Inhibitors of Phenylethanolamine N-Methyltransferase and Epinephrine Biosynthesis. 1. Chloro-Substituted 1,2,3,4-Tetrahydroisoquinolines

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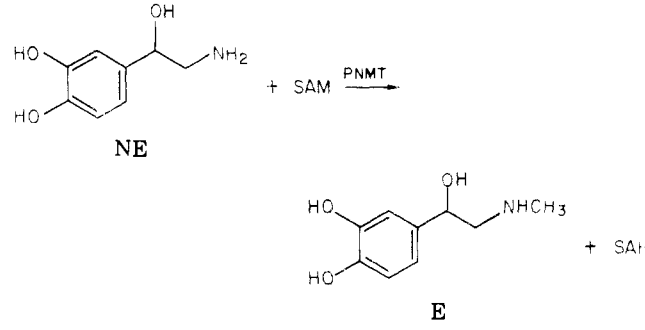
In a search for inhibitors of epinephrine biosynthesis as potential therapeutic agents, a series of 13 ring-chlorinated 1,2,3,4-tetrahydroisoquinolines was prepared. These compounds were tested initially for their ability to inhibit rabbit adrenal phenylethanolamine N-methyltransferase (PNMT) in vitro. Enzyme-inhibitor dissociation constants, determined for the six most potent members of the series, indicated the following order of decreasing potency: 7,8-Cl_2 > 6,7,8-Cl_3 > 7-Cl ≈ 8-Cl > 5,6,7,8-Cl_4 > 5,7,8-Cl_3. These compounds were subsequently examined for PNMT-inhibiting activity in intact rats and mice. 7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline (13, SK&F 64139) was the most potent member of the series both in vitro and in vivo and is currently undergoing clinical investigation.

Phenylethanolamine N-methyltransferase (PNMT), also referred to as norepinephrine N-methyltransferase (NMT, EC 2.1.1.28), catalyzes the terminal step in the biosynthesis of epinephrine (E).[1] As illustrated in Scheme I, this reaction involves the transfer of a methyl group from S-adenosylmethionine (SAM) to norepinephrine (NE) to afford E and S-adenosylhomocysteine (SAH). PNMT is located mainly in the adrenal medulla, but small quantities are also found in certain other organs,[1] including the mammalian brain stem and spinal cord where it is localized in areas concerned with the regulation of autonomic function, e.g., heart rate and blood pressure.[2-4] E is detected in the same anatomical regions.[5]

Under basal conditions, E stored in the adrenal medulla apparently is of minor consequence as a physiological regulator; its rate of secretion is low.[6,7] In response to stress, however, this hormone is released into the bloodstream in substantial quantities[8-10] and, together with NE released from sympathetic nerve terminals, produces physiological and behavioral[11] changes of possible adaptive benefit. In certain circumstances, e.g., in individuals with underlying disease conditions such as angina pectoris,[12,13] occlusive coronary disease,[14,15] or anxiety,[16,17] the physiological changes induced by the discharge of adrenal E may have adverse consequences. Further, although the physiological manifestations of NE and E are somewhat similar, there are meaningful differences in the response of target organs to these adrenergic catecholamines.[18-21] Thus, an inhibitor of PNMT that prevents E biosynthesis, or one that alters the E/NE ratio, might find therapeutic utility.

Scheme I



Several means for inhibiting E biosynthesis seem plausible.[22] Most obviously such an action might be produced by inhibitors of the enzyme that are chemically related to its natural substrates, i.e., SAM and NE. SAH[23] and other compounds related to SAH and SAM[24] inhibit PNMT;

(1) J. Axelrod, *J. Biol. Chem.*, **237**, 1657 (1962).
(2) T. Hökfelt, K. Fuxe, M. Goldstein, and O. Johansson, *Brain. Res.*, **66**, 235 (1974).
(3) J. M. Saavedra, M. Palkovits, M. J. Brownstein, and J. Axelrod, *Nature (London)*, **248**, 695 (1974).
(4) J. M. Saavedra, H. Grobecker, and J. Axelrod, *Science*, **191**, 483 (1976).
(5) S. Koslow and M. Schumpf, *Nature (London)*, **251**, 530 (1974).
(6) S. Udenfriend, J. R. Cooper, C. T. Clark, and J. E. Baer, *Science*, **117**, 663 (1953).
(7) J. D. Spira and K. Bron, *J. Clin. Endocrinol. Metab.*, **33**, 436 (1971).
(8) J. Malmejac, *Physiol. Rev.*, 44, 186 (1964).
(9) R. Gordon, S. Specter, A. Sjoerdsma, and S. Udenfriend, *J. Pharmacol. Exp. Ther.*, **153**, 440 (1966).
(10) R. Kvetnansky and L. Mikulaj, *Endrocrinology*, **87**, 738 (1970).
(11) W. C. Holz, R. G. Pendleton, W. T. Fry, and C. A. Gill, *J. Pharmacol. Exp. Ther.*, **202**, 379 (1977).
(12) J. Hamer and E. Sowton, *Am. J. Cardiol.*, **18**, 354 (1966).
(13) W. Raab, *Am. J. Cardiol.*, **9**, 576 (1962).
(14) S. Bondurant, *Am. J. Cardiol.*, **24**, 612 (1969).
(15) L. Ceremuzynski, J. Staszewska-Barczak, and K. Herbaczynska-Cedro, *Cardiovas. Res.*, **3**, 190 (1969).
(16) U. S. von Euler, *Clin. Pharmacol. Ther.*, **5**, 398 (1964).
(17) R. J. Wurtman, *Recent Adv. Biol. Psychiatry*, **9**, 350 (1966).
(18) A. M. Lands and T. G. Brown, Jr., in "Drugs Affecting the Peripheral Nervous System", Vol. 1, A. Burger, Ed., Marcel Dekker, New York, 1967, p 399.
(19) G. B. West, *J. Pharm. Pharmacol.*, **7**, 81 (1955).
(20) J. A. Wilkens and N. Sapeika, *J. Atheroscler. Res.*, 4, 346 (1964).
(21) H. W. Scott, D. H. Riddell, and S. K. Brockman, *Surg. Gynecol. Obstet.*, **120**, 707 (1965).
(22) C. Kaiser and R. G. Pendleton, *Intra-Sci. Chem. Rep.*, **8**, 43 (1974).
(23) T. Deguchi and J. Barchas, *J. Biol. Chem.*, **246**, 3175 (1971).
(24) R. T. Borchardt, Y. S. Wu, and B. S. Wu, *J. Med. Chem.*, **21**, 1307 (1978), and references cited therein.

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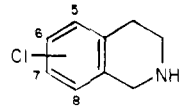
Table I. Chloro-Substituted 1,2,3,4-Tetrahydroisoquinolines



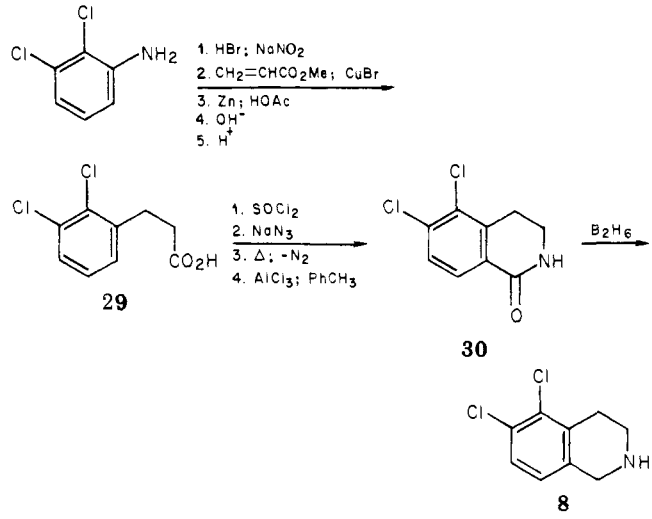
no.	position of Cl substn	method[a]	yield, %	recrystn solv(s)	mp, °C	formula[b]	in vitro PNMT inhibn, %[c] 10^{-4} M	in vitro PNMT inhibn, %[c] 10^{-6} M
3				MeOH-EtOAc	196-197[d]	$C_9H_{11}N\cdot HCl$	73	3
4	5	B	31	MeOH-Et_2O	171-172	$C_9H_{10}ClN\cdot C_4H_4O_4$[e]	81	6
5	6	B	40	MeOH-Et_2O	229-231[f]	$C_9H_{10}ClN\cdot HCl$	81	19
6	7	A	33	MeOH-Et_2O	213-214.5	$C_9H_{10}ClN\cdot HCl$	98	60
7	8	A	67	MeOH-EtOH-Et_2O	245-247	$C_9H_{10}ClN\cdot HCl$	96	52
8	5, 6			Me_2CHOH-MeOH-Et_2O	250-252	$C_9H_9Cl_2N\cdot HCl$	93	17
9	5, 7	A	47	MeOH	300 dec	$C_9H_9Cl_2N\cdot HCl$	95	24
10	5, 8	A	55	MeOH	311 dec	$C_9H_9Cl_2N\cdot HCl$	89	15
11	6, 7	A	29	EtOH	269-274[g]	$C_9H_9Cl_2N\cdot HCl$	98	43
12	6, 8	A	43	MeOH-EtOH	296 dec	$C_9H_9Cl_2N\cdot HCl$	76	6
13	7, 8	A	48	MeOH-Et_2O	225-227	$C_9H_9Cl_2N\cdot HCl$	98	97
14	5, 7, 8	B	50	MeOH-EtOAc	178-179	$C_9H_8Cl_3N\cdot C_4H_4O_4$[e]	97	73
15	6, 7, 8	B	52	MeOH-Et_2O	193-194	$C_9H_8Cl_3N\cdot C_4H_4O_4$[e]	96	86
16	5, 6, 7, 8	B	50	MeOH-EtOAc	>300	$C_9H_7Cl_4N\cdot HCl$	95	74

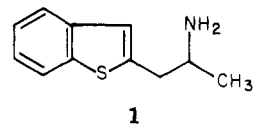
[a] See Experimental Section: "Chemistry. General Methods". [b] All compounds were analyzed for C, H, N; analytical values were within ±0.4% of calculated values. [c] See Experimental Section: "Pharmacology. Methods A. Primary Assay". [d] Reported mp 196-197 °C, R. Forsyth, C. I. Kelly, and F. L. Pyman, *J. Chem. Soc.*, **127**, 1659 (1925). [e] Maleate. [f] Reported mp 236-237 °C, L. W. Deady, N. Pirazada, and R. D. Topsom, *Chem. Commun.*, 799 (1971). [g] Sealed capillary tube.

however, the majority of PNMT inhibitors[22,25-40] are structurally related to NE.

In a search for potent, selective, and orally active inhibitors of PNMT, aralkylamines are of particular interest, as earlier studies[22,25,26] show that phenethylamines are more effective inhibitors of PNMT than are their phenylethanolamine counterparts. In the course of this investigation, it was observed that incorporation of the aminoalkyl side chain of certain β-heteroarylethylamines into a fused piperidine ring greatly enhances PNMT-inhibitory potency. Thus, at a concentration of 10^{-4} M, 2-(2-aminopropyl)thianaphthene (1) produces only 33% inhibition of rabbit adrenal PNMT in vitro,[41] whereas 3-methyl-1,2,3,4-tetrahydro[1]benzothieno[3,2-c]pyridine (2, SK&F 7698)[40,42,43] is significantly more potent; it has an IC_{50} of 7×10^{-7} M in this procedure. In addition, 2 selectively

Scheme II





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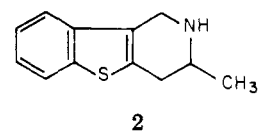

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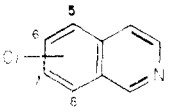
(25) R. W. Fuller and J. M. Hunt, *Biochem. Pharmacol.*, **14**, 1896 (1965).
(26) R. W. Fuller, J. Mills, and M. M. Marsh, *J. Med. Chem.*, **14**, 322 (1971).
(27) G. L. Grunewald, J. M. Grindel, W. C. Vincek, and R. T. Borchardt, *Mol. Pharmacol.*, 11, 694 (1975).
(28) L. R. Krakoff and J. Axelrod, *Biochem. Pharmacol.*, **16**, 1384 (1967).
(29) R. W. Fuller, B. B. Molloy, W. A. Day, B. W. Roush, and M. M. Marsh, *J. Med. Chem.*, **16**, 101 (1973).
(30) R. W. Fuller, B. W. Roush, H. D. Snoddy, and B. B. Molloy, *J. Med. Chem.*, **16**, 106 (1973).
(31) R. W. Fuller, B. W. Roush, H. D. Snoddy, W. A. Day, and B. B. Molloy, *J. Med. Chem.*, **18**, 304 (1975).
(32) R. W. Fuller, B. W. Roush, S. K. Hemrick, H. D. Snoddy, and B. B. Molloy, *Life Sci.*, **22**, 1421 (1978).
(33) R. W. Fuller and B. B. Molloy, *Biochem. Pharmacol.*, **26**, 446 (1977).
(34) R. W. Fuller, B. W. Roush, and B. B. Molloy, *Adv. Enzyme Res.*, **12**, 311 (1974).
(35) N. P. Jensen, S. M. Schmitt, T. B Windholz, T. Y. Shen, L. R. Mandel, B. Lopez-Ramos, and C. C. Porter, *J. Med. Chem.*, **15**, 341 (1972).
(36) L. R. Mandel, C. C. Porter, F. A. Kuehl, Jr., N. P. Jensen, S. M. Schmitt, T. B. Windholz, T. R. Beattie, J. A. Carty, B. G. Christensen, and T. Y. Shen, *J. Med. Chem.*, **13**, 1043 (1970).
(37) R. G. Pendleton, I. B. Snow, C. Kaiser, J. Wang, and H. Green, *Biochem. Pharmacol.*, **21**, 2967 (1972).
(38) R. W. Fuller, K. W. Perry, and S. K. Hemrick, *Brain Res.*, **158**, 240 (1978); R. W. Fuller, B. B. Molloy, and S. K. Hemrick, *Biochem. Pharmacol.*, **28**, 528 (1979).
(39) R. G. Pendleton, C. Kaiser, and G. Gessner, *J. Pharmacol. Exp. Ther.*, **197**, 623 (1976).
(40) R. G. Pendleton, C. Kaiser, G. Gessner, E. Finlay, and H. Green, *J. Pharmacol. Exp. Ther.*, **190**, 551 (1974).
(41) C. Kaiser and R. Pendleton, unpublished results.
(42) J. Y. Lew, T. Miyamoto, and M. Goldstein, *Biochem. Pharmacol.*, **25**, 1433 (1976).
(43) R. J. Katz and B. J. Carroll, *Psychopharmacology*, **57**, 39 (1978).

Table II. Chloro-Substituted Isoquinolines



no.	position of Cl-substn	method[a]	yield, %	recrystn solv(s)	mp, °C	formula[b]
17	5		20	MeOH-EtOAc	204-205	$C_9H_6ClN \cdot HCl$
18	6	C	11	MeOH-Et_2O	>320[c]	$C_9H_6ClN \cdot HCl$
19	7		14	EtOH-Et_2O	215-216	$C_9H_6ClN \cdot HCl$
20	8	C	6	EtOH-Et_2O	191-193	$C_9H_6ClN \cdot HCl$
21	5, 7	C	54	n-C_6H_{14}	155-156	$C_9H_5Cl_2N$
22	5, 8	C	16	Me_2CO	117-118[d]	$C_9H_5Cl_2N$
23	6, 7	C	9	EtOH-Et_2O	227 dec	$C_9H_5Cl_2N \cdot HCl$
24	6, 8	C	<1	MeOH-EtOH-Et_2O	238 dec	
25	7, 8	C	49	EtOH	222-224	$C_9H_5Cl_2N \cdot HCl$
26	5, 7, 8	D	2	c-C_6H_{12}	178-179[e]	$C_9H_4Cl_3N$
27	6, 7, 8	C	6	MeOH-EtOAc	205-207	$C_9H_4Cl_3N \cdot CH_3SO_3H$
28	5, 6, 7, 8	D	2	c-C_6H_{12}	210-212	$C_9H_3Cl_4N$

[a] See Experimental Section: "Chemistry. General Methods". [b] All compounds for which formula is indicated were analyzed for C, H, N, and analytical values were within ±0.4% of calculated values. [c] Sublimes partially at 250 °C. [d] Reported (ref 48) mp 117-118 °C. [e] Reported (ref 48) mp 177-178 °C.

reduces the adrenal content of E following chronic oral administration to rats. Unfortunately, **2** lacks specificity. Particularly, it produces α-adrenergic receptor-blocking effects at concentrations approximating those required to inhibit PNMT.[40]

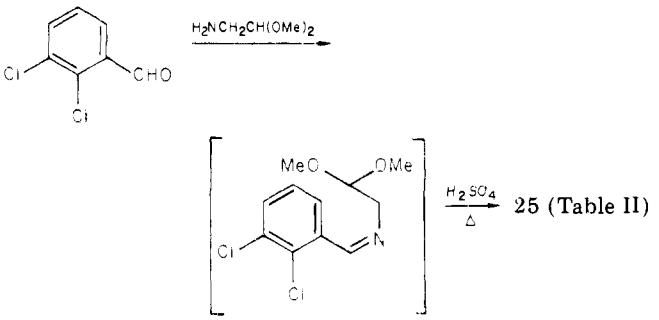
Similar incorporation of the aminoethyl side chain of β-phenethylamine into a fused ring system also results in markedly increased PNMT-inhibitory activity in vitro. At a concentration of 10^{-4} M, β-phenethylamine causes only 17% inhibition of rabbit adrenal PNMT in vitro,[22] whereas the ring-fused relative, 1,2,3,4-tetrahydroisoquinoline (**3**; Table I), produces 73% inhibition under identical conditions. Since appropriate chlorination of the aromatic ring of many NE-related PNMT substrates and inhibitors often results in compounds with strikingly increased potency,[22,26,29-38,44] the influence of aromatic chlorination of 1,2,3,4-tetrahydroisoquinoline was investigated. In this article are described the synthesis and PNMT-inhibitory properties of a series of chloro-substituted 1,2,3,4-tetrahydroisoquinolines.

Chemistry. Tetrahydroisoquinolines **3-16** (Table I), with the exception of **8**, were prepared from the corresponding isoquinolines **17-28** (Table II) by catalytic hydrogenation of the hydrochloride (method A) or by reduction of the free base with excess diborane in tetrahydrofuran (method B). The latter method of reduction of isoquinolines, which has not been previously reported, gave tetrahydroisoquinolines in 30-50% yield.

5,6-Dichloro-1,2,3,4-tetrahydroisoquinoline (**8**) was synthesized as outlined in Scheme II. Accordingly, Meerwein arylation of methyl acrylate with the diazonium salt derived from 2,3-dichloroaniline and cuprous bromide gave methyl 2-bromo-3-(2,3-dichlorophenyl)propionate. The bromine was removed by reduction with zinc in acetic acid and the ester saponified to give **29**. Curtius rearrangement of the acyl azide, obtained from **29** via the acid chloride, afforded an isocyanate which cyclized to the isoquinolinone **30** upon treatment with aluminum chloride. Diborane reduction of the isoquinolinone produced **8**.

Isoquinolines **18**, **20-25**, and **27** were obtained from appropriate chloro-substituted benzaldehydes via the Pomeranz-Fritsch reaction[45] as exemplified for the synthesis

Scheme III



of **25** in Scheme III. Condensation of chloro-substituted benzaldehydes with aminoacetaldehyde dimethyl acetal was quantitative, and the resulting benzylidenamines were generally employed for cyclization without purification. Cyclization and concomitant elimination of methanol to afford isoquinolines resulted upon treatment of the benzylidenamines with hot concentrated sulfuric acid. In the case of 3,4-dichloro-*N*-(2,2-dimethoxyethyl)benzylidenamine only a single isomer, i.e., 6,7-dichloroisoquinoline (**23**), was isolated. The range in yields (6-54%) of isoquinolines resulting from this procedure may be explained, in part, by the concurrent formation of phenyloxazoles.[46]

The 5- (**17**) and 7-chloroisoquinolines (**19**) were prepared from corresponding aminoisoquinolines via the Sandmeyer reaction as previously described.[47] 5,7,8-Trichloro- (**26**) and 5,6,7,8-tetrachloroisoquinoline (**28**) were obtained by modification of the previously reported direct chlorination of isoquinoline.[48]

Results

The results of testing tetrahydroisoquinolines **3-16** at 10^{-4} and 10^{-6} M in an in vitro test for inhibition of rabbit adrenal PNMT-catalyzed conversion of NE to E[49] are tabulated in Table I. In all cases, the PNMT-inhibitory potency of 1,2,3,4-tetrahydroisoquinoline (**3**) was enhanced by chlorination of the aromatic ring. This increase was slight for the 5- (**4**) and 6-monochlorinated (**5**) derivatives;

(44) R. W. Fuller, S. K. Hemrick, and B. B. Molloy, *Res. Commun. Chem. Pathol. Pharmacol.*, 18, 577 (1977).
(45) W. J. Gensler, *Org. React.*, 6, 191 (1951).
(46) E. V. Brown, *J. Org. Chem.*, 42, 3208 (1977).
(47) R. H. F. Manske and M. Kulka, *Can. J. Res., Sect. B*, 27, 161 (1949).
(48) M. Gordon and D. E. Pearson, *J. Org. Chem.*, 29, 329 (1964).
(49) R. G. Pendleton and I. B. Snow, *Mol. Pharmacol.*, 9, 718 (1973).

Table III. K_i Values and in Vivo PNMT Inhibition Data for Some Ring-Chlorinated Tetrahydroisoquinolines

no.	K_i, nM[a]	% inhibn of [3H]E biosynthesis, 50 mg/kg, po, t.i.d., rats[b]	decrease in adrenal E/NE ratio, mg/kg, po, b.i.d., mice[c]	
			MAD[d]	MID[e]
6	29	78	12.5	7.5
7	30	45	7.5	2.5
13	3	*f*	5.0	2.5
14	59	16		100.0
15	20	25		50.0
16	52		100.0	25.0

[a] See Experimental Section: "Pharmacology. Method A. Secondary Assay". [b] See Experimental Section: "Pharmacology. Method B". [c] See Experimental Section: "Pharmacology. Method C". [d] Minimum active dose. [e] Maximum inactive dose. [f] ED_{50} = 3.

however, 7- (6) and 8-monochlorinated (7) derivatives were strikingly more potent than the unsubstituted parent 3 at both concentrations. Among dichlorinated derivatives 8–13, greatest potency enhancement was observed with the 7,8-dichloro compound 13, which caused almost complete inhibition of PNMT at 10^{-6} M. Chloro substitution at positions 7 and 8 is synergistic; 13 was markedly more potent than either the 7- or 8-monosubstituted derivatives, 6 and 7, respectively. 6,7-Dichloro-1,2,3,4-tetrahydroisoquinoline (11) was significantly less effective than the 7,8-isomer 13; 11 had PNMT-inhibitory potency approximately equal to that of the 7-monochloro derivative 6. At a concentration of 10^{-6} M, other dichlorinated tetrahydroisoquinolines, 8–10 and 12, were markedly less effective as in vitro inhibitors of rabbit adrenal PNMT than was 13. The 6,8-dichloro compound 12 was only marginally more potent than 1,2,3,4-tetrahydroisoquinoline (3). Tri- (14 and 15) and tetrachlorotetrahydroisoquinolines (16), each of which retains chloro substituents at positions 7 and 8, were somewhat less potent than 13 at 10^{-6} M.

To more definitively measure PNMT-inhibitory potency for structure–activity comparison, enzyme–inhibitor dissociation constants (K_i values) were determined for the six most potent chloro-substituted tetrahydroisoquinolines in Table I using a modified in vitro test for inhibition of rabbit adrenal PNMT in which phenylethanolamine was the substrate methylated.[50] The results of this study, presented in Table III, permit a more accurate ranking of the potent inhibitors. The rank order of PNMT-inhibitory potency of ring-chlorinated tetrahydroisoquinolines in this secondary in vitro test, i.e., **13 > 15 > 6 ≈ 7 > 16 > 14**, is slightly different from that determined in the primary procedure, i.e., **13 > 15 > 16 ≈ 14 > 6 > 7**, and may be due to variability in the latter assay in which the coefficient of variation is about 9%. Clearly, however, the most potent inhibitor in both in vitro tests is 13.

Each of the tetrahydroisoquinolines in Table III was active following oral administration in at least one of two tests that measure PNMT-inhibitory activity in vivo. These tests measure the inhibition of adrenal [3H]epinephrine biosynthesis and decreases in the endogenous adrenal epinephrine/norepinephrine ratio, respectively, and are described under Experimental Section. The inhibition of [3H]epinephrine biosynthesis test measures the ability of a compound to inhibit conversion of [3H]NE to [3H]E in the adrenal medulla of rats. The rank order of potency of the chlorinated tetrahydroisoquinolines examined in this procedure, **13 > 6 > 7 > 15 > 14**, paralleled the K_i values, except for 15 which was less potent than expected; 13 had a unit dose ED_{50} of about 3 mg/kg, po.[39] In the adrenal epinephrine/norepinephrine ratio test, the content of E and NE in mouse adrenal glands is determined after chronic oral administration of the test compound. A decrease in the E/NE ratio is indicative of PNMT inhibition.[39] In contrast to the large differences in the potencies of 6 and 7 vs. 13 in vitro and in the inhibition of [3H]E biosynthesis test, these compounds were approximately equipotent in reducing the E/NE ratio. The tetrachloro derivative 16 was significantly less potent, and 14 and 15 were ineffective at unit doses of 100 and 50 mg/kg, respectively.

Discussion

The data presented in Table IV indicate that the in vitro PNMT-inhibitory potency of ring-chlorinated tetrahydroisoquinolines in all cases is greater than that of the correspondingly substituted phenethylamines[26] or benzylamines.[29] The potency of the tetrahydroisoquinolines, which incorporate a secondary amino group, is even more striking when one considers that N-alkylation of PNMT-inhibitory aralkylamines generally decreases their potency.[26,29] It may be significant that 13, the most potent member of the tetrahydroisoquinoline series, combines the features of both 3,4-dichlorophenethylamine and 2,3-dichlorobenzylamine, compounds that are among the most potent in their respective series. Further, 11, whose structure incorporates the features of 3,4-dichlorophenethylamine, but not of 2,3-dichlorobenzylamine, is a much less potent in vitro inhibitor of PNMT than is 13. These comparisons suggest that the hetero ring in the tetrahydroisoquinolines holds the chloro, aryl, amino, and methylene moieties in a relative orientation, accessible to a lesser extent from chlorophenethylamines and chlorobenzylamines that is highly favorable for interaction with the enzyme's NE recognition site. The effect of chloro substitution on potency may reflect interaction with loci of bulk tolerance and/or lipophilicity in the binding site. These factors, which combine to make 13 the most potent PNMT inhibitor reported to date, are not applicable at other NE binding sites, e.g., monoamine oxidase and catechol *O*-methyltransferase, or at adrenergic receptors where 13 is practically devoid of activity.[39]

The in vivo potency of 7,8-dichloro-1,2,3,4-tetrahydroisoquinoline (13) as an inhibitor of adrenal PNMT may reflect its high affinity for the enzyme, coupled with a degree of lipophilicity consistent with good oral absorption and facile transport into the adrenal medulla, and freedom from rapid metabolic inactivation; 13 has been the subject of extensive pharmacological testing;[39,43,50–54] it is presently undergoing clinical investigation.[55]

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analyses were performed by the Analytical and Physical Chemistry Section of Smith Kline & French Laboratories. Where

(50) R. G. Pendleton, G. Weiner, and B. Jenkins, *Res. Commun. Chem. Pathol. Pharmacol.*, 17, 201 (1977).

(51) R. G. Pendleton, G. Gessner, and B. Jenkins, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **295**, 127 (1976).

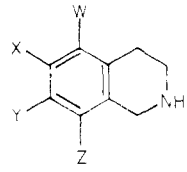
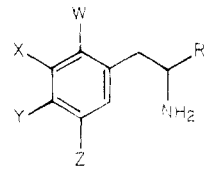
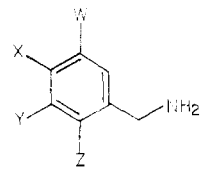
(52) R. G. Pendleton, G. Weiner, B. Jenkins, and G. Gessner, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **297**, 245 (1977).

(53) J. H. Khalsa, G. Gessner, J. Sawyer, H. Sarau, and R. G. Pendleton, *Pharmacologist*, 18, 209 (1976).

(54) R. G. Pendleton, J. Khalsa, G. Gessner, and J. Sawyer, *Nauyn-Schmiedeberg's Arch. Pharmacol.*, **299**, 219 (1977).

(55) J. W. Dubb, R. M. Stote, F. Alexander, A. P. Intoccia, M. Geczy, and R. G. Pendleton, *Clin. Pharmacol. Ther.*, **25**, 837 (1979).

Table IV. Comparison of in Vitro PNMT-Inhibitory Potency of Ring-Chlorinated Tetrahydroisoquinolines, Phenethylamines, and Benzylamines

substitution								
W	X	Y	Z	no.	IC_{50}, M[a]	R[b]	IC_{50}, M	IC_{50}, M
H	H	Cl	H	6	2×10^{-7}	CH_3	3×10^{-4} [c]	9×10^{-6} [d]
H	H	H	Cl	7	2×10^{-7}	CH_3	6×10^{-5} [c]	2×10^{-5} [d]
H	H	Cl	Cl	13	1×10^{-8}	H	2×10^{-5} [c]	6×10^{-7} [d]
Cl	H	Cl	Cl	14	3×10^{-7}	H[e]	1×10^{-4}, 80% [f]	
H	Cl	Cl	Cl	15	1×10^{-7}	CH_3[g]	1×10^{-4}, 39.9% [f]	
Cl	Cl	Cl	Cl	16	2×10^{-7}			

[a] Determined by in vitro PNMT inhibition test (secondary assay) as described under Experimental Section. [b] The presence of a methyl group on the α carbon of the side chain of a phenethylamine generally has little influence on PNMT-inhibitory potency (ref 22). [c] Reference 26. [d] Reference 29. [e] J. M. Osbond, G. A. Fothergill, H. Bruderer, and A. Rheiner, Jr., British Patent 1 109 924 (1968); *Chem., Abstr.*, 69, 51816c (1968). [f] Percent inhibition at indicated concentration (M) determined in the in vitro PNMT inhibition test (primary assay) as described under Experimental Section. [g] Reference 22.

analyses are reported by symbols of elements, results were within ±0.4% of calculated value. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. NMR spectra were recorded with either a Perkin-Elmer R-24 or R-32 spectrometer (Me_4Si). IR and NMR spectral data were obtained for all numbered or named compounds and were judged to be consistent with the assigned structures. 2-Chloro- and 4-chlorobenzaldehyde and 2,4-, 3,4-, and 3,5-dichlorobenzaldehyde were purchased. 2,3-Dichlorobenzaldehyde was prepared as described.[56] 2,5-Dichloro- and 2,3,4-trichlorobenzaldehyde were prepared as described under Experimental Section.

Chemistry. General methods are exemplified by specific examples.

Method A. 7,8-Dichloro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (13). A mixture of **25** (34 g, 0.14 mol) and PtO_2 (3.4 g) in 300 mL of MeOH at 25 °C was reduced with H_2 (60 psi) until uptake was completed. The mixture was filtered and the filtrate was evaporated to yield 33 g of crude **13**, mp 215–220 °C. [For other compounds prepared by this method (Table I), the crude product was purified by recrystallization of an appropriate salt from the indicated solvent.] Crude **13** was acetylated by heating with NaOAc (11.3 g, 0.14 mol), 200 mL of HOAc, and 50 mL of Ac_2O for 1 h on the steam bath. The mixture was poured into H_2O, made basic with NH_4OH and extracted with $CHCl_3$. Concentration of the $CHCl_3$ solution gave a residue which was recrystallized twice from 2-propanol to give 18.6 g (54%) of 2-acetyl-7,8-dichloro-1,2,3,4-tetrahydroisoquinoline, mp 100.5–102 °C. Anal. ($C_{11}H_{11}Cl_2NO$) C, H, N. The amide was suspended in 950 mL of 12 N HCl, and the mixture was refluxed for 4 h. The solution was evaporated and the residue crystallized to yield 16.7 g of a colorless, crystalline solid, **13**.

Method B. 5-Chloro-1,2,3,4-tetrahydroisoquinoline Maleate (4). A solution of 5-chloroisoquinoline (3.3 g, 0.02 mol) in 15 mL of THF and 120 mL of 1 M diborane (0.12 mol) in THF were refluxed in a N_2 atmosphere for 2.5 h and cooled. Excess diborane was destroyed by careful addition of MeOH, and the mixture was concentrated, treated with 10% aqueous HCl, and refluxed for 1 h. The mixture was cooled and extracted with EtOAc to yield the crude product, which was converted to the maleate and crystallized to give 1.8 g of **4**.

Method C. 7,8-Dichloroisoquinoline Hydrochloride (25). A mixture of 2,3-dichlorobenzaldehyde (79 g, 0.5 mol) and aminoacetaldehyde dimethyl acetal (53 g, 0.5 mol) in 800 mL of C_6H_6 was refluxed azeotropically until H_2O formation was completed. The solvent was evaporated to give a quantitative yield of 2,3-dichloro-*N*-(2,2-dimethoxyethyl)benzylidenamine. The benzylidenamine (50 g, 0.19 mol) was added dropwise to 600 mL of concentrated H_2SO_4 stirred at 140 °C. The temperature was maintained below 145 °C by the concurrent, gradual addition of a total of 200 mL of concentrated H_2SO_4. The mixture was stirred for 0.5 h at 140–150 °C and then it was *carefully* poured onto crushed ice. The acidic mixture was made strongly alkaline by the *careful* addition of NaOH (1200 g) in 1.2 L of H_2O with stirring and external cooling. The basic mixture was extracted with Et_2O. The ethereal solution was washed with brine, dried over Na_2SO_4, and evaporated to give 23.3 g of 7,8-dichloroisoquinoline. The isoquinoline was converted to the hydrochloride **25** (22 g).

Method D. 5,7,8-Trichloroisoquinoline (26). Chlorine gas (63 g, 0.89 mol) was bubbled through a stirred melt (105–110 °C) of isoquinoline (36 g, 0.28 mol) and aluminum chloride (75 g, 0.56 mol) during 18 h. The hot mixture was *very carefully* poured onto 1 L of crushed ice, made strongly basic with 40% aqueous NaOH, and filtered. The filter cake was refluxed with 600 mL of acetone and filtered. Recrystallization of the solid twice from cyclohexane gave 1.5 g of **26**.

5,6,7,8-Tetrachloroisoquinoline (28) was also prepared by method D. In this case, Cl_2 (96 g, 1.35 mol) was bubbled through a stirred melt (110–115 °C) of isoquinoline (36 g, 0.28 mol) and aluminum chloride (75 g, 0.56 mol) for 12 h and worked up as described for **26**. The aqueous alkaline solution was extracted with toluene, and the extracts were evaporated to yield 52 g of a solid. The solid was chromatographed on silica gel eluted with cyclohexane containing increasing amounts of EtOAc. Fractions containing **28** were combined, evaporated, and recrystallized three times from cyclohexane to give 1.5 g of **28**.

Methyl 2-Bromo-3-(2,3-dichlorophenyl)propionate. A mixture of 2,3-dichloroaniline (162 g, 1 mol), 2 L of acetone, and 320 mL of 48% hydrobromic acid stirred at −5 °C was treated over 30 min with a solution of sodium nitrite (84 g, 1.22 mol) in 200 mL of H_2O. Methyl acrylate (860 g, 10 mol) and cuprous bromide (0.15 g, 0.5 mmol) were added. The internal temperature was allowed to rise in a carefully controlled manner; at 7 °C the evolution of N_2 was vigorous. When the rate of N_2 evolution decreased, the mixture was stirred at 25 °C for 30 min to complete the reaction. The mixture was evaporated, and the residue was partitioned between H_2O and toluene to give 312 g (99%) of crude methyl 2-bromo-3-(2,3-dichlorophenyl)propionate, which was used in the next reaction without purification.

3-(2,3-Dichlorophenyl)propionic Acid (29). Crude methyl 2-bromo-3-(2,3-dichlorophenyl)propionate (312 g, ca. 1 mol) in 1325 mL of HOAc was stirred and treated with Zn dust (131 g, 2 mol) in portions over 30 min. The mixture was stirred for an additional 30 min and filtered, and the filter cake was washed with HOAc. After the filtrate was evaporated, the residue was partitioned between $CHCl_3$ and H_2O to give 233 g (99%) of crude methyl 3-(2,3-dichlorophenyl)propionate, which was refluxed with 1.6 L of 10% aqueous NaOH for 2 h, cooled, treated with decolorizing carbon, and filtered. The filtrate was acidified with 3 N HCl and filtered. The dry filter cake was recrystallized from

(56) G. Lock, *Monatsch. Chem.*, **90**, 683 (1959).

toluene to give 93.3 g (43%) of 3-(2,3-dichlorophenyl)propionic acid, mp 117–118 °C, and 54.6 g (25%) of a second crop, mp 109–116 °C (lit.[57] mp 114 °C).

5,6-Dichloro-3,4-dihydro-1(2*H*)-isoquinolinone. (30). A mixture of **29** (21.9 g, 0.1 mol) and 0.4 mL of DMF in 190 mL of toluene was treated with $SOCl_2$ (15.1 g, 0.13 mol) and then stirred at 55–60 °C for 2.5 h. The mixture was cooled, filtered, and evaporated. The acid chloride in 350 mL of Me_2CO was added, dropwise, to a mixture of NaN_3 (18.2 g, 0.28 mol) in 60 mL of H_2O stirred at 0 °C. The mixture was stirred for an additional 10 min, poured into ice–water, and extracted with toluene. The toluene extract was dried over $MgSO_4$ in the cold, filtered, and heated gradually on the steam bath. After 30 min the toluene was evaporated and 21.5 g (99%) of the crude isocyanate was added to a mixture of $AlCl_3$ (27.8 g, 0.21 mol) and 200 mL of tetrachloroethylene. The mixture was stirred at 70–75 °C for 3 h, cooled, carefully treated with 42.5 mL of 3 N HCl, and filtered to give 10.4 g (50%) of **30**, which was used without purification. A small sample was recrystallized from ethanol, mp 202–204 °C. Anal. ($C_9H_7Cl_2NO$) C, H, N.

5,6-Dichloro-1,2,3,4-tetrahydroisoquinoline Hydrochloride (8). A mixture of **30** (9.3 g, 0.043 mol) and 50 mL of dry THF was stirred at 5 °C and treated with 150 mL of 1 M BH_3. The mixture was refluxed for 18 h, cooled, and carefully treated with 8 mL of H_2O and then with 40 mL of 3 N HCl. The mixture was filtered to yield 6.1 g of **8**. An additional 3.3 g of **8** was obtained from the filtrate by basification, extraction with $CHCl_3$, evaporation, and treatment with ethereal HCl. Recrystallization gave 8.3 g (81%) of **8**.

2,5-Dichlorobenzaldehyde. 2,5-Dichlorobenzyl alcohol (35.4 g) and manganese dioxide (272 g) in 700 mL of CH_2Cl_2 were stirred for 24 h. The mixture was filtered and the CH_2Cl_2 evaporated to give 26.2 g (78%) of 2,5-dichlorobenzaldehyde, mp 57–59 °C (lit.[58] mp 54–56 °C).

2,3,4-Trichlorobenzaldehyde. 2,3,4-Trichloroaniline was diazotized and the diazonium salt was treated with cuprous cyanide as described[59] to give 2,3,4-trichlorobenzonitrile, which was hydrolyzed with refluxing hydrochloric acid to yield 2,3,4-trichlorobenzoic acid, mp 189 °C, lit.[60] mp 187–188 °C. The acid was reduced with diborane in THF to give 2,3,4-trichlorobenzyl alcohol, mp 106–106.5 °C. Anal. ($C_7H_5Cl_3O$) C, H. The alcohol was oxidized with manganese dioxide, as described for the preparation of 2,5-dichlorobenzaldehyde, to give 2,3,4-trichlorobenzaldehyde, mp 83–83.5 °C (lit.[6] mp 83 °C).

Pharmacology. Method A. In Vitro PNMT Inhibition Test. Primary Assay. This test was performed as previously described.[39,49] Lyophilized, partially purified rabbit adrenal PNMT was obtained commercially from Gallard-Schlesinger Co., where it was prepared by previously described methods.[61] The enzyme was solubilized in potassium phosphate buffer and the reaction run in 300 μL constituted as follows: PNMT, 280 μg; phosphate buffer (pH 7.4), 50 nmol; (–)-NE, 9 nmol; and [*methyl*-^{14}C]SAM (ca. 20 000 dpm), 9 nmol. The reaction was run for 15 or 30 min at 37 °C and then it was terminated by the addition of 1 N HCl (200 μL). Approximately 1 g of solid NaCl was then added and the solution was extracted with 6 mL of acid-washed NaCl-saturated butanol. One milliliter of the butanol layer, containing labeled E, was then added to 10 mL of an aqueous 2,5-bis[2′-(5′-*tert*-butylbenzoxazolyl)]thiophene (BBOT) phosphor, counted in a Tricarb liquid scintillation spectrophotometer for 10 min, and quantitated in terms of nanomoles of E produced. Inhibition values were obtained by comparing the decreased E production in samples containing concentrations of 10^{-4} and 10^{-6} M test compound with controls and are the average of four determinations.

Secondary Assay. This test was performed as previously described.[50] Rabbit adrenal PNMT was solubilized in potassium phosphate buffer, and the reaction was conducted in a total volume of 300 μL constituted as follows: PNMT, 42 μg; potassium phosphate buffer (pH 7.9), 10 μmol; DL-phenylethanolamine, 90 nmol (3×10^{-4} M); [*methyl*-^{14}C]SAM (ca. 20 000 dpm), 9 nmol (3×10^{-5} M). The reaction was run for 15 min at 37 °C and was terminated by the addition of 0.5 mL of 0.5 M borate buffer, pH 10. The ^{14}C product was extracted into 6 mL of toluene–isoamyl alcohol (97:3). One milliliter of the organic layer was added to 10 mL of liquid scintillation cocktail, counted in a Nuclear-Chicago scintillation counter for 10 min, and quantitated in terms of the nanomoles of *N*-methylphenylethanolamine produced. Percent inhibition was determined by comparison of the quantity of *N*-methylphenylethanolamine formed in the presence of various concentrations of test compound with controls. Concentrations causing 50% inhibition of PNMT, IC_{50}, were derived graphically from at least four such measurements, one of which produced less than 50% inhibition. In each case, the slope of the curve approached 0.576. K_i values for **6**, **7**, and **13–16** were calculated from the IC_{50} values as described.[50] The phenylethanolamine K_m value used in this calculation was 95 nM.

Method B. Inhibition of Tritiated Epinephrine Biosynthesis. The details of this procedure have been previously reported.[39] Male Charles River rats, weighing 190 to 260 g, were divided into groups of five and dosed orally with three doses of inhibitor in 1% methylcellulose or vehicle over a 24-h period. The dose volume was 10 mL/kg. One hour after administration of the third dose of the inhibitor, each animal was injected intravenously with 50 μCi of (–)-[7-^{3}H]NE, specific activity 5–10 Ci/μmol. Each rat received a total NE dose of approximately 1 μg. Fifteen minutes after the [^{3}H]NE injection, the animals were sacrificed, and the adrenals were removed, homogenized in 5 mL of 0.4 N $HClO_4$, and assayed for both endogenous and radioactive NE and E content. Percent decrease in E biosynthesis was determined by comparison of adrenal [^{3}H]E content of drug-treated animals with that of controls. In each case, the total number of counts present in the tissue in the form of E and NE did not differ significantly from control levels. An ED_{50}, i.e., the unit dose of compound causing 50% inhibition of E biosynthesis, was estimated for **13** as previously described.

Method C. Adrenal Epinephrine/Norepinephrine Ratio. Male Charles River mice, weighing from 30 to 40 g, were divided into groups of five and were dosed orally with inhibitor homogenized in 1% methylcellulose or vehicle. The dose volume was 10 mL/kg. The animals were dosed twice a day at approximately 8 a.m. and 5 p.m. for 7 consecutive days, which included a final dose on the morning of the 8th day of dosing. Two hours after the conclusion of the chronic dosing period, the animals were sacrificed by decapitation. The adrenals were removed, weighed, and homogenized in 10 mL of 0.4 N $HClO_4$. An aliquot of the extract was passed through an alumina column at pH 8.2–8.6. The catechol derivatives were then eluted with 0.2 M HOAc. E and NE were determined as described previously.[62] A compound was presumed active as a PNMT inhibitor if it significantly ($p > 0.05$) decreased the endogenous adrenal E/NE ratio.[39] Unit minimum active doses (MAD) and maximum inactive doses (MID) were determined for **6**, **7**, and **13–16** (Table III).

(57) F. Mayer, H. Philipps, F. W. Ruppert, and A. T. Schmitt, *Chem. Ber.*, **61**, 1966 (1928).
(58) A. J. Boulton, P. B. Ghosh, and A. R. Katritsky, *J. Chem. Soc. B*, 1011 (1966).
(59) H. Koopman, *Recl. Trav. Chim. Pays-Bas*, **80**, 1075 (1961).
(60) H. C. Brimelow, R. L. Jones, and T. P. Metcalfe, *J. Chem. Soc.*, 1208 (1951).
(61) J. K. Saelens, M. S. Schoen, and G. B. Kovascics, *Biochem. Pharmacol.*, **16**, 1043 (1967).
(62) R. W. Weedon and A. R. Maass, *Adv. Autom. Anal., Technicon Int. Congr., 7th, 1976*, **2**, 263–268 (1977).