Inhibition of Adrenal Phenethanolamine N-Methyltransferase by Substituted Benzimidazoles

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A series of substituted 2-aminobenzimidazoles was synthesized and tested for their inhibitory effect *in vitro* on bovine adrenal phenethanolamine *N*-methyltransferase, the enzyme catalyzing the final step in the biosynthesis of epinephrine. The most potent inhibitors found were those with a free amino group in the 2 position and with Cl, NO_2, or CF_3 substituents in the 5 position or both the 5 and 6 positions. These compounds produced 18–55% inhibition of enzyme activity at 0.28 μg/ml. Several compounds were also found to inhibit the biosynthesis of adrenal epinephrine when administered ip to mice in 3 doses of 25–100 mg/kg per dose.

The final step in the biosynthesis of epinephrine is the transfer of a Me group from *S*-adenosylmethionine to the primary amine N of norepinephrine.[1] The reaction is catalyzed by phenethanolamine *N*-methyltransferase (PNMT), an enzyme present in relatively high content in the adrenal medulla of several mammalian species[2] with some activity detectable in heart[2] and brain.[3,4] The adrenal enzyme N-methylates a variety of naturally occurring and synthetic phenylethanolamine derivatives but not phenethylamines or indoleethylamines.[2] Its activity is markedly depressed following hypophysectomy. Enzyme activity can be restored to normal after administration of ACTH or dexamethasone suggesting the biosynthesis of epinephrine in the adrenal medulla is regulated by the pituitary–adrenocortical system.[5] However in the intact animal PNMT appears to be maximal since further stimulation of the adrenocortical system does not increase enzyme activity.[6]

PNMT is inhibited by a variety of amines including phenethylamines and phenylethanolamines,[7] *trans*-2-phenylcyclopropylamine, and other related compounds.[8] Inhibition of the enzyme by epinephrine could serve as a mechanism for regulating its activity and may be a means by which an increased rate of epinephrine production occurs when adrenal levels are depleted.[6] Relatively high concentrations of norepinephrine also inhibit PNMT but the physiological significance of this effect remains to be established.[6]

This report describes the inhibition *in vitro* of bovine adrenal medulla PNMT by substituted benzimidazoles. Many of these compounds produced more than 50% inhibition of enzyme activity at 28 μg/ml and some structures inhibited enzyme activity 30% when tested at 0.28 μg/ml. In follow-up studies *in vivo* several benzimidazoles were also found to selectively lower the epinephrine content of adrenal glands of mice without significantly altering their norepinephrine content.

Chemistry.—Most of the 2-aminobenzimidazoles were prepared by treating BrCN with the appropriate *o*-phenylenediamine. The preparation of a bis(trifluoromethyl)-*o*-phenylenediamine, which had previously been prepared by an extended sequence,[9] was greatly facilitated by the use of SF_4[10] to introduce CF_3. The 2 substituents of **21**, **22**, **24**, and **28** were introduced by treating a 2-chlorobenzimidazole with appropriate amines. In the case of **21** a 1-Me substituent was introduced prior to the introduction of the 2-amino group because of the possibility of obtaining an isomeric mixture if a 2-aminobenzimidazole were alkylated. It should be noted, however, that in the preparation of **26** direct alkylation gave only substitution at the 1 position.

Biological Methodology.—Steer adrenal medulla PNMT was prepared and assayed by minor modifications of the method of Axelrod.[2] The dialyzed 35–55% $(NH_4)_2SO_4$ fraction was centrifuged to remove insoluble material and the supernatant used as the enzyme source. Each incubation mixture contained 100 μmoles of potassium phosphate buffer, pH 7.9, 0.17 μmole of DL-normetanephrine or 0.018 μmole of DL-norepinephrine, 2–5 μmoles (0.1–0.2 μCi) of *S*-adenosylmethionine-*methyl*-^{14}C (New England Nuclear Corp.), 20–100 μg of enzyme protein, and 5 μl of inhibitor soln (in DMSO) in a final vol of 305 μl. Incubations were carried out at 37° for 30–60 min and the extraction of metanephrine-^{14}C or epinephrine was as described previously.[2] Aliquots of the organic phases were transferred to 20 ml of a PhMe–EtOH phosphor for determination of radioactivity in a Packard liquid scintillation spectrometer.

Compounds which were found to inhibit PNMT *in vitro* were tested for their ability to alter the adrenal content of epinephrine and norepinephrine in mice and rats. The drugs were suspended in water containing 0.5% Tween 80 and administered ip. Female mice of

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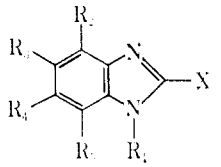
TABLE I
BENZIMIDAZOLE DERIVATIVES



No.	R_1	R_2	R_3	R_4	R_5	X	Mp, °C and ref
1	H	H	H	H	H	H	*a*
2	H	H	CH_3	CH_3	H	H	*a*
3	H	H	Cl	H	H	H	*b*
4	H	H	Cl	Cl	H	H	*a*
5	H	H	H	H	H	NH_2	*a*
6	H	H	CH_3	H	H	NH_2	*c*
7	H	H	OCH_3	H	H	NH_2	*d*
8	H	H	CH_3SO_2	H	H	NH_2	236–240
9	H	H	CH_3SO_2NH	H	H	NH_2	196–199
10	H	H	*n*-C_4H_9	H	H	NH_2	189–190
11	H	H	F	H	H	NH_2	172–176
12	H	H	Cl	H	H	NH_2	*e*
13	H	H	NO_2	H	H	NH_2	*f*
14	H	NO_2	H	H	H	NH_2	275–279
15	H	H	CF_3	H	H	NH_2	159–160.5[g]
16	H	H	CH_3	CH_3	H	NH_2	*a*
17	H	H	Cl	Cl	H	NH_2	*h*
18	H	Cl	H	Cl	H	NH_2	221–224
19	H	H	NO_2	NO_2	H	NH_2	~310 dec
20	H	H	CF_3	CF_3	H	NH_2	179–181.5
21	CH_3	H	Cl	Cl	H	NH_2	246–247
22	H	H	Cl	Cl	H	$N(CH_3)_2$	253–257
23	CH_3	H	Cl	Cl	H	$N(CH_3)_2$	72–74
24	H	H	CH_3	CH_3	H	$N(CH_3)_2$	251–254
25	H	H	CF_3	H	H	$NHC(O)CH_3$	270–275
26	CH_2Ph	H	CH_3	CH_3	H	NH_2	261.5–263[i]
27	H	H	Cl	Cl	H	CH_2NH_2	202–209
28	H	H	Cl	Cl	H	$NHNH_2$	190–200
29	H	H	OH	H	H	$NHC(O)CH_3$	279–283
30	H	H	CF_3	H	H	CF_3	198–199

[a] Commercially available. [b] A. F. Crowther, F. H. S. Curd, D. G. Davey, and G. J. Stacey, *J. Chem. Soc.*, 1260 (1949). [c] L. Joseph *J. Med. Chem.*, **6**, 601 (1963). [d] L. Basaglia and B. Mariani, *Ann. Chim. (Rome)*, **53**, 755 (1963) [*Chem. Abstr.*, **59**, 15411*d* (1963)]. [e] N. J. Leonard, D. Y. Custin, and K. M. Beck, *J. Amer. Chem. Soc.*, **69**, 2459 (1947). [f] S. S. Berg and E. W. Parnell, *J. Chem. Soc.*, 5275 (1961). [g] B. C. Bishop, A. S. Jones, and J. C. Tatlow, *ibid.*, 3076 (1964) gave mp 156–158°, but were unable to obtain analytical material. We found that sublimation at 150°(50 μ) or recrystallization from H_2O gave analytical material. [h] J. K. Horner and D. W. Henry, *J. Med. Chem.*, 11, 946 (1968). [i] A. M. Simonov, A. E. Pozharskii, and V. M. Marianovskii, *Indian J. Chem.*, **5**, 81 (1967) gave mp 254–255°.

the Carworth CF-1 strain (16–23 g) received 3 doses of test compound at 25–100 mg/kg per dose over a period of 2 days. In most experiments 3 groups of 5 mice per group were used. Physiological saline was administered to the control groups. Male Charles River rats (150–200 g) were treated as indicated. Four hours after the last dose the animals were decapitated and the adrenal glands removed and stored at −20° prior to assay.

The pooled adrenal glands from 5 mice or 3 rats were homogenized in *n*-BuOH and catecholamines were back extracted into 0.1 *M* phosphate buffer, pH 6.5. Aliquots of the extract were treated with I_2[11] for the development of fluorescent trihydroxyindole derivatives.[12] Fluorescence was determined at 2 sets of wavelengths—those optimal for epinephrine and norepinephrine, respectively. The solution of simultaneous equations using constant factors derived from the fluorescence readings of I_2-oxidized standard solns of norepinephrine and epinephrine led to numbers representing the concentrations of the two catecholamines in the adrenal glands.

In Vitro **Structure–Activity Relationships.**—The compounds evaluated in this study and their respective method of preparation are listed in Table I. The ability of substituted benzimidazoles to inhibit the methylation of normetanephrine by *S*-adenosylmethionine and partially purified steer adrenal PNMT *in vitro* is shown in Table II. The most potent inhibitors are those compounds with a free amino group in the 2 position and with Cl, NO_2, or CF_3 in the 5 position (**12**, **13**, **15**) or with these electron-withdrawing substituents in both the 5 and 6 positions (**17**, **19**, **20**). For example, 5,6-dichloro-2-aminobenzimidazole (**17**) produces 55% inhibition of enzyme activity when tested at 0.28 μg/ml. Compounds with electron-donating substituents in the 5 and 6 position are generally less active (**6**, **7**, **10**, **16**, **29**). Placing an electron-withdrawing substituent in the 4 position was not advantageous, as in the case of the 5 position, but resulted in a loss of ac-

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tivity (**14**, **18**). Substitution of the C-2 amino group was also detrimental to activity (**22**, **24**, **25**) and substitution of the ring N even more so (**21**, **26**). Replacement of the 2-amino group with a variety of other substituents[13] results in virtually complete loss in activity; compounds **27**, **28**, and **30** are exceptions.

TABLE II

INHIBITION OF ADRENAL PHENETHANOLAMINE N-METHYLTRANSFERASE *in Vitro*

No.	Concn (μg/ml)	Per cent inhibition of the methylation of— 5.7×10^{-4} *M* DL-Normetanephrine	6.0×10^{-5} *M* DL-Norepinephrine
1	28	29	
2	28	29	
3	28	30	
4	28	81	61
	2.8	38	0
5	28	57	
6	28	59	57
7	28	47	
8	28	21	
9	28	0	
10	28	17	
11	28	69	
	2.8	31	
12	28	84	75
	2.8	41	
	0.28	18	
13	28	87	85
	2.8	61	
	0.28	22	
14	28	38	
15	28	92	89
	2.8	72	
	0.28	40	
16	28	75	69
	2.8	46	22
17	28	95	94
	2.8	85	73
	0.28	55	
18	28	61	45
	2.8	24	
19	28	93	
	2.8	76	
	0.28	33	
20	28	90	79
	2.8	70	59
	0.28	28	
21	28	72	70
22	28	93	90
	2.8	76	
	0.28	33	
23	28	55	
	2.8	13	
24	28	57	
25	28	46	
26	28	0	
27	28	77	80
	2.8	55	
28	28	97	95
	2.8	44	
29	28	0	
30	28	65	
	2.8	10	

(13) Several dozen 2-substituted benzimidazoles, other than those reported in this article, were available from our sample collection and were tested.

Some of the compounds were also tested for their effect on the methylation of norepinephrine (Table II), the substrate considered to be the one occurring *in vivo*.[7] In general the compounds were slightly less effective when 6×10^{-5} *M* DL-norepinephrine replaced 5.7×10^{-4} *M* DL-normetanephrine as substrate in the assay.

In Vivo **Results and Discussion.**—A spectrum of PNMT inhibitors were tested in follow-up studies *in vivo* for their ability to lower adrenal epinephrine levels in mice. Most of the compounds were administered ip in 3 doses of 100 mg/kg each during 24 hr; some compounds were given in lower doses because of toxicity. Those inhibitors which yielded a ratio [(adrenal epinephrine/adrenal norepinephrine)$_{\text{treated mice}}$: (adrenal epinephrine/adrenal norepinephrine)$_{\text{control mice}}$] of 0.8 or less are considered active. The data in Table III demonstrate that several compounds lowered adrenal epinephrine *in vivo*. However, the relationship between activity *in vivo* and potency as enzyme inhibitors *in vitro* is poor (*cf.* Table II). 5,6-Dimethyl-2-aminobenzimidazole (**16**) was found to be the most active compound *in vivo*, producing as much as a 45% reduction in adrenal epinephrine in mice when administered in 3 doses of 50 mg/kg each. *In vitro* it produced only 22% inhibition of the methylation of norepinephrine at 2.8 μg/ml. Conversely 5,6-dichloro-2-aminobenzimidazole (**17**) produced up to 30% inhibition of adrenal epinephrine when administered in 3 doses of 100 mg/kg each while blocking norepinephrine methylation *in vitro* by 73% at 2.8 μg/ml. The apparent lack of correlation between *in vitro* and *in vivo* results may be attributed to differences in absorption, distribution, metabolism, and excretion of these compounds.

TABLE III

INHIBITION OF ADRENAL EPINEPHRINE FORMATION IN MICE (*in Vivo*)

No.	Dosage (mg/kg × 3 doses)	Activity[a]
3	100	0.76
7	100	0.78
8	100	0.78
12	100	0.71
13	100	0.71
15	100	0.58
16	50	0.55
	25	0.90
17	100	0.73
20	100	1.08
22	100	0.80
27	100	0.64
28	40	0.93

[a] (adrenal epinephrine/adrenal norepinephrine)$_{\text{treated mice}}$. (adrenal epinephrine/adrenal norepinephrine)$_{\text{control mice}}$.

To determine whether the *in vivo* activity of 5,6-dimethyl-2-aminobenzimidazole (**16**) was unique to the mouse, this compound was also tested for its effect on adrenal epinephrine formation in rats. The rat was found to be more sensitive than the mouse to the inhibitory action of this compound, for as shown in Table IV, 3 to 5 ip doses of only 25 mg/kg each reduced the epinephrine content to 25% of normal. Moreover 5 oral doses of 50 mg/kg produced a 40% reduction in adrenal epinephrine, indicating oral activity for at least one of the benzimidazoles.

TABLE IV

INHIBITION OF ADRENAL EPINEPHRINE FORMATION IN RATS BY 5,6-DIMETHYL-2-AMINOBENZIMIDAZOLE (16)

Dosage (mg/kg)	Route of administration	Activity[a]
25 × 5	Ip	0.68
25 × 3	Ip	0.24
25 × 3	Ip	0.25
50 × 5	Oral	0.59

[a] (adrenal epinephrine/adrenal norepinephrine)$_{\text{treated mice}}$ / (adrenal epinephrine/adrenal norepinephrine)$_{\text{control mice}}$

The *in vivo* activity of many of the compounds listed in Table III was found to be associated with toxicity. Anorexia with loss in weight, sedation, accumulation of abdominal fluid, decreased motor activity, and hemorrhaged adrenal glands were the toxic manifestations noted particularly when compounds were administered in doses exceeding 100 mg/kg. The adrenal hemorrhage may account for the change in the epinephrine–norepinephrine ratios. Studies with PNMT inhibitors other than benzimidazoles suggest these effects may be inseparable.[14] Substituted 2-aminobenzimidazoles had been reported to cause defects in the normal conductive processes in cat and rabbit heart *in vivo*.[15]

At present the utility of PNMT inhibitors in medicine is not established. Increased excretion of epinephrine has been associated with situations of anxiety and stress. An increase in plasma catecholamines has been noted following myocardial infarction and in patients undergoing cardiac catheterization.[16,17] Nestel[18] examined the relationship between stress, catecholamines, and blood pressure and found that in young patients with labile hypertension adrenaline excretion more than doubled under mental stress. Pitts and McClure[19] reported that injection of Na lactate into patients with anxiety neurosis produces symptoms and attacks of anxiety. These workers hypothesize that under stress there is an elevation in intracellular lactate ions in response to an increased release of adrenal epinephrine. The chronically anxious are particularly sensitive in part because of consistent overproduction of epinephrine. Increases in epinephrine excretion have also been noted in threatening situations of uncertainty, in emotional states, and a variety of other anxiety situations.[20,21]

Alterations in catecholamine metabolism in laboratory animals have been reported in a variety of stress situations, including exposure and acclimation to cold temperatures,[22] administration of CCl_4,[23] and administration and withdrawal of morphine.[24–26] It would be of interest to determine whether prior administration of PNMT inhibitors would block the selective overproduction of epinephrine observed under some of these experimental conditions.

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Experimental Section[27]

General Preparation of 2-Aminobenzimidazoles.—A 10-mmole portion of the required *o*-phenylenediamine and 15 mmoles of CNBr were stirred in 50 ml of 100–33% MeOH at room temperature[28] until the reaction was complete by tlc. Additional CNBr was added every 1 or 2 days. When the reaction was complete the solvent was removed *in vacuo* and the residue made basic with concd NH_4OH. The crude material was recrystallized from H_2O (DMF–H_2O in the case of **19**).

TABLE V

No.	Solvent	Time, days	Formula[a]
8	50% MeOH	2	$C_9H_{11}N_3O_2S$
14	MeOH	1	$C_7H_8N_4O_2$
18	33% MeOH	1	$C_7H_5Cl_2N_3$
19	33% MeOH	15	$C_7H_5N_5O_4$ · C_3H_7NO[b]
20	33% MeOH	4	$C_9H_5F_6N_3$

[a] All compounds were analyzed for C, H, N. [b] Analyzed with 1 mole of DMF; prepared by A. A. Pessolano of these laboratories.

4-(Methylsulfonyl)-*o*-phenylenediamine.—A 7.0-g portion of 4-(methylsulfonyl)-2-nitroaniline[29] in 500 ml of EtOH was hydrogenated at 3 atm in the presence of 1.0 g of 10% Pd–C. The catalyst was removed by filtration and washed with hot EtOH. The filtrates and washings yielded 3.5 g of product after recrystallization from EtOH, mp 157–159°. *Anal.* ($C_7H_{10}N_2O_2S$) C, H, N, S.

2-Carboxymethylamino-5-methylsulfonamidobenzimidazole.—A suspension of 10.0 g of 5-amino-2-carboxymethylaminobenzimidazole[30] in 175 ml of H_2O was stirred under N_2 and 5 ml of MesCl was added dropwise over a period of 4 min. After stirring 20 min, 175 ml of *i*-PrOH was added followed by careful addition of 6.15 g of $NaHCO_3$ in 50 ml of H_2O. The mixture was then refluxed 30 min and allowed to cool before 9.83 g of crude product was collected on a filter, mp 249–256° dec. Two recrystallizations from DMF–H_2O gave material melting at 285–289° dec. *Anal.* ($C_{10}H_{12}N_4O_4S$) C, H, N.

2-Amino-5-methylsulfonamidobenzimidazole (9).—A mixture of 1.06 g of 2-carboxymethylamino-5-methylsulfonamidobenzimidazole, 449 mg of NaOH, and 4.5 ml of H_2O was refluxed 20 hr. After dilution with 15 ml of H_2O the soln was decanted from insolubles and brought to pH 6 with 1 *N* HCl. The resultant precipitate was removed by filtration and the filtrate was brought to pH 7.2 and cooled in an ice bath. After standing 3 hr, 0.34 g of product was collected. *Anal.* ($C_8H_{10}N_4O_2S$) C, H, N, S.

2-Amino-5(6)-*n*-butylbenzimidazole (10).—A mixture of 101 mg of 2-propionamido-5(6)-*n*-butylbenzimidazole,[31] 450 mg of NaOH, and 5 ml of H_2O was refluxed 15 hr. After cooling, 74 mg of product was collected by filtration, mp 185–187°. Recrystallization from EtOH–H_2O gave analytical material. *Anal.* ($C_{11}H_{15}N_3$) C, H, N.

2-Amino-5(6)-fluorobenzimidazole (11).—A 10-g portion of 4-fluoro-2-nitroaniline[32] was hydrogenated in 400 ml of EtOH at

(27) Melting points were determined on a Kofler hot stage and are corrected. Compounds were routinely examined by ir and nmr spectroscopy, and tlc. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.
(28) We found that extending the time at room temperature gave better results than heating.
(29) V. A. Lavrishchev, Val L. Plakidin, and A. E. Kretov, *Izv. Vyssh. Uchebn. Zaved., Khim. Khim. Tekhnol.*, **3**, 127 (1960) [*Chem. Abstr.*, **54**, 17303*e* (1960)].
(30) Kindly provided by Dr. M. H. Fisher of these laboratories.
(31) Kindly provided by Mr. F. S. Waksmunski of these laboratories.
(32) K. Takatori, Y. Yamada, T. Arai, and R. Nakazawa, *Yakugaku Zasshi*, **78**, 108 (1958) [*Chem. Abstr.*, **52**, 11013*e* (1958)].

3 atm in the presence of 1 g of 10% Pd–C. The catalyst was removed by filtration and the filtrate was put under N_2[33] and cooled in an ice-bath before 6.8 g of CNBr was added. The mixture was then stirred for 1 hr without external cooling and 15 hr at 5° before being made basic with concd NH_4OH. After concn to a gum the mixture was extracted with 10:1 MeOH–$CHCl_3$ and the extract was chromatographed on silica gel using 4:1 $CHCl_3$–MeOH as an eluant. Further purification was achieved through conversion into the HBr salt followed by reconversion into the free base and recrystallization from H_2O to give 1.0 g of product. *Anal.* ($C_7H_7FN_3$) C, H, N.

4,5-Dinitro-1,2-bis(trifluoromethylbenzene).—A mixture of 7.0 g of 4,5-dinitrophthalic acid,[34] 75 g of SF_4, and 35 ml of HF was heated in a steel bomb for 8 hr at 140°. After evapn of the reagents at room temp the residue was extracted with $CHCl_3$. The $CHCl_3$ soln was filtered, dried, and concd to a residue which was triturated with hexane to give 8.35 g, mp 93–97°. Additional purification was achieved by recrystn from hexane and sublimation, mp 96–97°. *Anal.* ($C_8H_2F_6N_2O_4$) C, H, N.

4,5-Bis(trifluoromethyl)-*o*-phenylenediamine.—A soln of 10 g of $SnCl_2 \cdot 2H_2O$ in 40 ml of concd HCl was cooled to 0° and 200 g of 4,5-dinitro-1,2-bis(trifluoromethylbenzene) was added. The mixture was stirred 16 hr with initial intermittant cooling which kept the temp at <40°. The mixture was then cooled to 0° and the precipitate was collected. After washing with cold 6 *N* HCl the ppt was treated with NH_4OH to give 1.26 g, mp 64–65.5°. Sublimation gave material melting at 65–66°. *Anal.* ($C_8H_6F_3N_2$) C, H, N.

2,5,6-Trichlorobenzimidazole.—A 20-g portion of 5,6-dichlorobenzimidazolinone[35] was refluxed in 300 ml of $POCl_3$ for 17.5 hr. HCl gas was passed into the mixture for the first 1.5 hr. Excess $POCl_3$ was removed *in vacuo* and the residue was decompd cautiously with H_2O. The resultant mixture was extd with hot concentrated HCl (500 and 3 × 100 ml). The HCl ext were made slowly basic with concd NH_4OH. The first 0.2 g of ppt which sepd was discarded. Further addition of NH_4OH gave 1.7 g of crude product. Purification for analysis was achieved by sublimation at 90° (50 μ) for 2 days (discard sublimate) followed by sublimation at 115° (50 μ), mp 233–235°, resolidified at 238°. *Anal.* ($C_7H_3Cl_3N_2$) C, H, Cl, N.

2-Amino-5,6-dichloro-1-methylbenzimidazole (21).—A 1.44-g portion of 2,4,5-trichlorobenzimidazole was treated with 4.5 ml of 5 *N* NaOH and 1.5 ml of Me_2SO_4. After 2 hr 1.50 g of material which was pure by tlc was collected on a filter. An 0.80-g portion of this material was treated in a sealed tube at 200°/6 hr with 3 ml of concd NH_4OH. The resultant insoluble product was purified by preparative tlc on silica gel plates (10% MeOH–$CHCl_3$) followed by sublimation [130°(50 μ)]. *Anal.* ($C_8H_7Cl_2N_3$) C, H, Cl, N.

5,6-Dichloro-2-dimethylaminobenzimidazole (22).—A mixture of 1.7 g of 2,5,6-trichlorobenzimidazole and 8.0 ml of 40% $(CH_3)_2NH$ was heated for 6 hr at 155° in a sealed tube. After cooling and dilution with 75 ml of H_2O, 1.57 g of product was collected, mp 250–258°. Sublimation at 150°(50 μ) gave pure material. *Anal.* ($C_9H_9Cl_2N_3$) C, H, Cl, N.

5,6-Dichloro-2-dimethylamino-1-methylbenzimidazole (23).—To a cooled mixture of 0.64 g of **22**, 1.5 ml of 5 *N* NaOH, and 5.0 ml of H_2O was added 0.55 ml of Me_2SO_4. The mixture was stirred 12 hr with intermittent additions of NaOH and Me_2SO_4 and diluted with H_2O before a crude product was collected. This material was decolorized, dried in $CHCl_3$ soln, and evapd to a solid which was extracted (Et_2O). The residue from evapn of the Et_2O ext was recrystd twice from Et_2O to give 0.26 g of product. *Anal.* ($C_{10}H_{11}Cl_2N_3$) C, H, Cl, N.

5,6-Dimethyl-2-dimethylaminobenzimidazole (24) was prepared from 2-chloro-5,6-dimethylbenzimidazole[36] by the sample procedure used in the synthesis of **22**. *Anal.* ($C_{11}H_{15}N_3$) C, H, N.

2-Acetamido-5(6)-trifluoromethylbenzimidazole (25).—A mixture of 1.0 g of **15** and 6 ml of pyridine was cooled and 0.50 ml of Ac_2O was added. After 1 hr at room temperature and 1.5 hr in a steam bath the mixture was poured into ice–water. Crude product (1.05 g) was collected, mp 274–285°. Recrystn from MeOH gave analytical material. *Anal.* ($C_{10}H_8F_3N_3O$) C, H, F, N.

2-Amino-1-benzyl-5,6-dimethylbenzimidazole (26).—To a soln of 0.63 g of Na in 50 ml of abs EtOH was added 4.2 g of 5,6-dimethylbenzimidazole. The mixture was stirred 30 min and 3.0 ml of $PhCH_2Cl$ was added. After stirring 15 hr the mixture was refluxed 1 hr and filtered. The filtrate was concentrated *in vacuo* and triturated with H_2O to give 3.6 g of product, mp 259–261°. Recrystn from EtOH gave analytical material: nmr (DMSO). τ 2.79 (m, 5), 3.07 (s, 1), 3.20 (s, 1), 3.63 (s, 2), 4.8 (s, 2), 7.83 (s, 6). *Anal.* ($C_{16}H_{17}N_3$) C, H, N.

2-Aminomethyl-5,6-dichlorobenzimidazole (27).—A mixture of 17.6 g of 4,5-dichloro-*o*-phenylenediamine, 7.50 g of glycine, 120 ml of H_2O, and 80 ml of concd HCl was stirred and refluxed 14 hr under N_2. The solvent was removed *in vacuo* and the residue was added to H_2O. Precipitates were collected successively as the pH was raised to 4.0, 6.4, 7.0, and 9.2. At the highest pH, 1.03 g of product was obtained. Purification was achieved by sublimation. *Anal.* ($C_8H_7Cl_2N_3$) C, H, Cl, N.

2-Hydrazino-5,6-dichlorobenzimidazole (28).—A mixture of 0.56 g of 2,5,6-trichlorobenzimidazole and 5.6 ml of 99–100% hydrazine was heated in a sealed tube at 150° for 5 hr. After cooling, 0.53 g of crude product was collected. Sublimation at 150° (0.5 mm) gave 0.42 g. *Anal.* ($C_7H_6Cl_2N_4$) C, H, Cl, N.

2-Acetamido-5(6)-hydroxybenzimidazole (29).—A mixture of 100 g of dry pyridine·HCl and 10 g of **7** was heated at 200–275° for 2 hr. After cooling, 600 ml of H_2O and NH_4OH to make pH 9 were added and the mixture was extd continuously with EtOAC. Concentration of the extract gave 19 g of purple solid which yielded 3.5 g of crude product, mp 273–283°, by trituration with H_2O followed by MeOH. The material was purified by recrystn from MeOH. *Anal.* ($C_9H_9N_3O_2$) C, H; N: calcd 21.98, found, 21.51.

2,5(6)-Bis(trifluoromethyl)benzimidazole[37] (30).—A mixture of 5.16 g of F_3CCO_2H, 8.0 g of 4-trifluoromethyl-*o*-phenylenediamine,[38] and 90 ml of 4 *N* HCl was refluxed and stirred 2 hr. After cooling, 5.0 g of product was collected, mp 196–198°. Two recrystns from C_6H_6 gave analytical material. *Anal.* ($C_8H_4F_6N_2$) C, H, N, F.

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(33) This intermediate in solution was very air sensitive and was therefore not isolated.

(34) (a) H. Goldstein and J. P. Merminod, *Helv. Chim. Acta*, **35**, 1476 (1952). (b) T. Momose, A. Inaba, K. Inoue, K. Miyahara and T. Mori, *Chem. Pharm. Bull.*, **12**, 14 (1964). (c) We prepared this compound in a 2-step sequence from 4,5-dimethyl-4-nitroaniline by first converting it into the known [E. Noelting and G. Thesmar, *Ber.*, **35**, 628, (1902)] 1,2-dimethyl-4,5-dinitrobenzene by the procedure of K. J. Clark and G. I. Fray, *J. Chem. Soc.*, 894, (1960), and then oxidizing this intermediate quantitatively to the desired phthalic acid by heating in a sealed tube at 150°/10 hr as a 10% soln in concd HNO_3.

(35) R. L. Clark and A. A. Pessolano, *J. Amer. Chem. Soc.*, **80**, 1657 (1958).

(36) N. P. Bednyagina, G. N. Tyurenkova and I. V. Panov, *Zh. Obsch. Khim.*, **34**, 1575 (1964) [*Chem. Abstr.*, **61**, 8297*a* (1964)].

(37) This compound was prepared by K. Reber of these laboratories.

(38) W. B. Whalley, *J. Chem. Soc.*, 2792 (1950).