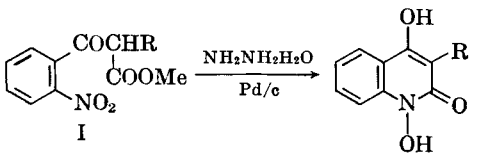
Antibacterial Activity of Some Quinolines Containing a Cyclic Hydroxamic Acid Group

By R. T. COUTTS*, W. N. PITKETHLY†, and D. G. WIBBERLEY†

A series of quinolines containing a cyclic hydroxamic acid group have been prepared and tested for antibacterial activity. Results show that the activity exhibited by 3-alkylquinoline hydroxamic acids is influenced by both the nature of the other substituents in the molecule and by the size of the alkyl group. One cyclic hydroxamic acid, 1,2-dihydro-1,4-dihydroxy-3-ethyl-2-oxoquinoline, showed some hypnotic activity.

CERTAIN CYCLIC hydroxamic acids have been shown to possess antimicrobial activity. Previous investigations in this field (1–3) have produced two new methods for the synthesis of such compounds initially required for antibacterial testing, but routine pharmacological screening indicated that 1,2-dihydro-1,4-dihydroxy-3-ethyl-2-oxoquinoline (II, R = Et), displayed hypnotic properties. Therefore, a series of 3-alkyl-1,2-dihydro-1,4-dihydroxy-2-oxoquinolines (II) (Table I) were prepared by reducing the appropriate 2-alkyl-2-

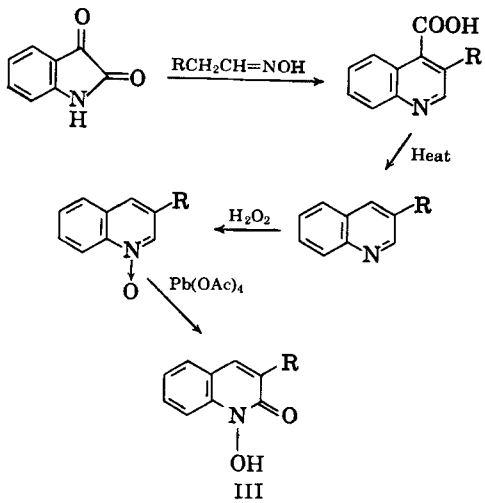


I II

(*o*-nitrobenzoyl)acetate (I) with hydrazine hydrate in the presence of palladium-charcoal.

The results of testing for hypnotic effect on mice were disappointing. Only one compound (II, R = Et) displayed such an effect at concentrations of one-eighth (and greater) of the LD_{50} dose. With the other compounds (II, R = Me, *n*Pr, isoPr, *n*Bu, and isoBu), the concentration required to produce hypnosis was too close to the LD_{50} concentration. The authors have been forced to conclude that the hypnotic effect is simply a measure of the toxicity of these compounds.

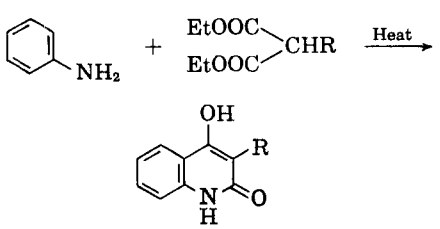
Compounds of type II were tested also for their *in vitro* antibacterial activity. This revealed that the presence of the hydroxamic acid group did not by itself confer antibacterial properties; the nature of the 3-alkyl side chain also had some influence on this activity (Table III). In view of this and of the reported (4) antibacterial properties of 1,2-dihydro-1-hydroxy-2-oxoquinoline (III, R = H), it seemed desirable to synthesize a variety of substituted quinolines with and without a hydroxamic acid group to compare the antibacterial properties of these compounds in an attempt to determine the most desirable molecular features for such activities. The syntheses of some of the compounds tested already have been reported. (See *Ref. to Prepn.*, Table III.) Two compounds possessing formula III were prepared. One of these, (III, R = Me), has been reported already in the literature, but its antibacterial properties were not described (5). In this preliminary investigation, this compound (III, R = Me) and its homolog (III, R = Et) have been synthesized by a series of reactions which involved the interaction of isatin and a suitable aldoxime to give a 3-alkylquinoline-4-carboxylic acid, which was decarboxylated, and the resulting 3-alkylquinoline successively oxidized with hydrogen peroxide, then lead tetraacetate, to give the required 3-alkyl-1,2-dihydro-1-hydroxy-2-oxoquinoline. The reaction sequence is shown in Scheme I.



III

Scheme I

The 3-alkyl-1,2-dihydro-4-hydroxy-2-oxoquinolines listed in Table III were obtained by the interaction of aniline with alkyl malonic esters.



EXPERIMENTAL

Preparation of 2-Alkyl-2-(o-nitrobenzoyl)acetates (I).—The potassium salt of methyl *o*-nitrobenzoylacetate was treated with a 50% excess of the appropriate alkyl halide by the previously reported method (1). All the products were yellow oils and distilled over the range 140–160° at 1.5–3 mm.

Anal.—Calcd. for $C_{13}H_{15}NO_5$ (I, R = *n*Pr): N, 5.28. Found: N, 5.23.

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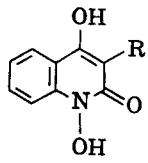
TABLE I.—3-ALKYL-1,2-DIHYDRO-1,4-DIHYDROXY-2-OXOQUINOLINES (II)



R	Formula	M.p.,[a] °C.	Yield	C,% Calcd.	C,% Found	H,% Calcd.	H,% Found	N,% Calcd.	N,% Found
n-C_3H_7	$C_{12}H_{13}NO_3$	195–196	68	65.73	65.69	5.94	5.97	6.40	6.40
iso-C_3H_7	$C_{12}H_{13}NO_3$	142–143	69	65.73	65.53	5.94	5.82	6.40	6.62
n-C_4H_9	$C_{13}H_{15}NO_3$	167–168	43	66.94	66.59	6.44	6.35	6.00	6.16
iso-C_4H_9	$C_{13}H_{15}NO_3$	158–159	54	66.94	66.55	6.44	6.54	6.00	6.15

[a] Melting points are corrected; they were determined by the capillary tube method after crystallization from 95% ethanol.

Anal.—Calcd. for $C_{13}H_{15}NO_5$ (I, R = isoPr): N, 5.28. Found: N, 5.15.

Anal.—Calcd. for $C_{14}H_{17}NO_5$ (I, R = *n*Bu): N, 5.02. Found: N, 5.28.

Anal.—Calcd. for $C_{14}H_{17}NO_5$ (I, R = isoBu): N, 5.02. Found: N, 4.98.

Preparation of 3-Alkyl-1,2-dihydro-1,4-dihydroxy-2-oxoquinolines (II).—Reduction of the 2-alkyl-2-(*o*-nitrobenzoyl)acetates with hydrazine hydrate and palladium-charcoal (1) yielded the cyclic hydroxamic acids recorded in Table I.

Preparation of 3-Methyl- and 3-Ethyl-quinoline-4-carboxylic Acids.—The Pfitzinger reaction of isatin (30 Gm.) with propionaldoxime (9.3 Gm.) and butyraldoxime (11.1 Gm.), respectively, using the methods of Ornstein (7) and Mulert (8), was employed. The 3-methylquinoline-4-carboxylic acid obtained (16 Gm.) melted at 250–252°; reported m.p. 254° (7). The 3-ethyl compound (20 Gm.) melted at 218–220°; reported m.p. 222° (8).

Preparation of 3-Methyl- and 3-Ethyl-quinoline.—A suspension of the 3-alkylquinoline-4-carboxylic acid (10 Gm.) and copper bronze powder (1 Gm.) in diphenyl ether (100 Gm.) was refluxed for 1 hr. or until evolution of carbon dioxide had ceased, and the alkyl quinoline had dissolved. The mixture then was diluted with dry ether, filtered, and diluted to approximately 1000 ml. with more ether. Dry hydrogen chloride was passed into the ether solution to precipitate the 3-alkyl quinoline hydrochlorides. Upon filtering the mixtures and then dissolving the residues in water, the quinolines were obtained by making the solution alkaline with sodium hydroxide and extracting with ether. Removal of the ether gave oils which were purified by distillation. 3-Methylquinoline (44.7% yield) boiled at 100°/1.5 mm.; 3-ethylquinoline (32.5% yield) boiled at 111°/1.5 mm. Reported b.p., 252–254°/735 mm. (9); 135–138°/12 mm. (10).

Preparation of 3-Methyl- and 3-Ethyl-quinoline-N-oxides.—The corresponding 3-alkylquinolines were oxidized with hydrogen peroxide using the method of Kaslow and Buchner (11) for the preparation of 3-(*p*-nitrophenyl)quinoline-1-oxide. The melting points of the 3-methyl and the 3-ethyl compounds were 48–49° and 83–84°, respectively.

Preparation of 1,2-Dihydro-1-hydroxy-3-methyl- and-3-ethyl-2-oxoquinolines.—The corresponding 3-alkylquinoline-*N*-oxides were treated with lead tetraacetate using the method of Ohta and Ochiai (12). The 3-methyl derivative (24% yield) melted at 182° after recrystallization from benzene. Reported m.p., 182° (5).

Anal.—Calcd. for $C_{10}H_9NO_2$: C, 68.56; H, 5.18; N, 8.00. Found: C, 68.35; H, 5.18; N, 7.72.

The 3-ethyl derivative (28% yield) melted at 148° after recrystallization from benzene.

Anal.—Calcd. for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40. Found: C, 69.13; H, 5.74; N, 6.82, 7.40.

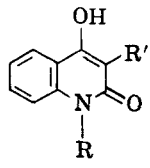
TABLE II.—PREPARED 3-ALKYL-1,2-DIHYDRO-4-HYDROXY-2-OXOQUINOLINES

R	Yield of Quinoline, Gm.	M.p., °C.	Remarks
Me	3.85	265°	Reported m.p. >275° (13) and 264° (14). Calcd.: N, 8.00. Found: N, 7.62.
Et	6.4	264°	Reported m.p. 259° (15).
*n*Pr	7.5	228°	Calcd. for $C_{12}H_{13}NO_2$: C, 70.91; H, 6.45; N, 6.89. Found: C, 70.99; H, 6.40; N, 6.56.
*n*Bu	4.8	192°	Calcd. for $C_{13}H_{15}NO_2$: C, 71.90; H, 6.91; N, 6.65. Found: C, 71.56; H, 7.30; N, 6.42.

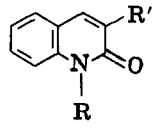
Preparation of 3-Alkyl-1,2-dihydro-4-hydroxy-2-oxoquinolines (IV, R = H, R′ = Alkyl).—Aniline (4 ml.), diethyl alkylmalonate (16 ml.), and diphenyl ether (30 Gm.) were refluxed in an apparatus which allowed for the continuous removal of ethanol produced during the reaction and at the same time permitted a high temperature (approximately 200°) to be maintained in the reaction flask. After the theoretical volume of ethanol (approximately 5 ml.) had been collected, the reaction mixture was cooled and diluted with light petroleum (boiling range 40–60°). The precipitate was a mixture of the desired product, uncyclized material and diamide. The by-products were separated readily by treating with sodium hydroxide solution in which only the required compound was soluble. Acidification of the filtrate gave the 3-alkyl-1,2-dihydro-4-hydroxy-2-oxoquinoline, which was purified by recrystallization from glacial acetic acid. (See Table II.)

Preparation of 1,2-Dihydro-1-hydroxy-2-oxoquinoline.—This was prepared from quinoline-*N*-oxide using the method of Ohta and Ochiai (12).

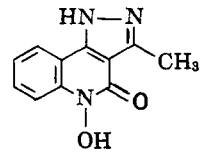
TABLE III.—ANTIBACTERIAL ACTIVITY OF VARIOUS QUINOLINE DERIVATIVES[a]



IV



V

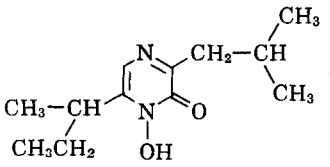


VI

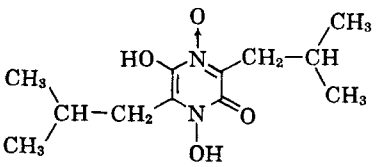
Compd.	Structure No.	R	R'	Ref. to Prepn.	M.I.C. (mg./100 ml. Broth)[b] *S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*P. vulgaris*
1[c]	IV	OH	H	(1)	...	...	...	...	...
2		OH	Me	(1)	0.3	0.6	20	20	...
3		OH	Et	(1)	10	20	20	20	...
4		OH	*n*Pr		...	...	...	...	...
5		OH	isoPr		5	10	20	20	20
6[c]		OH	*n*Bu		...	...	...	...	...
7		OH	isoBu		5	10	10	5	...
8		OH	—CH(CONHNH$_2$)CH$_3$	(3)	...	...	...	...	...
9		OH	CN	(2)	...	...	...	...	...
10		H	Me		...	40	...	...	...
11		H	Et		...	...	...	...	...
12		H	*n*Pr		40	...	...	...	...
13		H	*n*Bu		40	...	...	...	...
14[c]	V	OH	COOH	(2)	40	...	...	...	...
15[c]		OAc	COOEt	(2)	...	...	...	...	...
16		OH	COOEt	(2)	10	20	20	20	20
17[a]		OH	H	(4)	10	5	5	5	10
18		OH	Me		0.3	0.3	1.2	0.3	5
19		OH	Et		6	2.5	1.2	2.5	5
20		H	COOH		20	...	...	...	...
21	VI			(2)	...	...	...	...	...
Phenol					20	40	40	40	...

[a] The method of testing has a significant effect on antibacterial properties. Compound *17* was found to be five times as active against *S. aureus* and *E. coli* when tested by other workers (4). [b] M.I.C. greater than 40 mg./100 ml. of broth unless otherwise stated. [c] Slight inhibition over the range 5–40 mg./100 ml.

TABLE IV.—ANTIBACTERIAL ACTIVITY OF ASPERGILLIC ACID (VII) AND PULCHERRIMINIC ACID (VIII)



VII



VIII

	M.I.C., mg./100 ml. Broth *S. aureus*	*B. subtilis*	*E. coli*	*S. typhi*	*P. vulgaris*
Aspergillic acid	0.6	1.25	2.5	2.5	5
Pulcherriminic acid	5	5	40	10	10

Preparation of 1,2-Dihydro-2-oxoquinoline-3-carboxylic Acid.—This acid was prepared by hydrolysis of its ethyl ester (16).

RESULTS

Antibacterial Testing.—The general method of testing[1] entailed the addition of serial dilutions of the compounds (4 mg.) in alcohol (0.5 ml.) to tubes of nutrient broth (9.3 ml.). The test organism then was added (0.2 ml. of a 4-day culture) and the tubes incubated for 3 days, then inspected for inhibition of growth. Appropriate controls were performed also. The minimum and maximum concentrations of the substance being tested were 0.31 and 40 mg./100 ml. of broth.

Screening results (Table III) indicate that the presence in the molecule of a hydroxamic acid group does not by itself confer antibacterial properties (6). Compounds *1*, *4*, *6*, *8*, *9*, *14*, and *21* are cyclic

[1] Devised by Mr. A. Vickers, Mr. E. Jones, and Mr. D. Wilson, School of Pharmacy, Sunderland Technical College, Sunderland, County Durham, England.

hydroxamic acids with no antibacterial properties. It is significant to note, however, that all the compounds which do show activity (compounds *2, 3, 5, 7, 16, 17, 18, 19*) possess this grouping; in some cases, it is apparent that the activity is solely due to the presence of the hydroxamic group. For example, compound *16* shows some activity; the related compound *15* does not. Compound *18* was the most active of the compounds tested, whereas the closely related compound *10* had virtually no activity. The results also show that the nature of the substituents at positions 3 and 4 on the quinoline nucleus influences the antibacterial properties. From the limited number of examples examined, it would seem that an alkyl group is preferred at position 3, and the length and stereochemistry of this side chain contributes to the activity. The most active 3-substituted compounds (compounds *2, 3, 5, 7, 18, 19*) have a methyl, ethyl, isopropyl, or isobutyl group at this position. The only other 3-substituted quinoline hydroxamic acid which shows some activity has an ethoxycarbonyl group (compound *15*). Compounds possessing the greatest antibacterial activity (compounds *18* and *19*) are unsubstituted at position 4. An investigation is being conducted to discover if such a feature is desirable.

It can be concluded that factors in addition to the presence of a hydroxamic acid group are necessary for antibacterial activity; the degree of substitution and the nature of the substituents also are contributing factors.

Through Dr. J. C. MacDonald, National Research Council of Canada, Prairie Regional Laboratory, Saskatoon, Saskatchewan, samples of two naturally occurring cyclic hydroxamic acids, aspergillic acid (VII) and pulcherriminic acid (VIII), became available. Their *in vitro* antibacterial activities, determined by our general method of testing, are listed in Table IV. These results indicate that our test is more rigorous than that applied by previous authors (17–19). The activities of compounds *18* and *19* (Table III) compare favorably with those of aspergillic acid and pulcherriminic acid.

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2-Halogenoethylamines as Potential Folic Acid Antagonists I. Synthesis and Biological Activity of Ethyl-*N*-[1-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-(2-chloro)-propyl]-*p*-aminobenzoate

By A. M. TRIGGLE and D. J. TRIGGLE

The synthesis of the title compound (VII) is described. The key intermediate in this synthesis was prepared from 1-chloro-2,3-epoxypropane and ethyl-*p*-aminobenzoate. Preliminary biological data, including toxicity, and antitumor and folic reductase inhibitory actions, are presented.

THE USE OF nitrogen mustards as antitumor agents has met with varying degrees of success (1, 2). One of the principal disadvantages to the use of these agents in chemotherapy is their generally high toxicity to the host, which results from their undoubted ability to act, in many cases, as rather nonspecific alkylating agents. Nevertheless, the search for selectively and specifically acting (3) alkylating agents is of importance because it offers the hope that appropriately designed compounds will be able to inactivate irreversibly and specifically enzyme and other macromolecular systems of special importance to the tumor cell.

Previous studies have raised the possibility that certain 2-halogenoethylamines, *i.e.*, 4-*N,N*-(di-2-bromoethyl)aminobenzene sulfonamide (I), may produce tumor inhibition through inactivation of one or more stages of the folic acid pathway. As part of a general program intended to investigate the potential of 5-substituted pyrimidines as antitumor agents, the authors are engaged in the synthesis of 2-halogenoethylamines bearing an appropriately substituted pyrimidine nucleus (II). The synthesis and preliminary biological data for one of these compounds, ethyl-*N*-[1-(2-amino-4-hydroxy-6-methyl-5-pyrimidyl)-3-(2-chloro)propyl]-*p*-aminobenzoate (II, R_1 = OH, R_2 = Me, X = Cl, R_3 = H, R_4 = CO_2Et) are reported here. The synthetic route is outlined in Scheme I.

The key intermediate, ethyl-*N*-(2-hydroxy-3-chloropropyl)-*p*-aminobenzoate (III), was con-

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