$H_5C{\equiv}CCH_2C(CH_3)(CO_2C_2H_5)_2$, 83067-48-5; $C_2H_5C{\equiv}CCH_2CH(CH_3)CO_2C_2H_5$, 99783-80-9.

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Received September 30, 1985

Unsaturated Heterocyclic Amines as Potent Time-Dependent Inhibitors of Dopamine β-Hydroxylase

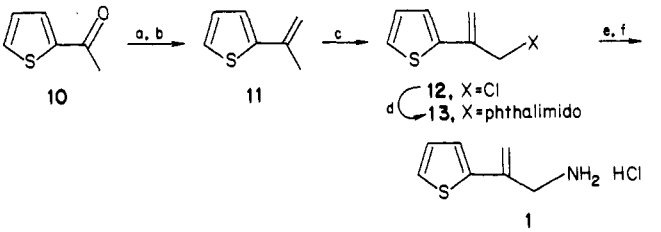
Sir:

Dopamine β-hydroxylase (DBH; EC 1.14.17.1), a copper-dependent monooxygenase, catalyzes the conversion of dopamine to norepinephrine in the peripheral sympathetic as well as in the central nervous systems.[1] The enzyme is easily inhibited by copper chelators, but these types of inhibitors lack selectivity; the most notable example is fusaric acid, which was studied in the clinic for the treatment of hypertension.[2,3] Recently, a number of enzyme-activated inhibitors of DBH have been reported in the literature: β-chlorophenethylamine,[4] 4-hydroxybenzyl cyanide,[5] 2-halo-3-(*p*-hydroxyphenyl)-1-propenes,[6] 1-phenyl-1-propyne (**9**),[7] and 2-phenylallylamine (**8**).[8] Despite their progressive increases in activity as time-dependent inhibitors, the most effective of these compounds remains in the millimolar potency range and none have been reported to exhibit antihypertensive activity.

We report that, contrary to previous belief,[9] certain heteroaromatic amines can serve as substrates[10] and as exceptionally potent time-dependent inhibitors of dopamine β-hydroxylase. Indeed, 2-(2-thienyl)allylamine hydrochloride (**1**) exhibited a greater than 1000-fold enhancement in activity over the corresponding phenyl analogue (**8**)[8] and has antihypertensive activity in the spontaneously hypertensive rat (SHR). In addition, we report that 3-phenylpropargylamine (**7**) is equipotent to **1** as a time-dependent inhibitor of DBH.

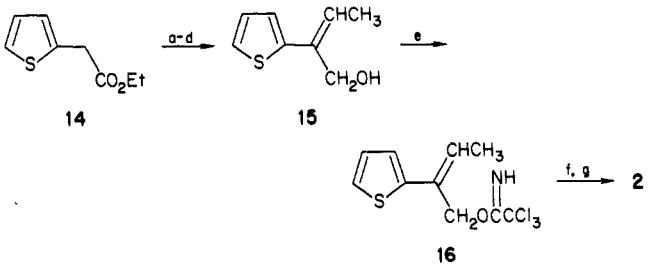
Chemistry.[11] 2-(2-Thienyl)allylamine (**1**) was prepared as outlined in Scheme I. 2-Acetylthiophene (**10**) was allowed to react with methylmagnesium bromide and the resulting alcohol dehydrated to 2-isopropylidenethiophene (**11**). The allylic chlorination procedure of Hori and Sharpless[12] was used to provide a mixture of allyl chloride **12** and vinyl chlorides, which was immediately allowed to react with potassium phthalimide. Deprotection of the highly crystalline phthalimide **13** provided the desired amine **1**. The 3-thienyl regioisomer **4** and the 2- and 3-furanylallylamines **5** and **6** were prepared by the same reaction sequences with the exception that the synthesis of **6** started with ethyl 3-furoate. This starting material was converted to the corresponding tertiary alcohol with 2 equiv of methylmagnesium bromide and dehydrated as in the preparation of **11**. *N*-Methylallylamine **3** was prepared by reaction of *N*-methyltrifluoracetamide with purified allyl chloride **12** (NaH/DMF/80 °C) and hydrolysis of the resulting allyl trifluoacetamide during workup (1 N NaOH).

Scheme I[a]



[a] (a) CH_3MgBr, (b) $KHSO_4$, (c) NCS, $(PhSe)_2$ (cat.), pyr (cat.), (d) potassium phthalimide/DMF, 90 °C, (e) $NH_2NH_2{\cdot}H_2O$/EtOH, (f) HCl/Et_2O.

Scheme II[a]



[a] (a) LDA/THF/−70 °C, then CH_3CHO, (b) MsCl/Et_3N/CH_2Cl_2/20 °C, (c) DBU/CH_2Cl_2/20 °C, (d) 2 *i*-Bu_2AlH/CH_2Cl_2/−70 °C, (e) CCl_3CN/CH_2Cl_2/DBU (cat.)/20 °C, (f) xylene, reflux, (g) KOH/EtOH/40 °C.

α-Methylallylamine **2** was synthesized according to Scheme II. 2-Thiopheneacetic acid ethyl ester **14** was deprotonated and the resulting enolate was trapped with acetaldehyde to furnish a mixture of diastereomeric alcohols. The crude alcohols were converted to a geometric mixture of olefin esters which were reduced to the corresponding allyl alcohols **15** with diisobutylaluminum hydride. Alcohols **15** were treated with trichloroacetonitrile in the presence of a catalytic quantity of DBU to furnish trichloroacetimidates **16**. Rearrangement was effected by using Overman's methodology[13] to provide the corresponding allyltrichloroacetamide. Base-promoted hydrolysis yielded the desired α-methylallylamine **2**.

3-Phenylpropargylamine (**7**) was prepared by the literature procedure.[14]

Biochemistry and Pharmacology. DBH was purified from beef adrenals following a described procedure.[15] The enzyme was homogeneous in SDS gel electrophoresis and

(1) Kaufman, S.; Friedman, S. *Pharm. Rev.* **1965**, *17*, 71.
(2) Pieschi, L.; Oehlke, J.; Schoretter, E.; Oehme, P. *Pharmazie* **1983**, *38*, 335.
(3) Hidaka, H. *Nature (London)* **1971**, *231*, 54.
(4) Mangold, J. B.; Klinman, J. P. *J. Biol. Chem.* **1984**, *259*, 7772.
(5) Colombo, G.; Rajaskekar, B.; Giedioc, D. P.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 1593.
(6) (a) Rajashekar, B.; Fitzpatrick, P. F.; Colombo, G.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 6925. (b) Fitzpatrick, P. F.; Flory, D. R.; Villafranca, J. J. *Biochemistry* **1985**, *24*, 2108.
(7) Colombo, G.; Villafranca, J. J. *J. Biol. Chem.* **1984**, *259*, 15017.
(8) May, S. W.; Mueller, P. W.; Padgette, S. R.; Herman, H. H.; Philips, R. S. *Biochem. Biophys. Res. Commun.* **1983**, *110*, 161.
(9) Creveling, C. R.; van der Schoot, J. B.; Udenfriend, S. *Biochem. Biophys. Res. Commun.* **1962**, *8*, 215.
(10) The 2- and 3-substituted thiophene and furan ethylamines serve as substrates for DBH with V_{max}/K_m values in the range of that for tyramine. These results will be discussed in the full paper.
(11) All new compounds gave satisfactory elemental analyses and IR, NMR, and mass spectra consistent with the assigned structures.
(12) Hori, T.; Sharpless, K. B. *J. Org. Chem.* **1979**, *44*, 4204.
(13) Overman, L. E. *J. Am. Chem. Soc.* **1976**, *98*, 2901.
(14) Klemm, L. M.; McGuire, T. M.; Gopinath, K. W. *J. Org. Chem.* **1976**, *41*, 2571.
(15) Aunis, D.; Murias-Portugal, M. T.; Mander, P. J. *Neurochemistry* **1975**, *24*, 425.

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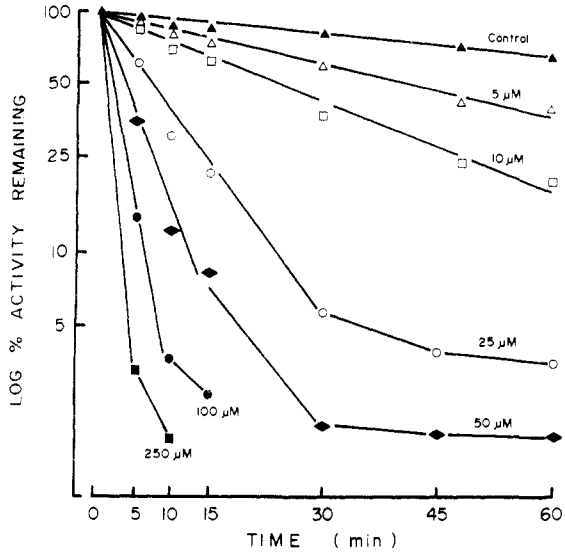


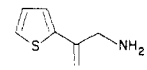
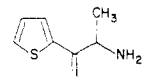
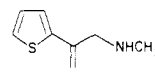
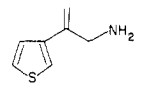
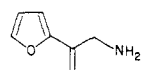
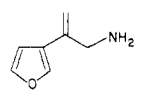
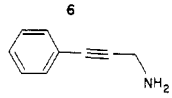
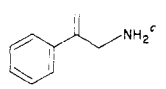
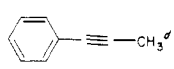
Figure 1. Time- and concentration-dependent inhibition of DBH by 1. Purified DBH was incubated with various concentrations of 1 under conditions described in the text. At indicated times, aliquots were withdrawn and tested for remaining enzyme activity with use of tyramine as substrate.

hydrophobic HPLC (Ultrasphere, TSK 5PW). It was analyzed by following the formation of octopamine from tyramine by reverse-phase HPLC with fluorescent detection (0.2 M acetate, pH 5, 20 mM sodium fumarate, 10 mM ascorbic acid, 1300 units catalase, 2.5 μM $CuSO_4$, 1 mM tyramine, under air at 37 °C). Under these conditions, this preparation had a specific activity of 7 μmol of octopamine formed (mg of protein)$^{-1}$ min^{-1}. Time-dependent inhibition was measured by incubating the enzyme with inhibitor under standard conditions, except for the absence of tyramine and diluting aliquots into the assay medium.

Upon incubation of purified DBH in air with compounds 1–7 there was time-dependent loss of enzyme activity. For instance, Figure 1 shows the pseudo-first-order decay (at least for 3 half-lives of enzyme activity) upon incubation of DBH with 1 at concentrations ranging from 5 to 250 μM. This inhibition is ascorbate dependent and is not reversed by dilution nor by extensive dialysis. The substrates tyramine and dopamine protect against inactivation of enzyme by 1. Furthermore, when incubation was carried out under nitrogen instead of air, there was no decrease of enzyme activity (not shown). The detailed mechanism will be studied further; nevertheless, the available evidence is consistent with an enzyme-activated type mechanism of inhibition as postulated for 8.[8] By plotting $t_{1/2}$ as a function of $1/I$ as in a Kitz and Wilson plot,[16] the kinetic parameters K_I and k_{cat} for compounds 1–7 can be estimated. These data as well as the calculated k_{cat}/K_I are reproduced in Table I. This value, which to a first approximation represents the rate of enzyme inhibition at concentrations of inhibitor (I) much smaller than K_I, was used to compare potencies.

It appears that compounds 1 and 7 are about equipotent and at least 1000 times more active than the reported phenylallylamine.[8] A clear structural relationship appears from Table I for compounds 1–6. Neither the nature of the heterocycle, the position of the allylamine unit on the ring, the side-chain substitution, nor amine alkylation had much influence on k_{cat} (or on half-life at saturating concentrations). This was not unexpected as the mechanism of activation of compounds 1–6, and the steps involved in the inhibition are presumably the same. However, these same parameters greatly influenced the apparent affinity of the compounds for the enzyme. The 2-thiophene derivative 1 has the highest affinity, substitution of a methyl group α to the amine decreased affinity by a factor of 10, while N-methylation was particularly detrimental as it decreased affinity over 60-fold. Moving the allylamine unit from position 2 to 3 on the thiophene ring caused a loss of affinity of 15-fold, while in the furan series the same shift caused a gain in affinity. Replacing the sulfur by oxygen decreased affinity.

Table I. Physical and Kinetic Constants of Dopamine β-Hydroxylase Inhibitors

compd[a]	K_I,[e] μM	k_{cat},[e] min^{-1}	k_{cat}/K_I, M^{-1} min^{-1}	mp, °C
 1	35	0.124	3540	140–145 dec
 2	360	0.058	161	182–183
 3	2150	0.098	44	158–160
 4	570	0.147	257	181–182
 5	410	0.130	317	150–151
 6	230	0.077	334	151–152
 7	21	0.07	3333	215[b]
 8	13000	0.04	3	
 9	~20000	0.06	6	

[a] Hydrochloride salts, with the exception of 9. [b] Lit.[14] mp 216–217 °C. [c] Kinetic constants obtained from ref 8. [d] Kinetic constants obtained from ref 7. [e] K_I and k_{cat} were calculated from plots of $t_{1/2}$ (50% DBH activity remaining at a given concentration of I, as in Figure 1) vs. $1/I$, where the y intercept = $0.693/k_{cat}$ and the x intercept = K_I.

The activity of the acetylenic amine 7 is in contrast to that of *trans*-cinnamylamine, which is a "poor substrate of DBH with indications of time-dependent inactivation",[8] since it is a potent time-dependent inhibitor of DBH. It should be noted that, very recently, the acetylenic hydrocarbon analogue of 7, 1-phenyl-1-propyne (9), was reported to be a time-dependent inhibitor of DBH.[7] The reported k_{cat} for 9 is 0.06 min^{-1} (0.07 for compound 7), while the K_I of 7 is 21 μM as opposed to approximately 20 000 μM for 9. This enormous difference in apparent affinity underlines the importance of the amine function for binding.

Compounds 1–7 demonstrated antihypertensive activity after intraperitoneal (ip) administration to SHR's (Table

(16) Kitz, R.; Wilson, T. B. *J. Biol. Chem.* **1962**, *237*, 3245.

Table II. Antihypertensive Activity of DBH Inhibitors in Conscious Spontaneously Hypertensive Rats

compd	dose, mg/kg, ip	n	max MBP,[a] mmHg	duration, h
1	10	4	−19 ± 11[b]	1
	30	5	−36 ± 28	43
	100	5	−34 ± 13	70
2	10	5		
	30	5	−16 ± 5	9
	100	4		
3	30	5	−23 ± 12	>1
	100	4	−16 ± 7	*c*
4	30	5	−18 ± 12	1
	100	4	−54 ± 20	2
5	10	4	−10 ± 6	1
	30	4	−26 ± 5	30
	100	6	−69 ± 29	79
6	30	5	−20 ± 15	6
	100	4	−21 ± 16	3
7	30	5	−13 ± 8	3
	100	4	*c*	

[a] Mean arterial blood pressure. [b] Mean ± SD. [c] Toxic.

II). Hypotension occurs rapidly after administration of all compounds but is of short duration. A second hypotensive response follows for **1** and **5**, which is slow in onset with long duration. The position of the allylamine unit on the ring, side-chain substitution, and amine alkylation influenced antihypertensive activity. Antihypertensive effects and duration were dose related for compounds **1** and **5** (2-substituted heterocycles) while 3-substituted heterocyclic compounds **4** and **6** only exhibited transitory hypotension. Side-chain substitution resulted in loss of activity (**2**) and/or toxicity (**3**). Compound **7** was also toxic. Studies are in progress to determine whether these antihypertensive effects can be correlated with inhibition of norepinephrine synthesis.

In conclusion, we have found that two classes of unsaturated amines represented by **1** and **7** exhibit potent time-dependent inhibition of DBH. Of equal importance is the finding that heteroaromatic furan and thiophene rings can be used in place of phenyl to produce dramatic increases in potency for the inhibition of this enzyme. The implications of these results for the study of new therapeutic approaches to diseases that may be interdicted by the inhibition of DBH is noteworthy.[17]

Acknowledgment. We thank Professors Sheldon W. May (Georgia Institute of Technology) and Michael Johnston (University of Chicago) for helpful discussions. In addition, we thank Eileen F. Oglesby for technical assistance.

Registry No. **1**, 99605-71-7; **1**·HCl, 99605-88-6; **2**, 99605-72-8; **2**·HCl, 99605-83-1; **3**, 99605-73-9; **3**·HCl, 99605-84-2; **4**, 99605-74-0; **4**·HCl, 99605-85-3; **4** (phthalimide), 99605-80-8; **5**, 99605-75-1; **5**·HCl, 99605-86-4; **5** (phthalimide), 99605-81-9; **6**, 99605-76-2; **6**·HCl, 99605-87-5; **6** (phthalimide), 99605-82-0; **7**, 78168-74-8; **7**·HCl, 30011-36-0; **8**, 28144-67-4; **8**·HCl, 56132-76-4; **9**, 673-32-5; **10**, 88-15-3; **11**, 30616-73-0; **12**, 99605-77-3; **13**, 99605-78-4; **14**, 57382-97-5; **15**, 99605-79-5; **16**, 99617-55-7; 2-(2-thienyl)-2-propanol, 5331-62-4; 2-(3-furanyl)-2-propanol, 6137-75-3; 3-isopropenylfuran, 6137-69-5; *N*-methyltrifluoroacetamide, 815-06-5; dopamine β-hydroxylase, 9013-38-1; trichloroacetonitrile, 545-06-2; potassium phthalimide, 1074-82-4; ethyl 3-furoate, 614-98-2.

(17) **Note Added in Proof.** After the submission of this manuscript, May and co-workers (Padgette, S. W.; Wimalasena, K.; Herman, H. H.; Sirimanne, S. R.; May, S. W. *Biochemistry* **1985**, *24*, 5826) reported **7** to be a potent time-dependent inhibitor of DBH with a k_{cat}/K_I = 1700 M^{-1} min^{-1} in their enzyme preparation and allude to a more complex process for its inactivation of DBH. In addition, they report the *p*-hydroxy analogue of **8** to be a potent time-dependent inhibitor of DBH.

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