Table I. Comparison of Antimalarial Activities of Quinine, Quinidine, and Their Derivatives[a]

	Dose, mg/kg (test minus control)[b]					
	640	320	160	80	40	20
9-Deoxyepiquinine-9-thiosulfuric acid $0.5H_2S_2O_3$ (2)	22.4 (*c*, 3)	15.9 (*c*, 3)	12.7 (*a*)	3.9	0.9	0.5
N-Quininium-*S*-thiosulfate · $1.5H_2S_2O_3$[c]	13.4 (*c*, 3)	12.7 (*a*)	10.3 (*a*)	4.9	0.5	0.5
Quinine · $0.5SO_4^{2-}$[d]	7.1 (*a*)	6.1	4.7	2.3	1.5	0.5
Quinidine · $0.5SO_4^{2-}$[e]	7.7 (*t*, 1)	4.0	2.4	2.2	1.2	
9-Deoxy-9-chloroquinine (1)	0.5	0.5	0.3	0.3	0.3	0.1
9-Deoxy-9-hydroquinidine (4)[f]		0.3		0.1		0.1
Quininone (5)[g,h]	0.9		0.7		0.7	
Quinidinone (5)[g,h]	0.2		0.2		0.0	
9-Methylquinidine (6)[i]	0.2		0.2		0.2	

[a]The compounds were evaluated against *Plasmodium berghei* KBG 173 malaria by administration to five male mice per dilution in a single subcutaneous dose 72 hr after infection.[8] [b]The mean survival time of the control animals was 6.1 days. Deaths from days 2–5 after drug administration are attributed to toxicity (*t*, number of dead mice). Compounds are classified as active (*a*) when the mean survival time of the treated mice is twice that of the controls, *i.e.*, test − control > 6.1 days, and curative (*c*, number of surviving mice) when one or more test animals live 60 days postinfection.[8] [c]This derivative was reported as a *N*-quininium-*S*-thiosulfate zwitterion[9] but was obtained by us as the sesquithiosulfate salt. [d]Source: S. B. Penick and Co., New York, N. Y. [e]Source: K and K Laboratories, Jamaica, N. Y. [f]Source: Dr. V. I. Stenberg, University of North Dakota. [g]Both samples are shown as structure **5** due to the probable epimerization at C-8 of quininone to form quinidinone as reported by Lyle and Gaffield.[4] The antimalarial activities of the two samples are not considered to be significantly different. [h]Source: Dr. G. R. Pettit, Arizona State University. [i]Source: Dr. D. E. Pearson, Vanderbilt University.

9-Deoxy-9-chloroquinine (1). To a mixture of 100 g (0.13 mol) of quinine · SO_4^{2-} · $2H_2O$ and 200 ml of $CHCl_3$ was added a solution of 10 g (0.25 mol) of NaOH in 100 ml of H_2O. This mixture was stirred at room temperature for 3 hr. The $CHCl_3$ layer containing the quinine free base was then separated, and the aqueous layer was extracted with additional $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O and dried. Anhydrous HCl was then bubbled into the solution to form quinine dihydrochloride. Thionyl chloride (132 g, 1.1 mol) was added to the mixture, which was then heated under reflux for 6 hr and cooled to room temperature. Water (30 ml) was added to decompose the excess $SOCl_2$, and the mixture containing the crude 1 · HCl in $CHCl_3$ was neutralized with 50 ml of 5 *N* NaOH solution. Compound 1 was extracted into the $CHCl_3$ layer which was washed with H_2O and dried. The resulting yellow solution was chromatographed on a 4 × 50 cm column of alumina (activity II–III) using $CHCl_3$–C_6H_6 (1:1) as the eluent. The product was recrystallized from EtOAc to yield 79 g (89%) of 1 as a white crystalline solid: mp 155–157° (lit.[3] 151°); $[\alpha]^{20}D$ +62° (*c* 0.5 in MeOH) (lit.[3] $[\alpha]^{20}D$ +62.0° (*c* 1.0 in EtOH)). *Anal.* ($C_{20}H_{23}ClN_2O$) C, H, N, Cl.

Monohydrochloride of 1. Concentrated HCl was added dropwise to a solution of 50 g (0.15 mol) of **1** in 150 ml of MeOH until pH 5 was reached. An additional 150 ml of MeOH was added, and the mixture was heated to dissolve the precipitated HCl salt. The hot solution was diluted with 300 ml of EtOAc and cooled to room temperature causing crystallization of the product. The yield of 9-deoxy-9-chloroquinine · HCl (from MeOH–EtOAc) was 43 g (78%): mp 218–219° dec (lit.[3] 219° dec); $[\alpha]^{20}D$ +10.3° (*c* 0.7 in H_2O) (lit.[3] $[\alpha]^{20}D$ +9.75° (*c* 1.0 in H_2O)). *Anal.* ($C_{20}H_{24}Cl_2N_2O$) C, H, N, Cl.

9-Deoxyepiquinine-9-thiosulfuric Acid (2) Hemithiosulfate. To 10 g (0.026 mol) of 1 · HCl in 60 ml of H_2O–MeOH (1:2) was added a solution of 6.5 g (0.026 mol) of $Na_2S_2O_3 \cdot 5H_2O$ in 20 ml of H_2O. This resulting solution was heated on a steam bath for 6 hr, and the precipitate which formed was collected and triturated with $CHCl_3$ to remove 3.6 g of unreacted **1**. The resulting light yellow solid was recrystallized from MeOH–H_2O (1:1) to yield 2.4 g (19%) of **2** as the hemithiosulfate: mp 197–199° dec; $[\alpha]^{20}D$ +288° [*c* 0.016 (4.6 (v/v) 10% aqueous HCl–MeOH)]; uv [4.2 × 10^{-5} *M*, 1:5 (v/v) 10% aqueous HCl–MeOH] λ_{max} 359 nm (*e* 5540), sh 330 (4100), 256 (21,100); ORD (*c* 0.01) $[\alpha]_{378}$ +1810, $[\alpha]_{332}$ −2050, $[\alpha]_{298}$ +39, $[\alpha]_{270}$ −130°; ν_{max} (KBr) 3448, 1230, and 1022 cm^{-1} *Anal.* ($C_{20}H_{24}O_4N_2S_2 \cdot 0.5H_2S_2O_3$) C, H, N, S.

ORD of quinine · HCl · $2H_2O$** (*c* 0.0077) had $[\alpha]D$ −52°; $[\alpha]_{364}$ −1650, $[\alpha]_{320}$ +1290, $[\alpha]_{272}$ sh −520, $[\alpha]_{270}$ −723°.

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**Source, Merck and Co., Rahway, N. J.

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Synthesis and Microbiological Properties of 3-Amino-1-hydroxy-2-indolinone and Related Compounds

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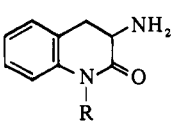
In our first study of the structure-activity relationships of hydroxamic acids in heterocyclic systems, the synthesis and antibacterial properties of 3-amino-3,4-dihydro-1-hydroxycarbostyril (**1**) were described.[1] Following this report, the 7-methoxy and 7-hydroxy derivatives of **1** were prepared and shown to have antibacterial activities equally



1, R = OH
3, R = H

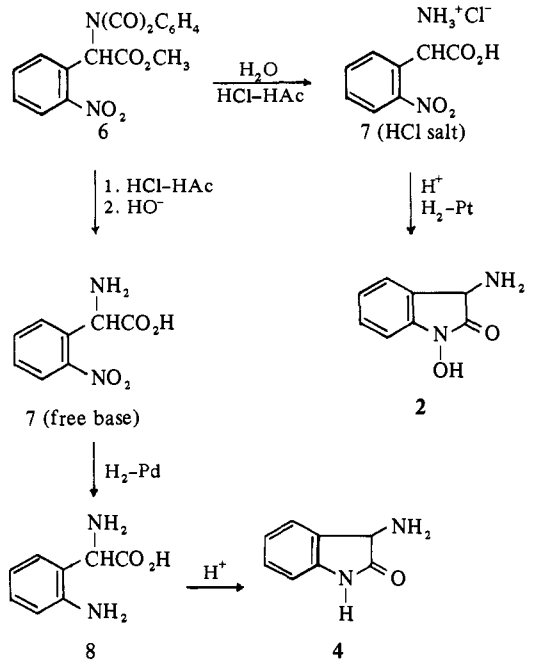
†Taken in part from the M.S. Thesis of D. R. Smith, Abilene Christian College, Abilene, Texas, May 1971.

as effective as the parent compound.[2] As growth inhibitors of *Escherichia coli* and *Leuconostoc dextranicum*, these carbostyrilhydroxamic acids were much more effective than their corresponding lactams.[2] In a recent study, the optically active isomers of **1** were prepared, and the L form inhibited the growth of *E. coli* and *L. dextranicum*, respectively, 100 and 400 times more effectively than the D form.[3]

These results prompted a further study of the structure-activity relationships of **1** which compares the effect of decreasing the size of the heterocycle by the $-CH_2-$ linkage at the 4 position upon the microbiological properties. Accordingly, this paper describes the synthesis of the lower condensed ring homolog of **1**, namely 3-amino-1-hydroxy-2-indolinone (**2**), and a comparative study of its antibacterial activities with those of **1** against *E. coli* and *L. dextranicum*. Since other compounds structurally related to **1** and **2** were of biological interest, the relative inhibitory activities of the homologous lactams, 3-amino-3,4-dihydrocarbostyril (**3**) and 3-amino-2-indolinone (**4**), were also included in this study.

In previous work, the carbostyrilhydroxamic acids were synthesized *via* reductive cyclization of the *o*-nitro-substituted phenylalanines.[1-3] For the synthesis of the indolinonehydroxamic acid (**2**) by this method, the hitherto unreported *o*-nitrophenylglycine (**7**) becomes the requisite amino acid.

The preparation of **7** was accomplished by a Gabriel synthesis in which methyl α-bromo-*o*-nitrophenylacetate (**5**) was condensed with potassium phthalimide in *N,N*-dimethylformamide to yield methyl α-phthalimido-*o*-nitrophenylacetate (**6**), followed by acid hydrolysis. Several attempts to prepare **7** from *o*-nitrobenzaldehyde *via* a Strecker synthesis were unsuccessful.



The catalytic hydrogenation of the hydrochloride salt of **7** under acidic conditions afforded **2** in good yield, whereas the catalytic hydrogenation of the free base of **7** under neutral conditions gave *o*-aminophenylglycine (**8**). In a separate experiment, **8** was converted to its lactam, 3-amino-2-indolinone (**4**), by spontaneous cyclization in acidic solution.

Compounds **2** and **4** were isolated as the hydrochloride salts. Both salts turn pink in the solid state and turn red in solution on exposure to air or heat. However, these compounds may be kept indefinitely under vacuum without decomposition. On treatment with ferric chloride reagent, **2** forms a deep blue ferric hydroxamate.

Table I. Relative Growth Inhibitory Properties of 3-Amino-1-hydroxy-2-indolinone Hydrochloride and Related Compounds

Compounds	MIC, μg/ml[a] *E. coli*	*L. dextranicum*
3-Amino-1-hydroxy-2-indolinone · HCl (**2**)	6	40
3-Amino-2-indolinone · HCl (**4**)	20	40
3-Amino-3,4-dihydro-1-hydroxycarbostyril · HCl (**1**)	1	1
3-Amino-3,4-dihydrocarbostyril · HCl (**3**)	60	600
o-Nitrophenylglycine (**7**)	>200	>200
o-Aminophenylglycine (**8**)	>200	>200
Aspergillic acid	10	2

[a] Minimum inhibitory concentration.

In microbiological studies, the indolinonehydroxamic acid (**2**) and the lactam **4** completely inhibit the growth of *E. coli* 9723 at concentrations of 6 and 20 μg/ml, respectively, as given in Table I. These two compounds are equally effective in inhibiting the growth of *L. dextranicum* 8086. Under the same assay conditions, compound **2** as a growth inhibitor is slightly more effective for *E. coli* and less effective for *L. dextranicum* than aspergillic acid,[4,5] a naturally occurring antibacterial hydroxamic acid which was used as a standard. Neither the *o*-nitro-substituted nor the *o*-amino-substituted phenylglycine is inhibitory to the growth of these microorganisms up to levels of 200 μg/ml.

In our earliest study,[1] it was found that the inhibition caused by the carbostyrilhydroxamic acid (**1**) was partially and noncompetitively reversed by histidine. This same effect of histidine upon the inhibitory activity of the indolinonehydroxamic acid (**2**) in both *E. coli* and *L. dextranicum* was also observed in the present study.

As a growth inhibitor of *E. coli* and *L. dextranicum*, 3-amino-2-indolinone (**4**) is 3 and 15 times as effective as 3-amino-3,4-dihydrocarbostyril (**3**). Even though the synthesis of the former compound *via* a different procedure[6] has been long-standing, this appears to be the first report of its antimicrobial properties and suggests that other biologically active compounds may be found among 2-indolinone derivatives (oxindoles).

For the homologous heterocyclic hydroxamic acids, the indolinonehydroxamic acid (**2**) is only 0.167 and 0.025 as effective as the carbostyrilhydroxamic acid (**1**) in inhibiting the growth of *E. coli* and *L. dextranicum*, respectively. Therefore, decreasing the size of the condensed ring system by the $-CH_2-$ linkage at the 4 position of **1** results in a decrease in the antibacterial activity.

This study introduces a new bioactive cyclic hydroxamic acid, 3-amino-1-hydroxy-2-indolinone (**2**), and indicates an additional structural feature which is associated with the potent inhibitory activities of 3-amino-3,4-dihydro-1-hydroxycarbostyril (**1**).

Experimental Section

General. Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental 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 for those elements were within ±0.4% of the theoretical values.

Methyl α-Bromo-o-nitrophenylacetate (5). A mixture of 20 g (0.09 mol) of methyl *o*-nitromandelate and 75 g (0.3 mol) of PBr_3 was stirred at 25° for 2 hr. The excess PBr_3 was removed from the reaction mixture with a current of air, and the residual oil was dissolved in a small amount of EtOH. Cooling of the solution gave a precipitate which was filtered to yield 17 g (65%) of product. An analytical sample was obtained by recrystallization from EtOH, mp 61–62°. *Anal.* ($C_9H_8NO_4Br$) C, H, N.

Methyl α-Phthalimido-o-nitrophenylacetate (6). A mixture of 11.2 g (0.05 mol) of **5** and 8.5 g (0.05 mol) of potassium phthalimide in 170 ml of DMF was stirred at 25° for 2 hr. The KBr was removed by filtration, and the filtrate was treated with 200 ml of $CHCl_3$ and 500 ml of H_2O. The $CHCl_3$ layer was separated and the aqueous layer was extracted twice with 200-ml portions of $CHCl_3$. The $CHCl_3$ extracts were combined, dried over anhydrous Na_2SO_4, and taken to dryness *in vacuo* to give a dark-colored oil. Treatment of the oil with hot EtOH effected the separation of a white solid. The precipitate was filtered, washed with H_2O, and dried *in vacuo* to yield 10.1 g (72%) of product. An analytical sample was obtained by recrystallization from EtOH, mp 151–152°. *Anal.* ($C_{17}H_{12}N_2O_6$) C, H, N.

***o*-Nitrophenylglycine (7).** A solution of 10.0 g (0.03 mol) of 6 in 20 ml of glacial AcOH and 30 ml of concentrated HCl was heated under reflux for 5 hr. The phthalic acid was removed by filtration and the filtrate was concentrated under reduced pressure. The residue was treated with a few milliliters of H_2O and the solution was adjusted to pH 7 with addition of concentrated NH_4OH. Evaporation of the solution gave an oily residue from which a solid formed on treatment with EtOH. The precipitate was filtered and washed with H_2O to give 2.2 g (38%) of product, mp 142–143°. *Anal.* ($C_8H_8N_2O_4$) C, H, N.

In a separate experiment, 18.5 g (0.05 mol) of 6 was hydrolyzed in 100 ml of refluxing concentrated HCl–AcOH (6:4) for 5 hr. After removal of the phthalic acid by filtration from the cooled reaction mixture, the filtrate was taken to dryness *in vacuo*. The resulting solid residue was recrystallized from MeOH–Et_2O to yield 10.45 g (83%) of *o*-nitrophenylglycine hydrochloride, mp 116–118° dec. This compound was used without further purification for the synthesis of **2**.

3-Amino-1-hydroxy-2-indolinone Hydrochloride (2). To 1.0 g (0.004 mol) of the HCl salt of 7 dissolved in 6 ml of 50% aqueous CH_3OH was added 1 ml of concentrated HCl and reduced at 3.18 kg/cm² H_2 pressure in the presence of 20 mg of 5% Pt on C for 30 min. The catalyst was removed by filtration and the filtrate was taken to dryness *in vacuo*. The residue was dissolved in a minimum amount of hot CH_3OH and treated with 2 vol of Et_2O. Chilling of the solution at −17° gave 0.54 g (62%) of product, mp 210–211° dec (turns pink at 165° and darkens at 200°). This compound gives a deep blue with $FeCl_3$ reagent. *Anal.* ($C_8H_8N_2O_2 \cdot HCl$) C, H, N.

***o*-Aminophenylglycine (8).** 7 (1 g, 0.006 mol) dissolved in 100 ml of H_2O was reduced at 3.18 kg/cm² H_2 pressure in the presence of 300 mg of Pd black for 12 hr. The catalyst was removed by filtration, and the filtrate was concentrated in volume *in vacuo*. Treatment of the concentrated solution with EtOH caused precipitation. Filtration gave 0.52 g (61%) of product, mp 204–205°. *Anal.* ($C_8H_{10}N_2O_2$) C, H, N.

3-Amino-2-indolinone Hydrochloride (4). To a 100-mg (0.0006 mol) sample of 7 in 10 ml of 75% aqueous MeOH was added 0.5 ml of concentrated HCl. After 1 hr the solution was taken to dryness *in vacuo*. The residue was recrystallized from CH_3OH–Et_2O. After filtration the precipitate was dried *in vacuo* to give 108 mg (97%) of product, mp 210–215° dec (turns pink at 175°, red at 200°; lit. mp 175° *via* different procedure[6]). *Anal.* ($C_8H_8N_2O \cdot HCl$) C, H, N.

Microbiological Assays. For the assays with *L. dextranicum* (ATCC 8086) a previously described assay procedure and basal medium[7] were used except that histidine, phenylalanine, and tyrosine were omitted; 0.2 μg/ml of calcium pantothenate and 0.02 μg/ml of pantethine were added; and the phosphate (salts A) concentration was increased fourfold. The assay procedure[8] and the inorganic salts–glucose medium[9] for *E. coli* (ATCC 9723) were the same as those previously described. In all assays the amount of growth was determined spectrophotometrically 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.

The compounds (10 mg) were dissolved in sterile H_2O (10 ml) at 25°. From these solutions, serial dilutions were made and added aseptically to the previously autoclaved assay tubes without heating. After inoculation, the assay tubes with *L. dextranicum* were incubated at 30° for 24 hr, and those with *E. coli* were incubated at 37° for 16 hr. For each assay, appropriate controls were performed and reproducible results of the minimum inhibitory concentrations of compounds were obtained on repeating the assay 12 times.

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Synthesis and Central Nervous System Depressant Activity of Some 2-Aryl-6-chloro-4-phenylquinazolines

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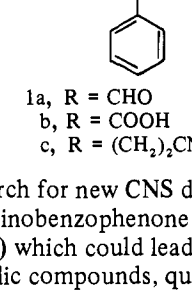
Six out of the seven 1,4-benzodiazepine type drugs introduced for clinical use as tranquilizers or sleep inducers bear two particular common structural features.[1] These features are the 5-phenyl and 7-chloro substituents. From a synthetic point of view, these six drugs may be conceived of as derived from 5-chloro-2-aminobenzophenone.

The structural moiety corresponding to 5-chloro-2-aminobenzophenone is also observed in other centrally acting nitrogen heterocyclic compounds. For example, the quinazolines **1a** and **1b** (R = CHO, CO_2H) are useful muscle relaxants,[2] while **1c** is useful as an anticonvulsant.[3] Consequently, in our search for new CNS depressants, we have used 5-chloro-2-aminobenzophenone hydrazone (**2**) in a reaction (Scheme I) which could lead theoretically to two types of heterocyclic compounds, quinazolines or benzotriazepines.



1a, R = CHO
b, R = COOH
c, R = $(CH_2)_2CN$

Chemistry. When 5-chloro-2-aminobenzophenone hydrazone (**2**) was treated with different aryl imido ester hydrochlorides, the only crystalline products obtained were the corresponding new 2-aryl-6-chloro-4-phenylquinazolines (**3a–g**, Table I). No benzotriazepine (**4**) was formed. To our knowledge, this is the first report of the use of this reaction