wise, with vigorous stirring. After the addition was completed, the mixture was stirred 2 hr. and filtered. The filter cake was washed with benzene and ether, and the filtrate was concentrated under vacuum, yielding 7.1 g. (98.2%) of an oil. The oil was dissolved in acetone (100 ml.) and added to a hot solution of fumaric acid (3.5 g.) in acetone (300 ml.), precipitating the fumarate salt of VIIIa. Recrystallization from acetone–methanol afforded 2.8 g. of product, m.p. 190–191°.

11-(3-Aminopropyl)-5,6-dihydro-11H-benzo[*a*]carbazole (VIIIb).—Reduction of VIIb (13.6 g., 0.05 mole) with $LiAlH_4$ (2.9 g., 0.08 mole) in the manner described for VIIIa afforded 12.1 g. of crude product. This was distilled, yielding 9.73 g. (71%) of product, which was collected at 218–228° (0.6 mm.). Treatment of the base in ethanol with an ethanolic solution of HCl gave the hydrochloride. Recrystallization from methanol yielded a product with m.p. 302–304°.

5-(3-Methylaminopropyl)-1,3,4,5-tetrahydrothiopyrano[4,3-*b*]-indole (XIa).—A 1-l. three-necked flask fitted as above was charged with anhydrous ether (250 ml.) and $LiAlH_4$ (2.2 g., 0.06 mole). To this was added, with stirring, a warm solution of Xa (4.7 g., 0.017 mole) in benzene (150 ml.). The reaction mixture was stirred at room temperature for 5 hr. Water (5 ml.) was added slowly and the mixture was allowed to stand overnight. The precipitate was filtered from the solution and washed well with ether. The combined filtrate and washings were washed with saline and dried over anhydrous $MgSO_4$. The solvent was removed, leaving 2.1 g. of base (50%), which was dissolved in absolute ethanol (20 ml.) and acidified with an ethanolic solution of HCl. Anhydrous ether was added until the solution became turbid. On cooling the solution, the hydrochloride crystallized out and was collected on a funnel, washed with ether, and dried. Recrystallization from ethanol afforded 1.7 g. (62%) of XIa, m.p. 190–192°.

11-(3-Methylaminopropyl)-5,6-dihydro-11H-benzo[*a*]carbazole (Xb).—Reduction of Xb (11.7 g., 0.38 mole) with $LiAlH_4$ (5 g., 0.13 mole) was carried out as described for Xa, yielding 10.5 g. (88.3%) of crude base. Conversion to the hydrochloride afforded 8.5 g. (74.8%), m.p. 200–201.5°.

Synthesis and Microbiological Properties of 3-Amino-3,4-dihydro-1-hydroxycarbostyril[1]

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3-Amino-3,4-dihydro-1-hydroxycarbostyril, synthesized by the catalytic hydrogenation of *o*-nitrophenylalanine hydrochloride, was compared with some structurally related compounds as growth inhibitors in several microbiological assays. Of these compounds, 3-amino-3,4-dihydro-1-hydroxycarbostyril inhibits the growth of *Escherichia coli*, *Leuconostoc dextranicum*, and *Lactobacillus arabinosus* at concentrations of 2 γ/ml. for each microorganism. In general, only a partial and noncompetitive reversal of 3-amino-3,4-dihydro-1-hydroxycarbostyril toxicity by certain protein hydrolysates and histidine was observed for each test organism, which suggests that other substituted derivatives of 3-amino-3,4-dihydrocarbostyrils may produce potent and noncompetitive antagonists.

In contrast to the strictly competitive-like response observed with phenylalanine in reversing the toxicity of *o*-aminophenylalanine for *Escherichia coli*, a noncompetitive reversal of the inhibitory effects of 3-amino-3,4-dihydrocarbostyril was demonstrated with phenylalanine for *E. coli* and *Leuconostoc dextranicum*.[3] In view of the biological results of 3-amino-3,4-dihydrocarbostyril with bacteria,[3] and its previously reported physiological activity,[4] further investigations with other substituted derivatives of 3-amino-3,4-dihydrocarbostyril seem warranted in an effort to produce noncompetitive antagonists as potential chemotherapeutic agents. Accordingly, a related derivative, 3-amino-3,4-dihydro-1-hydroxycarbostyril, was prepared and subsequently found to inhibit growth of several microorganisms, and only partial reversal of the inhibitions could be achieved with certain protein hydrolysates and with histidine. The method of synthesis as well as a preliminary report on its biologically antagonistic properties are herein presented.

Experimental[5]

Organic Syntheses. Ethyl 2-Acetamido-2-(*o*-nitrobenzyl)-cyanoacetate.—To a solution of 10 g. of ethyl acetamidocyanoacetate in 100 ml. of magnesium-dried ethanol containing 1.35 g. of sodium was added 10.1 g. of *o*-nitrobenzyl chloride and the solution was allowed to reflux for 3 hr. Sodium chloride was removed by filtration from the hot reaction mixture, and the filtrate was cooled overnight to yield 16 g. of crude material. Recrystallization from ethanol–water gave 15 g. of product, m.p. 150–151°.

Anal. Calcd. for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95. Found: C, 54.81; H, 4.80.

Hydrolysis of Ethyl 2-Acetamido-2-(*o*-nitrobenzyl)cyanoacetate.—A 10-g. sample of ethyl 2-acetamido-2-(*o*-nitrobenzyl)-cyanoacetate was hydrolyzed in the presence of 100 ml. of concentrated HCl for 5 hr. to yield 6.95 g. of *o*-nitrophenylalanine hydrochloride, m.p. 219–220° dec. (lit.[6] m.p. 222–223°).

A 4.0-g. sample of ethyl 2-acetamido-2-(*o*-nitrobenzyl)cyanoacetate was hydrolyzed in the presence of 4.0 g. of sodium carbonate for 6 hr. Acidification of the reaction mixture with hydrochloric acid and subsequent cooling yielded a crystalline material. Recrystallization from ethanol gave about 1 g. of N-acetyl-*o*-nitrophenylalanine, m.p. 205–206°.

Anal. Calcd. for $C_{11}H_{12}N_2O_5$: C, 52.38; H, 4.79. Found: C, 52.24; H, 5.23.

3-Amino-3,4-dihydro-1-hydroxycarbostyril Hydrochloride.—A 3.0-g. sample of *o*-nitrophenylalanine hydrochloride dissolved in 50% methanol (pH 1.5) was hydrogenated under 3.66 kg./cm.² of hydrogen pressure in the presence of 200 mg. of platinum black for 5 hr. The hydrogenated solution, after removal of the catalyst, showed a pH of 3.6. Reduction in volume of the filtrate *in vacuo* yielded a residue which was recrystallized from ethanol–water to yield 1.2 g. of product, m.p. 258–263° dec.

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(2) Taken in part from the M.S. Thesis of Ok Hi Park Choun, Abilene Christian College, August, 1963.

(3) A. L. Davis, R. Lloyd, J. Fletcher, L. Bayliss, and T. J. McCord, *Arch. Biochem. Biophys.*, **102**, 48 (1963).

(4) T. Sasaki and I. Otsuka, *J. Biochem.* (Tokyo), **12**, 429 (1930).

(5) All melting points were determined by the capillary technique and are corrected. The paper chromatograms were determined by the ascending techniques using the solvents indicated, and the spots were developed with ninhydrin reagent. The ultraviolet spectra were determined on a Bausch and Lomb Spectronic 505 spectrophotometer using water as solvent. The authors are indebted to D. Howell and D. Tharp for the elemental analyses.

(6) T. A. Connors, W. C. J. Ross, and J. G. Wilson, *J. Chem. Soc.*, 2994 (1960).

This compound gave a violet color with ferric chloride and a yellow color with ninhydrin. The R_f values of this compound in 65% pyridine and 1-butanol–acetic acid–water (4:1:1) were 0.75 and 0.48, respectively.

Anal. Calcd. for $C_9H_{10}N_2O_2 \cdot HCl$: C, 50.35; H, 5.16; N, 13.05. Found: C, 50.16; H, 5.50; N, 13.30.

A 200-mg. sample of 3-amino-3,4-dihydro-1-hydroxycarbostyril hydrochloride was dissolved in 12 ml. of water (pH 3.1). After careful addition of dilute NaOH to pH 6.6, the product started to precipitate, and the solution was allowed to cool in a refrigerator. Filtration yielded 60 mg. of 3-amino-3,4-dihydro-1-hydroxycarbostyril, m.p. 191–192° dec.

Anal. Calcd. for $C_9H_{10}N_2O_2$: C, 60.66; H, 5.65. Found: C, 60.23; H, 5.67.

3-Acetamido-1-acetoxy-3,4-dihydrocarbostyril.—To a 1.15-g. sample of 3-amino-3,4-dihydro-1-hydroxycarbostyril hydrochloride dissolved in water were added 10.1 ml. of 1 *N* NaOH and 3.4 ml. of acetic anhydride. Recrystallization of the resulting precipitate from dilute acetic acid yielded 1.1 g. of product, m.p. 149–150°. This compound showed negative results with ferric chloride and ninhydrin.

Anal. Calcd. for $C_{13}H_{14}N_2O_4$: C, 59.43; H, 5.38. Found: C, 59.48; H, 5.55.

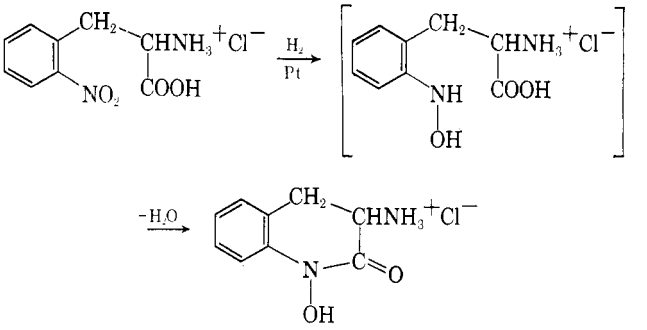
3-Acetamido-3,4-dihydro-1-hydroxycarbostyril.—A 300-mg. sample of 3-acetamido-1-acetoxy-3,4-dihydrocarbostyril and 300 mg. of sodium carbonate dissolved in water were refluxed in a stainless steel flask for 2 hr. The reaction mixture was acidified with concentrated HCl, reduced to dryness *in vacuo*, and the resulting residue was extracted with absolute ethanol. The salt was removed by filtration, and the resulting filtrate was set in the refrigerator overnight to yield 10 mg. of product, m.p. 201–202°. This compound gave a bluish violet color with ferric chloride but was not colored by ninhydrin.

Anal. Calcd. for $C_{11}H_{12}N_2O_3 \cdot H_2O$: C, 55.45; H, 5.95. Found: C, 55.30; H, 5.71.

Microbiological Assays.—For *E. coli* 9723, a previously described inorganic salts-glucose medium[7] was employed and the organism was incubated at 37° for about 16 hr. For the lactic acid bacteria, a previously reported amino acid medium[8] was modified by the addition of calcium pantothenate (0.2 γ/ml.), by the omission of histidine, phenylalanine, and tyrosine from the basal medium, and with additional modifications noted for each organism. For *Leuconostoc mesenteroides* 8293 and *L. dextranicum* 8086 the phosphate concentration was increased fourfold. L-Glutamine was added without heating to each of the sterile assay tubes. The lactic acid organisms were incubated at 30° for 20–30 hr. In all assays the amount of growth was determined 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. For *E. coli*, the data in Table III are recorded as absorbance readings which are related to the mg. of dry cells as calculated from a standard curve of mg. of dry cells per ml. *vs.* absorbance readings.

Results and Discussion

It has been previously observed that the catalytic hydrogenation of *o*-nitrophenylalanine (free base) under appropriate conditions yields *o*-aminophenylalanine, which readily cyclizes in acidic medium to form 3-amino-3,4-dihydrocarbostyril[3]; however, the isolated product which results from the same hydrogenation procedure using the hydrochloride salt of *o*-nitrophenylalanine was ultimately characterized as 3-amino-3,4-dihydro-1-hydroxycarbostyril hydrochloride. As indicated in the accompanying equations, the present course of hydrogenation apparently proceeds with formation of an intermediate hydroxylamino compound by partial reduction of the nitro group, followed by cyclization with the carboxyl group through dehydration to form 3-amino-3,4-dihydro-1-hydroxycarbostyril. The presence of a hydroxy group in this compound was indicated by formation of a diacetyl derivative,



and by its ability to produce a characteristic violet color with ferric chloride reagent. Since this compound was not observed to undergo any of the typical identification tests for phenolic compounds (*i.e.*, coupling with diazonium salts to form azo derivatives), the hydroxy group was assigned to the 1-position at the nitrogen atom rather than one of the remaining substituent positions on the benzene ring.[9] Such a structural assignment constitutes a cyclic hydroxamic acid for which the ferric chloride reaction is also characteristic.

The ultraviolet spectrum of 3-amino-3,4-dihydro-1-hydroxycarbostyril is comparable to the ultraviolet spectrum of 3-amino-3,4-dihydrocarbostyril at pH 2, but they are distinguishable by an appreciable difference at pH 7 and pH 10 as recorded in Table I. In neutral and alkaline solution, the spectrum of only the 1-hydroxy compound is changed; however, the original spectrum is regained upon acidification. Further, the biological properties of the two derivatives are different, as subsequently described.

TABLE I
ULTRAVIOLET SPECTRA OF SUBSTITUTED 3,4-DIHYDROCARBOSTYRILS

Compound	pH	λ_{max}, mμ (log ε)	λ_{min}, mμ (log ε)
3-Amino-3,4-dihydrocarbostyril	2	254 (4.415)	226 (3.644)
	7	255 (4.362)	226 (3.673)
	10	255 (4.245)	226 (3.644)
3-Amino-3,4-dihydro-1-hydroxycarbostyril[a]	2	257 (4.111)	226 (3.449)
	7	290 (3.825)	240 (3.554)
	10	290 (3.859)	240 (3.590)

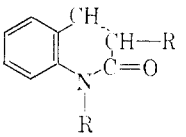
[a] Shoulder at 267 mμ at pH 7 (log ε 3.737) and pH 10 (log ε 3.773).

Under the specific assay conditions for each of the microorganisms described in the Experimental section, amino-3,4-dihydro-1-hydroxycarbostyril is inhibitory to the growth of *E. coli* 9723, *L. dextranicum* 8086, *L. mesenteroides* 8293, and *Lactobacillus arabinosus* 17-5 at concentrations of 2, 2, 2, and 6 γ/ml., respectively. Using *E. coli* and *L. dextranicum* as the model test organisms, a representative comparison of the biological effects of 3-amino-3,4-dihydro-1-hydroxycarbostyril with some structurally related compounds is presented in Table II. 3-Amino-3,4-dihydrocarbostyril is only about 0.033 and 0.0033 as active as its hydroxy derivative in the *E. coli* and *L. dextranicum* assays, respectively. No significant inhibitory effects were observed in these organisms up to concen-

(7) E. H. Anderson, *Proc. Natl. Acad. Sci. U. S.*, **30**, 120 (1946).
(8) J. M. Ravel, L. Woods, and W. Shive, *J. Biol. Chem.*, **206**, 391 (1954).
(9) T. Hashimoto, *Proc. Japan Acad.*, **24**, 15 (1948); *Chem. Abstr.*, **46**, 9102e (1952), reported the 6- and 8-hydroxy compounds.

trations of 60 γ/ml. of the 1-acetoxy and 1-hydroxy substituted derivatives of 3-acetamido-3,4-dihydrocarbostyril.

TABLE II
RELATIVE BIOLOGICAL ACTIVITIES OF SOME 3,4-DIHYDROCARBOSTYRIL DERIVATIVES



3,4-Dihydrocarbostyril derivative		Microorganism, γ/ml.[a]	
R	R′	*Escherichia coli*	*Leuconostoc dextranicum*
H	NH_2	60[b]	600[c]
HO	NH_2	2[d]	2[d]
HO	$NHCOCH_3$	>60[e]	>60[e]
CH_3CO_2	$NHCOCH_3$	>60[e]	>60[e]

[a] Minimal concentration required for complete inhibition of growth. [b] Reversed in a noncompetitive-like manner by phenylalanine. [c] Not reversed to an appreciable extent by phenylalanine. [d] Reversed in a noncompetitive-like manner by histidine. [e] The solubility of the compounds in the assay medium precluded testing them at higher concentration levels.

Preliminary attempts to reverse the growth inhibitions of *E. coli* by 3-amino-3,4-dihydro-1-hydroxycarbostyril using extracts and hydrolysates of natural materials indicated that only partial reversal of the inhibitor could be achieved by high concentrations of acid-hydrolyzed casein, and no appreciable reversing effects were observed with other hydrolysates (*e.g.*, yeast, corn, soy, and gelatin) and extracts of liver and yeast. In subsequent experiments, the biological effects of each of the known amino acids present in casein hydrolysate were studied to demonstrate that histidine only was effective in partially reversing the toxicity of 3-amino-3,4-dihydro-1-hydroxycarbostyril in a noncompetitive manner, as indicated in Table III. A constant inhibition index[10] was not observed; *e.g.*, for a 100-fold increase (from 0.06 to 6 γ/ml.) in the level of histidine, no more than a 10-fold increase (from 2 to 20 γ/ml.) in antagonist concentration is required for complete inhibition of growth for *E. coli*. For *L. arabinosus* and *L. dextranicum*, it was similarly demonstrated that histidine partially and noncompetitively reverses the growth inhibition of 3-amino-3,4-dihydro-1-hydroxycarbostyril.

TABLE III
REVERSAL OF 3-AMINO-3,4-DIHYDRO-1-HYDROXYCARBOSTYRIL INHIBITION BY HISTIDINE[a]

3-Amino-3,4-dihydro-1-hydroxycarbostyril, γ/ml.	L-Histidine, γ/ml.					
	None	0.06	0.2	0.6	2.0	6.0
	Absorbance readings[b]					
0	0.66	0.68	0.68	0.69	0.69	0.67
0.6	0.43	0.54	0.48	0.56	0.69	0.67
2	0	0.23	0.30	0.27	0.43	0.53
6	0	0.04	0.06	0.09	0.21	0.21
20	0	0	0	0	0	0

[a] Test organism, *E. coli* 9723 incubated at 37° for 16 hr. [b] A measure of culture turbidity in which absorbance readings of 0.10, 0.20, 0.30, 0.50, and 0.70 are equivalent to 0.05, 0.10, 0.15, 0.26, and 0.37 mg. of cells/ml., respectively.

These initial studies indicate that certain substituted derivatives of 3,4-dihydrocarbostyril can produce antagonists which are not appreciably affected by known metabolites and natural reversing agents (extracts and hydrolysates), and are potentially useful chemotherapeutic agents. Additional studies to determine whether the primary effect of 3-amino-3,4-dihydro-1-hydroxycarbostyril involves merely an antagonism of histidine biosynthesis, histidine incorporation into protein, or interaction with some activating enzyme having a specialized function in histidyl transfer are contemplated.

(10) Ratio of concentration of inhibitor to metabolite (substrate) necessary for preventing appreciable growth for the specified incubation period.