	C,H,N,O,HCI	>10 <sup>-5</sup>			
ςµ ι	-CH <sub>2</sub> CO <sub>2</sub> H	10	146-147	85	i-PrOH	Ci,H,N,O,HCI	>10-5	$>10^{-4}$		
	-C0,H	7	232-233	85	AcOH	C, H, N, O, HC	$5.3 \times 10^{-7}$	$>10^{-4}$		>10-4
4 -	ноо- 100 н	01 0	239-241	87	AcOH	C <sub>12</sub> H <sub>12</sub> N <sub>2</sub> O <sub>3</sub> ·HCl	$3.0  imes 10^{-9}$	>10-4	>10-4	>10-4
4 -			225-227	70.5		C <sub>13</sub> H <sub>14</sub> N <sub>2</sub> O <sub>3</sub>	$2.7 \times 10^{-8}$			
		N C	163-164	19	Et OH	C <sub>1</sub> ,H, N <sub>2</sub> O,	$3.2  imes 10^{-8}$			
<b>*</b> -	OUD, UU, H	N 0	162-164	44 7	CHJCN	C13H14N2O4·HCI	$9.5 \times 10^{-8}$	>10-4		>10-4
	CONTCH )	N C	132-133	74.5	EtOAc	C <sub>1</sub> <sup>3</sup> H <sub>1</sub> , N <sub>3</sub> O <sub>2</sub>	$8.6 \times 10^{-7}$	>10-4	>10-4	
4	OCH CONH	40	192-110	43	EtUAc	CIAH, NO CAHO	$1.1 \times 10^{-6}$	>10-4	>10-4	
4-1	CN CN	1 01	112-114	7 <del>1</del> 7 7	EtOH/Et O		0.0 X 10 5			>10-*
4-	CHN <sup>4</sup> <sup>h</sup>	2	196-197	34	EtOH/H,O	C.,H.,N.O	$1.5 \times 10^{-8}$	>10-4		
4	CONHCOCH	5	164 - 165	13	2-butanone	ĊĹĹĦĹĴŇĴŎ,	$6.3 \times 10^{-7}$	) 		
4 -	CUNHCUC, H,	~ ~	152 - 154	10	MeOH/EtOAc	C <sub>19</sub> H <sub>1</sub> , N <sub>3</sub> O <sub>3</sub>	$1.8 \times 10^{-7}$			
- - -		2 1	56-57	36.5'	EtOAc/PE	C.I.H.N.O.	$>5 \times 10^{-5}$			
4-1		2 0	0/T-00 01-03	202	EtOAc PLOA - /DP	C1H15NO2	$4.0 \times 10^{-6}$	>10-4		
4-	NHCONH	1 C.	199-201	03 89 5			2 01 X Q<			
4-	SO, NH,	101	147.5-148.5	19	LT20H	$C_1 H_1 A_1 A_2 C_2$	0.4 × 10 ·			
in.	nidazole			1 			$67 \times 10^{-4}$	$40 \times 10^{-3}j$	>10-2	
			N	VIK.	34.787)		$1.5 \times 10^{-8} \ k$	>10-4 j	>10-4	$2.0 \times 10^{-6}$
			CH30	Voo u find	ometheoin)		/ 10-4		1 6 \ 10-7	
				<sup>, со</sup> гн (ши сн <sub>3</sub>	Ошемасш)		. 01~		, 01 X C T	

Thromboxane Synthetase Inhibitors

Table I. [(Aryloxy)alkyl]-1*H*-imidazoles

Preferred conformations of 14 were calculated by using the CHEMLAB molecular modelling system,<sup>27</sup> and N-1 to carboxyl carbon distances were measured for conformers within 3 kcal of the minimum. In this case a range of 8.1–8.8 Å was found. There is presumably a specific binding site near the active site of the enzyme to accommodate the carboxyl group of the PGH<sub>2</sub> substrate, and it seems reasonable to suppose that the carboxyl group of 14 interacts with this site.

None of the compounds examined had any significant effect on PGI<sub>2</sub> synthetase at  $10^{-4}$  M. Compound 14 and the amide derivatives 5, 18, and 19 also had no effect on cyclooxygenase at  $10^{-4}$  M, although the unsubstituted compound 1 showed some activity at  $10^{-5}$  M. Activity against cyclooxygenase has been reported previously for imidazoles with N-alkyl groups containing greater than six carbons.<sup>16</sup> Compound 1 also caused marked inhibition of rat adrenal mitochondrial steroid  $11\beta$ -hydroxylase at  $10^{-5}$ M, but introduction of a carboxylic acid substituent as in 13, 14, and 17 virtually abolished this activity. The amide 5 retained activity against  $11\beta$ -hydroxylase, but activity was reduced in 20.

Because of its potency and selectivity, compound 14 (UK-37,248, dazoxiben) was selected for further evaluation. Fifteen minutes after intravenous administration of 0.3 mg/kg to anaesthetized rabbits, thromboxane production was inhibited by 75%.<sup>28</sup> The same dose of the acids 15 and 17 and the tetrazole 22 produced less than 30% inhibition after the same time. Oral administration of 3 mg/kg of 14 to conscious dogs produced a high level of inhibition of thromboxane production for up to 6 h,<sup>28</sup> and a similar picture was seen after oral administration of 100-or 200-mg doses to human volunteers.<sup>29</sup> Unlike 31, there is no evidence for inhibition of other cytochrome P-450 enzymes in vivo.

In summary, we have found that in a series of N-[(aryloxy)alkyl]-1H-imidazoles inhibitory activity against thromboxane synthetase can be increased by introduction of a carboxylic acid group at a suitable distance from the imidazole ring. In all the cases examined, the carboxyl group has the additional advantage of reducing activity against other cytochrome P-450 enzymes such as adrenal steroid 11 $\beta$ -hydroxylase. None of the substituted [(aryloxy)alkyl]imidazoles examined had significant activity against either PGI<sub>2</sub> synthetase or cyclooxygenase.

#### **Experimental Section**

**Enzyme Assays.** (a) Cyclooxygenase. Cyclooxygenase was prepared from ram seminal vesicle microsomes (RSVM) by the method of Takeguchi et al.<sup>30</sup> Test compounds or solvents were preincubated with RSVM (50–100  $\mu$ g) protein and 50 mM sodium phosphate buffer (pH 7.8) for 5 min prior to addition of 0.1 mM arachidonic acid (AA) ammonium salt (total volume 0.2 mL). After incubation for a further 1 min at 22 °C, the PGH<sub>2</sub> formed was determined by bioassay using a rabbit aorta spiral to measure contraction.<sup>14</sup>

(b) Thromboxane Synthetase. TxA<sub>2</sub> synthetase was prepared from human platelets by the method of Needleman et al.<sup>3</sup> Pretreatment of the platelet microsomes with indomethacin (30  $\mu$ M) for 30 min at 0 °C was carried out to inactivate the cyclooxygenase also present. The substrate PGH<sub>2</sub> was generated immediately

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prior to each assay by incubating 0.1 mM AA with RSVM for 1 min at 22 °C and rapidly cooling the incubate to 0 °C. Compounds or solvents were preincubated for 2 min in ice with the indomethacin-pretreated microsomes (100–200  $\mu$ g of protein) and 20 mM sodium phosphate buffer (pH 7.8). The generated PGH<sub>2</sub> (0.05 mL) was added (total volume 0.105 mL), and incubation was continued for 1 min in ice before termination by addition of 0.5 mL of EtOH.

The incubation mixtures were centrifuged for 2 min, aliquots of the supernatant were diluted (1:20) with Isogel Tris buffer (10 mM Tris/140 mM NaCl/0.1% w/v gelatin, pH 7.4), and 0.1-mL aliquots of the diluted samples were assayed in triplicate for  $TxB_2$  with a specific RIA.<sup>27</sup>

Incubations were carried out at different concns of test compounds to enable construction of dose-response curves.

(c)  $\mathbf{PGI}_2$  Synthetase.  $\mathbf{PGI}_2$  synthetase was prepared from porcine aorta by the method of Moncada et al.<sup>4</sup> The  $\mathbf{PGH}_2$ synthetase present was deactivated with indomethacin as described in (b). Incubations were carried out at 22 °C and pH 7.5, and incubation with  $\mathbf{PGH}_2$  was carried out for 30 s before termination with EtOH. Otherwise the procedure was identical with that described in (b). After centrifugation and dilution of the supernatant (1:50), 0.1-mL aliquots were assayed in triplicate for 6-keto  $\mathbf{PGF}_{1\alpha}$  with a specific RIA.<sup>27</sup>

(d) Steroid 11 $\beta$ -Hydroxylase. The adrenals of three male rats (Charles River strain) were homogenized in 33 vol of medium (10 mM HEPES/250 mM sucrose/2 mM EGTA, pH 7.4), and the homogenate was centrifuged at 900g for 10 min. The pellet was resuspended in the same volume of medium and centrifuged as before at 2-4 °C. The combined supernatants were centrifuged at 10000g for 15 min at 2-4 °C. The mitochondrial pellet was resuspended in the original volume of homogenizing medium and recentrifuged at 10000g for 15 min. The washed mitochondria were suspended in 20 vol of homogenizing medium and used within 30 min.

The incubation mixture consisted of 10 mM HEPES, pH 7.4, 250 mM sucrose, 1 mM MgCl<sub>2</sub>, 5 mM KH<sub>2</sub>PO<sub>4</sub>, 4 mM sodium malate, 50  $\mu$ M 4-[<sup>14</sup>C]-11-deoxycorticosterone (sp act. 0.1  $\mu$ Ci/ $\mu$ M), and test drug in a total volume of 1 mL. After a 5-min preincubation at 37 °C in a shaking water bath, mitochondria suspension (100  $\mu$ L) was added and the mixture was incubated for 10 min after which 5 mL of CH<sub>2</sub>Cl<sub>2</sub> was added. Unlabeled 11deoxycorticosterone and corticosterone were added (20  $\mu$ g), and the contents were transferred to capped tubes with  $0.5 \text{ mL of } H_0O$ and 1 mL of CH<sub>2</sub>Cl<sub>2</sub>. The tubes were rotated for 20 min and then centrifuged at 1000g for 5 min at room temperature. The lower  $CH_2Cl_2$  layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and evaporated under N<sub>2</sub>. The residue was redissolved in 75  $\mu$ L of CH<sub>2</sub>Cl<sub>2</sub> and applied to silica TLC plates that were developed in  $CHCl_3/MeOH$  (97:3 v/v). The areas corresponding to substrate and product were scraped into scintillation vials, and after addition of 1 mL of MeOH and 10 mL of toluene Omnifluor scintillant, the radioactivity in the samples was counted.

**Chemistry.** All melting points are uncorrected and were obtained on an Electrothermal capillary melting point apparatus. The structures of all compounds were confirmed by their IR and 60-MHz NMR spectra. The IR spectra were recorded on a Perkin-Elmer 197 or 237 spectrophotometer, and the <sup>1</sup>H NMR spectra were obtained on a Perkin-Elmer R12B spectrometer using  $Me_4Si$  as internal standard.

[4-(2-Chloroethoxy)phenyl]acetamide (34). A mixture of (4-hydroxyphenyl)acetamide (98.8 g, 0.65 mol), [(toluene-sulfonyl)oxy]ethyl chloride (150.5 g, 0.65 mol), anhydrous  $K_2CO_3$  (98.0 g, 0.7 mol), and 2-butanone (2 L) was heated under reflux with stirring for 16 h and then cooled and filtered. The filtrate was evaporated to give an oily solid that was stirred with a small volume of ether, and the mixture was filtered. The isoluble residue from the reaction mixture was washed with water, combined with the other solid, and crystallized from ethanol to give 34: yield 70.0 g (55%); mp 173–175 °C. Anal. ( $C_{10}H_{12}CINO_2$ ) C, H, N.

**3-(2-Chloroethoxy)benzonitrile (35).** Sodium hydride (2.40 g, 0.05 mol of 50% dispersion in mineral oil) was added portionwise to a stirred solution of 3-cyanophenol (5.95 g, 0.05 mol) in dry DMF (50 mL), and the mixture was warmed to 100 °C for 10 min and then cooled. [(Toluenesulfonyl)oxy]ethyl chloride (11.75 g,

Table II. Physical Data for (2-Chloroethoxy)aryl Intermediates

no.	R	mp or bp, °C	% yield	mol formulaª
36	2-CO <sub>2</sub> C <sub>2</sub> H <sub>5</sub>	167-71/12mm	51	C <sub>11</sub> H <sub>13</sub> ClO <sub>3</sub>
37	$4-CH_2CO_2C_2H_5$	$70-72^{b}$	66	$C_{12}H_{15}ClO_3$
38	$4-OCH_2CO_2C_2H_5$	138-40/0.1mm	37	$C_{12}H_{15}ClO_4$
	11 1	1 10 0	1 77 6 75	00.00.00

<sup>a</sup>All compounds were analyzed for C and H. <sup>b</sup>From 60-80 °C petroleum ether.

0.05 mol) was added, and the mixture was heated on a steam bath for 3 h and then cooled and poured into water (ca. 250 mL). The mixture was extracted several times with ether, and the combined extracts were washed well with water and dried (Na<sub>2</sub>SO<sub>4</sub>). Filtration and evaporation of the solvent gave an oil that was chromatographed on silica gel. Elution with hexane gave mineral oil, and further elution with ether/hexane (1:1) gave pure product. The product-containing fractions were combined and evaporated, and the residue was distilled to give **35**: yield 4.14 g (46%); bp 141–143 °C (0.6 mm); mp 42–44 °C. Anal. (C<sub>9</sub>H<sub>8</sub>ClNO), C, H, N.

Other novel (2-chloroethoxy)aryl intermediates were prepared similarly, and physical data are summarized in Table II.

Ethyl 4-(3-Bromopropoxy)benzoate (39). A mixture of ethyl 4-hydroxybenzoate (8.80 g, 0.05 mol), 1,3-dibromopropane (15.14 g, 0.075 mol), anhydrous  $K_2CO_3$  (15 g), and 2-butanone (150 mL) was heated under reflux with stirring for 72 h. The mixture was cooled and filtered, and the residue was washed well with 2-butanone. The combined filtrate and washings were evaporated to give an oil that was dissolved in ethyl acetate. The solution was washed with 1 N NaOH and water and dried (Na<sub>2</sub>SO<sub>4</sub>). Filtration and evaporation gave an oil that was distilled to give 39: yield 5.69 g (40%); bp 128–132 °C (0.1 mm). Anal. ( $C_{12}H_{15}BrO_3$ ) C, H.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzamide (5). Sodium hydride (14.4 g, 0.3 mol of 50% dispersion of mineral oil) was added portionwise to a stirred solution of imidazole (20.4 g, 0.3 mol) in dry DMF (100 mL), and the mixture was warmed to 100 °C for 10 min. A solution of 4-(2-chloroethoxy)benzamide (60.0 g, 0.3 mol) in the minimum volume of dry DMF was added, and the mixture was heated on a steam bath for 5.5 h. The solvent was evaporated, and the residue was poured into water. The mixture was extracted several times with chloroform, and the combined extracts were washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was triturated with a little ether to remove mineral oil, and the solid was crystallized from ethyl acetate containing a trace of methanol to give 5: yield 35.2 g (51%); mp 148-149 °C. Anal. ( $C_{12}H_{13}N_3O_2$ ), C, H, N.

This method was used for the preparation of compounds 1-4, 6-10, 25, and 26. In most cases the crude product was purified by chromatography (silica gel/CHCl<sub>3</sub>).

4-[2-(1*H*-Imidazol-1-yl)ethoxy]nitrobenzene (25). Treatment of imidazole (6.80 g, 0.1 mol) with NaH (4.8 g of 50% dispersion, 0.1 mol) followed by 4-(2-chloroethoxy)nitrobenzene (20.15 g, 0.1 mol) in dry DMF (100 mL) by the above method gave a crude product that was chromatographed on silica gel. Elution with EtOAc first gave mineral oil followed by a solid that was crystallized from MeOH to give 33: yield 4.90 g (26%); mp 205-206 °C (lit.<sup>31</sup> mp 204-205 °C). Anal. (C<sub>9</sub>H<sub>7</sub>N<sub>3</sub>O<sub>2</sub>), C, H, N.

Further elution with EtOAc gave a solid that was crystallized from EtOAc/PE to give 25: yield 8.50 g (36.5%); mp 56–57 °C. Anal.  $(C_{11}H_{11}N_3O_3)$ , C, H, N.

2-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid Hydrochloride (11). A solution of 2 (5.10 g, 0.02 mol) in 5 N HCl (30 mL) was heated on a steam bath for 8 h and then evaporated. The residue was crystallized from 2-propanol to give 11·HCl: yield 3.08 g (58%); mp 138-139 °C. Anal. ( $C_{12}N_{12}N_2O_3$ ·HCl), C, H, N.

This method was used to prepare compounds 12 and 17 (Table I) from the esters 4 and 8, respectively.

4-[3-(1*H*-Imidazol-1-yl)propoxy]benzoic Acid (15). A mixture of 9 (free base) (0.49 g, 0.0018 mol), KOH (0.20 g), ethanol (1 mL), and water (5 mL) was heated under reflux for 2 h and then evaporated to small bulk. The solution was filtered, and the filtrate was acidified with acetic acid. The precipitate was filtered off, washed with water, and dried to give 15: yield 0.31 g (70.5%); mp 225-227 °C. Anal. ( $C_{13}H_{14}N_2O_3$ ) C, H, N.

This method was also used for the preparation of 16 from the ester 6.

3-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid (13). A mixture of compound 10·HCl salt (2.0 g, 0.008 mol) and 5 N NaOH (15 mL) was heated on a steam bath for 6 h. The solution was made acidic with dilute HCl and evaporated. The residue was extracted with hot acetic acid, and the solution was filtered and evaporated. The residue was crystallized from acetic acid to give 13·HCl: yield 1.82 g (85%); mp 232-233 °C. Anal. ( $C_{12}H_{12}N_2O_3$ ·HCl) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzoic Acid Hydrochloride (14). A solution of 5 (4.50 g, 0.019 mol) in 5 N HCl (20 mL) was heated on a steam bath for 2 h and then allowed to cool. The solid was filtered off and crystallized from acetic acid to give 14·HCl: yield 4.55 g (87%); mp 239-241 °C. Anal.  $C_{12}H_{12}N_{2}$ -O<sub>3</sub>·HCl C, H, N.

**N-Methyl-4-[2-(1***H***-imidazol-1-yl)ethoxy]benzamide (18).** Thionyl chloride (1.0 mL) was added dropwise to a stirred mixture of 14-HCl (1.0 g, 0.0037 mol) and dry DMF (5 mL) at room temperature. The solution was stirred for 5 h and then added dropwise to a stirred solution of 40% aqueous methylamine (20 mL). The mixture was evaporated, the residue was dissolved in water, and the solution was made just basic with NaHCO<sub>3</sub> and then reevaporated. The residue was extracted with hot ethyl acetate, and the extract was filtered and evaporated. The residue was crystallized from ethyl acetate to give 18: yield 0.68 g (74.5%); mp 132–133 °C. Anal. (C<sub>13</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

Compound 19 (Table I) was prepared similarly.

**4-[2-(1H-Imidazol-1-yl)ethoxy]benzonitrile** (21). A solution of **5** (1.0 g, 0.0043 mol) in acetic anhydride (5 mL) was heated under reflux for 3 h and then evaporated. The residue was dissolved in ethyl acetate, and the solution was washed with NaHCO<sub>3</sub> solution and then dried (Na<sub>2</sub>SO<sub>4</sub>). Evaporation of the solvent gave an oil that was chromatographed on silica gel. Elution with CHCl<sub>3</sub> first gave some impurity followed by pure 21 as an oil: yield 0.45 g (49%); IR 2220 cm<sup>-1</sup> (C=N). A portion of the oil was dissolved in a small volume of EtOH, and an excess of an ether solution of fumaric acid was added. The solid was filtered off and crystallized from EtOH/Et<sub>2</sub>O to give 21.<sup>1</sup>/<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>, mp 112–114 °C. Anal. (C<sub>12</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>.<sup>1</sup>/<sub>2</sub>C<sub>4</sub>H<sub>4</sub>O<sub>4</sub>) C, H, N.

**5-[4-[2-(1***H***-Imidazol-1-yl)ethoxy]phenyl]-1***H***-tetrazole (22). A mixture of 21 (2.1 g, 0.0092 mol), NaN<sub>3</sub> (3.25 g, 0.05 mol), and NH<sub>4</sub>Cl (2.67 g, 0.02 mol) in dry DMF (25 mL) was heated on a steam bath for 22 h and then evaporated. The residue was extracted with CHCl<sub>3</sub>, and the extract was evaporated. The residue was crystallized from aqueous EtOH to give 22: yield 0.81 g (34%); mp 196–197 °C. Anal. (C\_{12}H\_{12}N\_6O) C, H, N.** 

**N-Acetyl-4-[2-(1H-imidazol-1-yl)ethoxy]benzamide (23).** Acetic acid (1.80 g, 0.03 mol) was added to a solution of 1,1'carbonylbis(1H-imidazole) (4.86 g, 0.03 mol) in dry DMF (10 mL), and the solution was stirred for 5 min. 5 (4.60 g, 0.02 mol) was added, and the solution was heated under reflux for 3 h and then evaporated. The residue was treated with NaHCO<sub>3</sub> solution, and the mixture was extracted several times with ethyl acetate. The combined extracts were washed with water, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated to give an oil that was chromatographed on silica gel. Elution with CHCl<sub>3</sub> gave some impurity, and further elution with CHCl<sub>3</sub>/MeOH (50:1) gave a solid that was crystallized from 2butanone to give 23: yield 0.90 g (13%); mp 164-165 °C. Anal. (C<sub>14</sub>H<sub>15</sub>N<sub>3</sub>O<sub>3</sub>) C, H, N.

This method was also used for the preparation of 24 using benzoic acid in place of acetic acid.

[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenoxy]acetamide (20). A mixture of 8 (1.0 g, 0.0034 mol), ethanol (10 mL), and concentrated ammonia solution (20 mL) was allowed to stand at room temperature for 20 h, and the resulting solution was evaporated. The residue was crystallized from 2-butanone to give 20: yield 0.38 g (42%); mp 123-124 °C. Anal. ( $C_{13}H_{15}N_3O_3$ ) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzeneamine (27). Iron powder (6.0 g) was added portionwise to a warm solution of 25

<sup>(31)</sup> Forsyth, R.; Pyman, F. L. J. Chem. Soc. 1930, 397.

<sup>(32)</sup> U.S. Patent 3 534 061, 1970; Chem. Abstr. 1971, 74, P31755g.

(3.70 g, 0.016 mol) in 5 N HCl (60 mL), and the mixture was warmed on a steam bath for 20 min. It was then cooled and made basic with dilute NaOH solution, and the mixture was shaken with CHCl<sub>3</sub> and filtered. The CHCl<sub>3</sub> layer of the filtrate was separated, dried (Na<sub>2</sub>SO<sub>4</sub>), and evaporated. The residue was crystallized from ethyl acetate/petroleum ether (bp 60–80 °C) to give **27**: yield 2.25 g (69%); mp 91–93 °C. Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O) C, H, N.

[4-[2-(1*H*-Imidazol-1-yl)ethoxy]phenyl]urea (28). A solution of KCNO (0.30 g, 0.0037 mol) in water (1 mL) was added to a solution of 27 (0.60 g, 0.0029 mol) in 1 N HCl (3.0 mL). The mixture was allowed to stand at room temperature for 15 min, and the precipitate was filtered off, washed with water, and crystallized from water to give 28: yield 0.60 g (82.5%); mp 199-201 °C. Anal. ( $C_{12}H_{14}N_4O_2$ ) C, H, N.

4-[2-(1*H*-Imidazol-1-yl)ethoxy]benzenesulfonamide (29). PCl<sub>5</sub> (2.08 g, 0.01 mol) was added cautiously to chlorosulfonic acid (2.91 g, 0.025 mol), and the mixture was cooled to 0 °C. 1 (1.88 g, 0.01 mol) was added portionwise, and then the mixture was heated on a steam bath for 10 min and cooled. It was poured onto a mixture of crushed ice and excess concentrated NH<sub>3</sub> solution, and the gummy solid was filtered off and sucked dry. The solid was chromatographed on silica gel. Elution with CHCl<sub>3</sub>/MeOH (4:1) gave a gum that crystallized on trituration with a little EtOH. The solid was crystallized from EtOH to give 29: yield 0.50 g (19%); mp 147.5–148.5 °C; 60-MHz <sup>1</sup>H NMR (Me<sub>2</sub>SO-d<sub>6</sub>) 4.37 (m, 2 H, CH<sub>2</sub>), 4.45 (m, 2 H, CH<sub>2</sub>), 6.95 (s, 1 H, imidazole H<sup>5</sup>), 7.14 (d, J = 8.9 Hz, 2 H, benzene CH), 7.28 (s, 2 H, SO<sub>2</sub>NH<sub>2</sub>), 7.30 (s, 1 H, imidazole H<sup>4</sup>), 7.74 (s, 1 H, imidazole H<sup>2</sup>), 7.79 (d,

#### J = 8.9 Hz, 2 H, benzene CH). Anal. (C<sub>11</sub>H<sub>13</sub>N<sub>3</sub>O<sub>3</sub>S) C, H, N.

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Registry No. 1, 30170-83-3; 2 maleate, 75912-61-7; 3, 75912-58-2; 4·HCl, 75912-62-8; 5, 75912-57-1; 6 fumarate, 75912-66-2; 7, 75912-59-3; 8 fumarate, 75912-68-4; 9, 74226-04-3; 9-HCl, 97315-33-8; 10·HCl, 75922-17-7; 11·HCl, 75912-72-0; 12·HCl, 75912-73-1; 13·HCl, 75912-75-3; 14·HCl, 74226-22-5; 15, 75912-95-7; 16, 97315-34-9; 17.HCl, 75922-18-8; 18, 75912-78-6; 19 fumarate, 75912-80-0; 20, 75912-99-1; 21, 75912-76-4; 21  $^{1}/_{2}$  fumarate, 97315-35-0; 22, 75912-77-5; 23, 75912-90-2; 24, 75912-91-3; 25, 75912-69-5; 26, 75912-70-8; 27, 75912-83-3; 28, 75912-85-5; 29, 75912-82-2; 30, 288-32-4; 33, 2301-25-9; 34, 97315-36-1; 35, 97315-37-2; 36, 97315-38-3; 37, 97315-39-4; 38, 97315-40-7; 39, 4-HOC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CONH<sub>2</sub>, 74226-00-9; 17194-82-0; CH<sub>3</sub>C<sub>6</sub>H<sub>4</sub>SO<sub>2</sub>O(CH<sub>2</sub>)<sub>2</sub>Cl, 80-41-1; 3-HOC<sub>6</sub>H<sub>4</sub>CN, 873-62-1; 4- $HOC_6H_4CO_2C_2H_5$ , 120-47-8;  $Br(CH_2)_3Br$ , 109-64-8; 4-Cl-(CH<sub>2</sub>)<sub>2</sub> $OC_6H_4CONH_2$ , 36616-30-5; 4-Cl(CH<sub>2</sub>)<sub>2</sub> $OC_6H_4NO_2$ , 3383-72-0; CH<sub>3</sub>NH<sub>2</sub>, 74-89-5; (CH<sub>3</sub>)<sub>2</sub>NH, 124-40-3; CH<sub>3</sub>CO<sub>2</sub>H, 64-19-7; C<sub>6</sub>H<sub>5</sub>CO<sub>2</sub>H, 65-85-0; NH<sub>3</sub>, 7664-41-7; ClSO<sub>3</sub>H, 7790-94-5; 2-HOC<sub>6</sub>H<sub>4</sub>CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, 118-61-6; 4-HOC<sub>6</sub>H<sub>4</sub>CH<sub>2</sub>CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, 17138-28-2; 4-HOC<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>CO<sub>2</sub>C<sub>2</sub>H<sub>5</sub>, 20872-28-0; TxA<sub>2</sub> synthetase, 60832-04-4; PGI<sub>2</sub> synthetase, 65802-86-0; cyclooxygenase, 39391-18-9; steroid  $11\beta$ -hydroxylase, 9029-66-7.

# Studies on the 1-Methyl-4-phenyl-2,3-dihydropyridinium Species 2,3-MPDP<sup>+</sup>, the Monoamine Oxidase Catalyzed Oxidation Product of the Nigrostriatal Toxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

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The nigrostriatal toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is biotransformed by brain monoamine oxidase (MAO) to an unstable dihydropyridinium intermediate that reacts with cyanide ion to form an  $\alpha$ -cyanotetrahydropyridine adduct and, in the absence of cyanide ion, undergoes disproportionation to the 1-methyl-4-phenylpyridinium species MPP<sup>+</sup> and MPTP. Comparison of the HPLC retention times, diode array UV, and chemical ion mass spectral characteristics of these products with those of synthetic standards led us to propose the 1-methyl-4-phenyl-2,3-dihydropyridinium species 2,3-MPDP<sup>+</sup> and 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine as tentative structure assignments for the dihydropyridinium metabolite and the cyano adduct, respectively. Results presented in this paper confirm the first assignment and establish that, although the proposed 6-cyano adduct is initially formed, the product that was isolated from the mitochondrial incubation mixtures of MPTP and sodium cyanide actually is the isomeric 2-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. On the basis of the selective incorporation of deuterium into these products, we provide rational mechanistic interpretations of the disproportionation of MPTP leads to the formation of a variety of reactive molecules that are potentially cytotoxic to nigrostriatal cells.

The cyclic allylamine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, 1) causes the selective degeneration of the dopaminergic nigrostriatal system in a number of species including mice,<sup>1-3</sup> monkeys,<sup>4,5</sup> and humans<sup>6,7</sup> and

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leads to a Parkinsonian-like syndrome in monkeys and humans. Both in vivo<sup>8</sup> and in vitro<sup>9</sup> studies have led to the characterization of the 1-methyl-4-phenylpyridinium species MPP<sup>+</sup> ( $3^+$ ) as a principal brain metabolite of the

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