Experimental Section§

N-Acetyl-(*o*-nitrophenyl)-DL-alanine. *o*-Nitrophenyl-DL-alanine (60.0 g) was treated with 26.1 g of Ac_2O in aq 1 *N* NaOH soln at 0.5° to yield 49.0 g (80%) of product after acidification, mp 203–205° (lit. mp 205–206° *via* different procedure[4]).

N-Acetyl-(*o*-nitrophenyl)-D- and -L-alanine Brucinates. A mixt of 32.0 g of racemic *N*-acetyl-*o*-nitrophenylalanine, 50.0 g of recrystd brucine hydrate, and 600 ml of MeOH was continuously heated with stirring until soln was effected. The soln was concd to about 0.6 of its original vol by evapn of the MeOH *in vacuo*. After standing at −17° overnight, a cryst ppt was formed, filtered, washed with a small portion of MeOH, and dried to weigh 37.7 g. Recrystn from a minimum amt of boiling MeOH gave 31.0 g (77%) of the brucine salt of *N*-acetyl-(*o*-nitrophenyl)-D-alanine, mp 210–212° $[\alpha]^{21}D$ −26.0° (*c* 1, H_2O) and −14.2° (*c* 1, MeOH). *Anal.* ($C_{34}H_{38}N_4O_9$) C, H.

The resoln mother liquor was concd to about 0.8 of its vol by removal of MeOH *in vacuo*, and stored at −17° overnight. There was recovered 4.0 g of ppt which melted over a wide range. In order to avoid any contamination of the more sol brucinate salt by small and varying amts of the D isomer, this crop was discarded. The resulting filtrate was reduced to about 0.8 of its vol by evapn *in vacuo*, chilled at −17° overnight, and yielded 25.7 g of the *N*-acetyl-(*o*-nitrophenyl)-L-alanine brucinate. The product was dissolved in a minimum amt of boiling MeOH treated with Norite, and recrystd to yield 21.7 g (54%), mp 188–191°, after drying *in vacuo* over P_2O_5; $[\alpha]^{21}D$ −12.0° (*c* 1, H_2O) and −7.3° (*c* 1, MeOH). *Anal.* ($C_{34}H_{38}N_4O_9$) C, H.

N-Acetyl-*o*-nitrophenyl-D-alanine. A 30.9-g sample of *N*-acetyl-*o*-nitrophenyl-D-alanine brucinate was treated with 200 ml of 1 *N* NH_4OH, and the resulting mixt was extd with 100 ml of $CHCl_3$. The $CHCl_3$ layer was sepd from the aq layer, and the latter was extd twice with 40-ml portions of $CHCl_3$ to remove brucine. The aq ammonical layer was taken to dryness *in vacuo*, and the residue was dissolved in 50 ml of H_2O. The resulting soln was adjusted to pH 1 with addn of concd HCl to form a ppt. This was collected on a filter, washed with cold H_2O, and dried to yield 10.9 g (90%) of product. A sample, when recrystd from EtOH–H_2O, had mp 201–202°; $[\alpha]^{21}D$ −42.0° (*c* 1, CH_3OH). *Anal.* ($C_{11}H_{12}N_2O_5$) C, H.

N-Acetyl-*o*-nitrophenyl-L-alanine. The same prep procedure as described above was repeated using 21.6 g of *N*-acetyl-*o*-nitrophenyl-L-alanine brucinate, which was decompd with NH_3 and freed of alkaloid to give 7.1 g (84%) of product. After recrystn from EtOH–H_2O, the compd was analytically pure, mp 201–202°, $[\alpha]^{21}D$ +42.0° (*c* 1, MeOH). *Anal.* ($C_{11}H_{12}N_2O_5$) C, H.

o-Nitrophenyl-D-alanine Hydrochloride (I). A soln of 8.87 g of *N*-acetyl-*o*-nitrophenyl-D-alanine in 90 ml of concd HCl was heated under reflux for 3 hr. After the reaction mixt was allowed to chill at −17° overnight, crystals of the HCl salt separated. Filtration, washing with cold H_2O, and drying gave 5.04 g (58%) of the product. An analytical sample was obtd by recrystn from MeOH–Et_2O, mp 223–224° dec; $[\alpha]^{21}D$ −16.5° (*c* 0.5, H_2O) and $[\alpha]^{21}D$ −41.1° (*c* 0.5, 1 *N* HCl). *Anal.* ($C_9H_{10}N_2O_4 \cdot HCl$) C, H.

o-Nitrophenyl-L-alanine hydrochloride (II) was prepd exactly as described for the D isomer except that 5.40 g of *N*-acetyl-*o*-nitrophenyl-L-alanine was employed. There was recovered 3.32 g (63%) of product, which was purified by recrystn from MeOH–Et_2O, mp 223–224° dec; $[\alpha]^{21}D$ +16.3° (*c* 0.5, H_2O) and $[\alpha]^{21}D$ +41.6° (*c* 0.5, 1 *N* HCl). *Anal.* ($C_9H_{10}N_2O_4 \cdot HCl$) C, H.

D-3-Amino-3,4-dihydro-1-hydroxycarbostyril (III). Using a similar procedure previously described for the synthesis of racemic 3-amino-3,4-dihydro-1-hydroxycarbostyril,[4] a 1.0-g sample of *o*-nitrophenyl-D-alanine hydrochloride (I) was hydrogenated in the presence of Pt on C to give the hydrochloride of III, which was subsequently converted with NH_4OH to yield 560 mg (77%) of III, mp 202–203° dec, $[\alpha]^{21}D$ +63° (*c* 1, 0.1 *N* HCl). *Anal.* ($C_9H_{10}N_2O_2$) C, H, N.

L-3-Amino-3,4-dihydro-1-hydroxycarbostyril (IV). Repeating the same condns of catalytic hydrogenation as above a 500-mg sample of *o*-nitrophenyl-L-alanine hydrochloride (II) was converted to 290 mg (80%) of product, mp 202–203° dec, $[\alpha]^{21}D$ −64° (*c* 1, 0.1 *N* HCl). *Anal.* ($C_9H_{10}N_2O_2$) C, H, N.

o-Aminophenyl-D-alanine (V). Using the method previously described for the synthesis of racemic *o*-aminophenylalanine,[7] 500 mg of the free base of I was hydrogenated to give 230 mg (51%) of product, mp 160–163° dec, $[\alpha]^{21}D$ +55.1° (*c* 0.5, 50% MeOH). *Anal.* ($C_9H_{12}N_2O_2 \cdot 0.5H_2O$) C, H, N.

o-Aminophenyl-L-alanine (VI). By the same procedure, 400 mg of the free base of II was converted to 190 mg (55%) of product, mp 165–166° dec, $[\alpha]^{21}D$ −54.2° (*c* 0.5, 50% in MeOH). *Anal.* ($C_9H_{12}N_2O_2$) C, H, N.

D-3-Amino-3,4-dihydrocarbostyril Hydrochloride (VII). A procedure was used similar to that previously described for the synthesis of racemic 3-amino-3,4-dihydrocarbostyril hydrochloride,[7] with the exception that V was not isolated. A 370-mg sample of the free base of I was hydrogenated to give a soln of V. The catalyst was removed by filtration and the filtrate was treated with 1 ml of concd HCl. The resulting soln was taken to dryness *in vacuo* to give a solid residue. Recrystn from MeOH–Et_2O gave 245 mg (70%) of product, mp 322–323° dec, $[\alpha]^{21}D$ +127.0° (*c* 0.5, H_2O). *Anal.* ($C_9H_{10}N_2O \cdot HCl$) C, H, N.

L-3-Amino-3,4-dihydrocarbostyril Hydrochloride (VIII). In a similar manner, 230 mg of the free base of II was converted to 150 mg (69%) of product, mp 322–323° dec, $[\alpha]^{21}D$ −125.0° (*c* 0.5, H_2O). *Anal.* ($C_9H_{10}N_2O \cdot HCl$) C, H, N.

Microbiological Assays. For *E. coli* 9723 and *L. dextranicum* 8086 a similar assay procedure was used as described previously.[4] In all assays the amt of growth was detd photometrically at 625 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer in terms of absorbance readings of the turbid culture medium against a blank of uninoculated medium set at 0 absorbance.

§Melting points were determined on a Thomas-Hoover apparatus and are corrected. Optical activity readings were taken with a Schmidt-Haensch precision polarimeter. Elementary analyses were performed by the M-H-W Laboratories, Garden City, Mich. Where analyses are indicated only by symbols of the elements, analytical results obtained by those elements were within ±0.4% of the theoretical values.

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Inhibition of Dopamine β-Hydroxylase by 5-Phenoxymethyl-2-oxazolidinethiones

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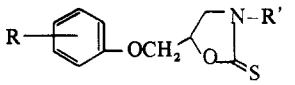
The bioconversion of dopamine (DA) to norepinephrine (NE) in the CNS and in the sympathetic nerve network is catalyzed by dopamine β-hydroxylase, a Cu-containing enzyme.[1] Inhibition of this enzyme in the brain results in the depletion of endogenous NE while DA stores remain intact.[2,3] Although several different classes of dopamine β-hydroxylase inhibitors have been reported, each of which acts through the chelation of the Cu^{+2}, none has yet found clinical use. Their principal utility to date has been as pharmacologic tools in attempts to elucidate the respective roles of DA and NE on behavior and mental function.

The inhibition of dopamine β-hydroxylase *in vitro* and *in vivo* with alkyl and aromatic thioureas was recently described.[4] Several groups of investigators have noted the *in vivo* dopamine β-hydroxylase inhibitory activity of various mono- and disubstituted dithiocarbamates[5-8] and the resulting alterations in behavioral responses in laboratory animals concurrent with the depletion of brain NE.[7,9,10] The iso-

Table I. Dopamine β-Hydroxylase Inhibition by 5-Phenoxymethyl-2-oxazolidinethiones[a]

Compd	R	R'	*In vitro* inhibition % of control	*In vivo* inhibition Rat brain norepinephrine after 200 mg/kg, ip[b] % of control 2 hr	4 hr	16 hr
						
1	H	H	73			
2	*p*-Cl	H	72		54	37
3	*m*-Cl	H	46	97	54	53
4[e,f]	2,5-Cl_2	H	49	86	77	78
5	*m*-Br	H	21	112		
6	*m*-F	H	100			
7	*m*-CH_3	H	37	78	49[c]	
8	*m*-$(CH_3)_3C$	H	56	79		
9	*p*-OCH_3	H	61			
10	*m*-OCH_3	H	76			
11	*o*-CF_3	H	36	79	34[d]	
12	*m*-CF_3	H	52	100	82	72
13[e,g]	*m,m'*-$(CF_3)_2$	H	48			
14	*m*-CF_3	CH_3	59	95	100	100
15	*p*-C_6H_5	H	44			
16[e,h]	*m*-CF_3	$CH_2CH_2N(C_2H_5)_2$	100			
Benzyloxyamine hydrochloride			44			
1-Phenyl-3-(2-thiazolyl)-2-thiourea (I)			8	38	25	14

[a] Inhibitor concn for each assay was $1 \times 10^{-4} M$; substrate (dopamine-*2-t*) concn was also $1 \times 10^{-4} M$. [b] Each value is the result of three detns unless indicated otherwise; control norepinephrine levels were 0.35 ± 0.01 μg/g wet weight of brain tissue (n = 17). [c] Two rats died. [d] One rat died. [e] Prepd as in reference 4. [f] Mp 158–159.5° (C, H, S). [g] Mp 148.5–150.5° (C, H). [h] Mp 57–59° (sinters 55°) (C, H, N, S).

steric relationship of the NC(S)O moiety in the oxazolidinethione to the thioureas [NC(S)N] and to the dithiocarbamates [NC(S)S] suggested that these compounds might also possess significant dopamine β-hydroxylase activity.

This report describes the *in vitro* dopamine β-hydroxylase inhibitory activity of several 5-phenoxymethyl-2-oxazolidinethiones, the synthesis of which has been previously reported.[11] The effect of these compounds upon brain NE levels and upon spontaneous motor activity is also noted.

Experimental Section†

Dopamine β-hydroxylase was isolated from bovine adrenals and purified through the $(NH_4)_2SO_4$ step as described by Friedman and Kaufman.[12] Inhibition of enzyme activity utilizing dopamine-*2-t* as substrate was detd as previously described.[4]

All compds were suspended in 0.25% aq methylcellulose immediately prior to ip administration to Upjohn Sprague–Dawley male rats (140–200 g). The max vol injected was 1 ml. Rats were sacrificed by decapitation, and brains were removed and placed on Dry Ice. NE was extd from individual whole rat brains and assayed.[13,14]

In the mouse feeding study, groups of 8 CF-1 male mice were weighed and housed as a group and then fed a stock ground diet (Upjohn B.A. diet). In addn to a control group, a single group was placed on a diet contg one of the 2-oxazolidinethione derivs in a fixed concn of 1% (w/w; 500 mg in 50 g). The feeding program ran for 24 hr after which food consumption was recorded. At the conclusion of the feeding trial, locomotor activity was measured with 3 sets of 2 mice from each group of 8. Motor activity was measured for a single 10-min period after a 10-min acclimation period in circular actophotometers (Woodard Research Corp.) each equipped with 6 light beams and 6 photoelectric cells.

Results

Effect of the compds upon dopamine β-hydroxylase activity *in vitro* and upon rat brain NE levels *in vivo* is summarized in Table I. *In vitro* compds **5**, **7**, and **11** were the most effective of the 5-phenoxymethyl-2-oxazolidinethiones tested. This inhibition, which was not competitive for increasing substrate concns, compares favorably with that produced by benzyloxyamine, also a noncompetitive inhibitor of dopamine β-hydroxylase.[15] However, this inhibition was much less than that produced by 1-phenyl-3-(2-thiazolyl)-2-thiourea (I).[4] Compds **6** and **16** were not inhibitory at $1 \times 10^{-4} M$ and were not tested further. Insufficient compd was available for addnl study of **1**, **10**, **13**, and **15**.

Rat brain levels of NE were essentially unaltered by any of the oxazolidinethiones 2 hr after a dose of 200 mg/kg (ip). This lack of a depleting effect contrasts with the marked depletion detected with I. Four hr after the 200 mg/kg dose, **11**, although toxic (1 of 3 rats died), was as effective as I in depleting brain NE. Compds **2**, **3**, and **7** were active depletors of rat brain NE at 4 hr, but none was as effective as I. At 16 hr NE levels were depleted to less than 40% of control with **2** and to 53% of control with **3**; less marked was the NE depletion with **4** and with **12** at 16 hr.

Since *in vivo* inhibition of brain dopamine β-hydroxylase results in reduced motility in mice,[10,16,17] we determined the effects of the oxazolidinethiones upon spontaneous motor activity of mice fed a diet containing compd in a fixed concn. These results are summarized in Table II. Several of the compds markedly decreased food intake. However, only **4** and **11** as well as I significantly reduced spontaneous motor activity.

Discussion

The relatively small number of compds and the limited number of structural modifications in this series does not permit an extensive analysis of structure-activity relationships. A clear-cut effect of substitution upon *in vitro* inhibitory activity was the addn of the $Et_2N(CH_2)_2$ moiety to the N in the oxazolidinethione ring which erased the enzyme inhibition of either **12** (NH) or **14**, the N-Me deriv. The inhibitory activity of the benzyloxyamine isostere of dopamine upon this enzyme was also decreased greatly by substitution on the N.[15]

†The research described in this report involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Table II. Effect of 5-Phenoxymethyl-2-oxazolidinethiones on Mouse Food Intake and Motor Activity[a]

Compd	Food intake, g/24 hr	Drug intake, mg/kg per 24 hr	Motor activity counts/10 min % of control
2	7	459	78
3	8	548	91
4	4	268	47[b]
5	13	858	83
7	20	1307	69
9	27	1765	99
11	11	714	49[b]
12	9	616	67
I	12	540	44[b]
Control	29		100[c]

[a] All drugs added as 1% of diet; see Experimental Section. [b] Significantly different from control, $p < 0.05$. [c] Spontaneous motor activity in control mice was 587 ± 122 counts/10 min (±S.D.); $n = 6$.

In vitro enzyme inhibitory activity was not predictive of *in vivo* activity as detd by depletion of NE concns from rat brain. Compd **5**, the most inhibitory of these oxazolidinethiones *in vitro*, did not deplete rat brain NE and did not affect food intake or spontaneous motor activity in mice. This lack of correlation between *in vitro* enzyme inhibition and *in vivo* effects may be due to several factors including (1) variable absorption of these compds after either ip (rat) or oral (mouse) administration, and (2) taste of the drug in the diet. Faiman, *et al.*, in detg the antithyroid activity of several 5-substituted 2-oxasolidinethiones in rats, found marked variability in the effect of these compds when ingested as part of the diet as compared to the effects of parenterally administered drug.[18] A similar difficulty in absorption of these compds by rats after ip administration could explain the latency in the onset of norepinephrine depleting activity. Absorption studies with **12** in rats showed max blood levels of drug 2 hr after dosing with 25 mg and the blood levels remained relatively constant over the 2- to 24-hr interval.[19] Although blood levels of circulating oxazolidinethione were not measured in this study, sustained blood levels would provide an explanation for the significant inhibition of brain dopamine β-hydroxylase as reflected in the depletion of brain NE at 16 hr with **2**, **3**, **4**, and **12**. The adverse effect of taste of drug when added to the diet cannot be detd. This factor may be responsible for the decreased food intake (**2**, **3**, and **12**) without an impairment of spontaneous activity.

Previous mention has been made of the antithyroid activity of numerous substituted 2-oxazolidinethiones.[18,20,21] Compd **12** also demonstrated thyroid toxicity in chronic studies in both rats (10 mg/kg per day) and in dogs (100 mg/kg per day).[22] The antithyroid effects of the remainder of these oxazolidinethiones have not been detd.

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Preparation of a Camoquine Derivative with Quaternary Carbon Side Chain

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Since an enzymatic degradation of the terminal alkylamino function of chloroquine or camoquine might be the mechanism of plasmodial resistance to antimalarials, we synthesized a novel analog of camoquine: 4-[3′-(α,α-dihydroxymethylaminomethyl)-4′-hydroxyphenylamino]-7-chloroquinoline (VI), with the terminal amino function attached to a quaternary C. The nonbiodegradability of an amino function adjacent to a quaternary C is well known.[1] Though antimalarial compounds with quaternary side chains have been made previously,[2] compounds with a quaternary C adjacent to the terminal amino function have not been reported.

Chemistry. *p*-Nitrophenol (I) was chloromethylated to the corresponding chloromethyl derivative (II)[3] which when treated with $NaNO_2$ or $AgNO_2$ at low temp gave the nitro compound III. Using $AgNO_2$ the yield of III was 75% as compared with a 50% yield when $NaNO_2$ was used. The low reaction temp (0° or less) was designed to obviate the formation of nitrate ester.[4] The facile nitration of the alkyl chloride II appears to contradict the generally held view[5] that only alkyl bromides or iodides are suitable for the preparation of the nitro derivatives. III in dioxane reacted readily with HCHO in the presence of $Ca(OH)_2$ to give 2-nitro-2-(2-hydroxy-5-nitrophenyl)propane-1,3-diol (IV) (yield 75%). The yield of IV was lower when $(C_2H_5)_3N$ was used in place of $Ca(OH)_2$. Reduction of IV with Zn and H_2SO_4 gave the diamine V which, without isolation, was condensed with 4,7-dichloroquinoline to yield VI.

Though Balcom and Furst[6] have studied the reduction of aromatic nitro groups by NH_2NH_2 and Raney Ni, there are apparently no reports of similar reductions of the aliphatic nitro function, possibly because of the instability of aliphatic nitro compounds under the basic conditions of the experiment.[7] Thus when III, a compound with an aliphatic nitro side chain, was reduced by this method and condensed