Preparation of Certain Biologically Active Anthraquinone Derivatives Containing Nitrogen

By ALFRED C. CORE† and ERNST R. KIRCH

A new approach to the synthesis of benzo[g]quinoline-5,10-diones is presented and discussed. Five benzo[g]quinoline-5,10-diones and two amino-hydroxyanthraquinones were prepared and screened for antibacterial activity. Some inhibition of growth of one or more common organisms was demonstrated by most of the compounds tested.

SINCE SOME naturally occurring hydroxyanthraquinones as well as other compounds of similar structure have been shown to possess antibacterial activity (1–3), it was felt that a series of such compounds might be tested for like activity. In addition, since many of the known antibiotics contain nitrogen, either in the form of an amine or within one of the rings of the compound (as in the actinomycins), it was desired to investigate a series of benzo[g]quinoline-5,10-diones for similar activity.

For this purpose the compounds (hitherto not synthesized) prepared and screened for antibacterial activity were 6,9-dihydroxybenzo[g]-quinoline-5,10-dione(I), 6-hydroxy-9-chlorobenzo[g]quinoline-5,10-dione(II), 6,9-dichlorobenzo[g]quinoline-5,10-dione(III), 1-hydroxy-4-dimethylaminoanthraquinone(IV), bis-(4-chlorophenyl) quinolinate(V).

It was desired to have a compound which had been prepared previously and which could serve as a reference compound for comparison of physical and chemical properties of the new compounds. The particular reference compound chosen was 2-hydroxybenzo[g]quinoline-5,10-dione-4-carboxylic acid, which was prepared from 3-amino-2-naphthoic acid according to the method of Etienne and Staehelin (4, 5). As reported by these authors, the compound when heated decarboxylated to form 2-hydroxybenzo[g]quinoline-5,10-dione, which in turn sublimed.

Since such treatment led to appreciable decomposition of the compound with resulting loss of material, it was decided to carry out the decarboxylation under reduced pressure.

However, when the material was heated *in vacuo*, the product collected was not the expected decarboxylated compound. Instead it was found that at low pressure the carboxylic acid could be sublimed unchanged.

No attempt was made to synthesize compounds bearing substituents on the benzenoid ring by this method, since the yields for all steps after formation of 1-acetylbenzindoxyl were good only if the quantities of starting material were kept within a range of from 100–500 mg.

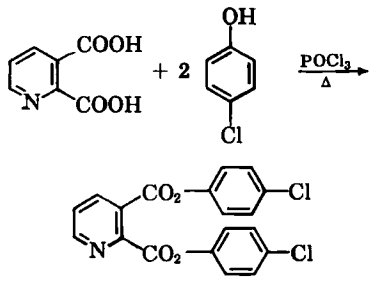
As an alternative it was hoped that a method might be found for condensation of quinolinic acid, its acid chloride or anhydride with a suitably substituted aromatic nucleus.

Attempts to condense the acid or its derivatives with activated aromatic ring systems using aluminum chloride (6), polyphosphoric acid (7), or sulfuric acid (8), were unsuccessful. No tractable products other than starting material were obtained.

Phosphoryl chloride acts as a Lewis acid which might serve to promote the formation of the desired benzo[g]quinolinediones. Further support for its use lay in the finding that aged phosphoryl chloride usually contains polyphosphoric acid (9) which might be expected to further the cyclization.

Treatment of *p*-chlorophenol and quinolinic acid with phosphoryl chloride resulted in the formation of a product which proved to be bis-(4-chlorophenyl) quinolinate.

Formation of the ester may be represented by



The use of a combination of phosphoryl chloride and polyphosphoric acid has been reported (10, 11). Assuming polyphosphoric acid could arise only from hydrolysis of the

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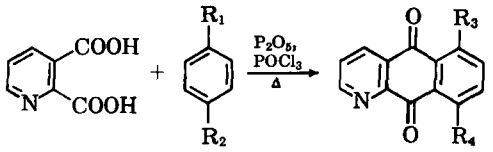
phosphoryl chloride in aged samples of the latter, phosphorus pentoxide should react with any phosphoric acid—arising either from hydrolysis or as the result of reaction with the quinolinic acid—to produce polyphosphoric acid.

Treatment of a 2.5:1 molar ratio mixture of quinolinic acid and hydroquinone monomethyl ether with the mixture of phosphoryl chloride and phosphorus pentoxide under reflux conditions led to the formation of 6,9-dihydroxybenzo[g]-quinoline-5,10-dione (I). This same method (with but slight modifications) was used in the preparation of the other benzo[g]quinoline-5,10-diones.

A general equation for these syntheses is



I. R_1 = —OCH_3; R_2 = —OH, —OCH_3; R_3 = R_4 = —OH
II. R_1 = R_3 = —OH; R_2 = R_4 = —Cl
III. R_1 = R_2 = R_3 = R_4 = —Cl

When the same reaction was run using the dimethyl ether of hydroquinone in place of the monomethyl ether an identical product was formed, as confirmed by physical constants and mixed melting point determination. Product identity was confirmed because the ether underwent cleavage to the phenolic compound when treated with phosphoryl chloride under reflux conditions. That cleavage occurred after ring closure was suggested by the somewhat better product yield when the dimethyl ether was used. Such an increase in yield could be attributed to the greater stability toward oxidation of the ether, hydroquinone itself tending to undergo oxidation to the quinone, which would be expected to be less amenable to attack by the electrophilic agent.

Treatment of *p*-chlorophenol and quinolinic acid under the same conditions led to the formation of a mixture of products. Since more than 94% of the total yield consisted of one isomer, it was felt that this compound was 6-hydroxy-9-chlorobenzo[g]quinoline-5,10-dione. Such an assumption was made on the basis that such reactions as have been reported which involve a Friedel-Crafts reaction between quinolinic acid and a benzenoid nucleus have led only to the formation of substituted picolinic acids rather than nicotinic acids (6); and further, that reactions involving phthalic acid condensations with substituted phenols to form substituted benzoylbenzoic acids have produced reactions *ortho* to the hydroxyl group when *p*-chlorophenol, *o*-cresol, or *p*-cresol were used (12).

It was possible to synthesize the 6,9-dichloro compound by the use of *p*-dichlorobenzene. In this case the yield was somewhat lower than when phenolic compounds were used. This is not surprising when one considers that the latter are in general much more amenable to nucleophilic attack.

BIOLOGICAL TESTING[1]

Due to the similarity in structure to some of the antibiotics currently in use, it was hoped that the compounds which were prepared might show some bacteriostatic activity. With this in mind, it was decided to screen the compounds for activity against several common organisms.

Since a rapid screening was desired, it was felt that a method similar to the original disk method (13) might well be used. Although the procedure was not quantitative, some quantitation could be achieved by comparison of the effects produced by the test compound with those of a known antibiotic.

A technique was employed whereby small filter paper disks were impregnated with alcoholic solutions of the compounds, and after streaking the agar plates with the test organism, the disks were imbedded in the surface of the agar. In this way it was possible to demonstrate activity of a limited nature by most of the compounds tested.

While nearly all of the compounds displayed at least slight activity, only two could be considered significant. These were 1-hydroxy-4-dimethyl*aminoanthraquinone* and 6,9-dihydroxybenzo[g]-quinoline-5,10-dione. Neither, however, was as effective as tetracycline under the conditions employed.

EXPERIMENTAL

Syntheses

2-Hydroxybenzo[g]-quinoline-5,10-dione-4-carboxylic Acid.—This compound was synthesized according to the method of Etienne and Staehelin (4, 5), starting with 3-amino-2-naphthoic acid. As reported by these authors, decarboxylation occurred upon heating, and the 2-hydroxybenzo[g]-quinoline-5,10-dione sublimed, observed m.p. 341–343° (uncorrected). Reported m.p. 349–351° (5). If heated to 230–240° at 0.3 mm. Hg pressure, the acid sublimed without decomposition.

1-Hydroxy-4-dimethylaminoanthraquinone.—The acid sulfate of *p*-aminophenol was prepared by dissolving the phenol in an excess of 10% sulfuric acid and precipitating the salt by the addition of ethanol. After recrystallization from absolute ethanol, the melting point was found to be 308–310° (uncorrected).

A mixture of 7.0 Gm. (0.034 mole) of the salt, 11.3 Gm. (0.076 mole) phthalic anhydride, and 1.5

[1] The authors are indebted to Dr. Leon LeBeau, Department of Microbiology, College of Medicine, University of Illinois, and to Dr. Carmen Mascoli, Department of Microbiology, West Virginia University, for their assistance and advice in the testing procedures.

Gm. boric acid with 30.0 ml. concentrated sulfuric acid was heated slowly in an oil bath to a temperature of 160°, and maintained at this temperature for 8 hours. At the end of this time, the melt was cooled and poured on about 200 Gm. chipped ice with constant stirring. The reaction mixture was then carefully neutralized to approximately pH 7 by the addition of sodium bicarbonate. The mixture was evaporated to dryness, the residue powdered and extracted with chloroform using a Soxhlet extractor. The chloroform extract was evaporated to dryness and the crude product (7.5 Gm. = 93%) purified by recrystallization from 95% ethanol, followed by sublimation at 3 mm. Hg, and a bath temperature of 140°. The bright red needles darkened slightly at 168–170° and melted at 212–213° (uncorrected). Reported m.p., 215° (14).

A solution of 200 mg. (0.0084 mole) of 1-hydroxy-4-aminoanthraquinone in 10 ml. absolute methanol was treated with 5 Gm. (0.035 mole) methyl iodide and 0.1 Gm. anhydrous potassium carbonate. After refluxing on a steam bath for 6 hours, the solvent and excess methyl iodide were allowed to evaporate.

The dry residue was purified by sublimation under reduced pressure at 160–180°. Overall yield 155 mg. (69.7%) of orange needles, m.p. 204–205° (uncorrected).

Anal.—Calcd. for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.82; H, 4.93; N, 5.19.

Bis-(4-chlorophenyl) Quinolinate.—A mixture of 2.1 Gm. (0.013 mole) quinolinic acid and 6.4 Gm. (0.050 mole) *p*-chlorophenol was treated with 13.4 Gm. phosphoryl chloride, and the mixture refluxed 6 hours. After cooling, the reaction mixture was poured into ice water and the excess phosphoryl chloride allowed to decompose. The reddish-brown oily product was separated from the aqueous layer and treated with solid sodium carbonate. The resulting oily green material was dissolved in chloroform, the solution dried over anhydrous sodium sulfate, and the chloroform evaporated. The still-oily residue was dissolved in a small amount of boiling methanol and filtered. Water was added dropwise until the solution began to cloud. The mixture was agan heated to boiling, filtered, and the filtrate cooled in an ice bath. The resulting dark green crystals were removed by suction filtration and dried. Repeated recrystallization from methanol and water gave 2.1 Gm. dark green needles, m.p. 121–122° dec. (uncorrected).

Anal.—Calcd. for $C_{19}H_{11}NO_4Cl_2$: C, 58.78; H, 2.86; N, 3.61; Cl, 18.27. Found: C, 58.65; H, 2.91; N, 3.58; Cl, 18.20; sapon. equiv., 192.

6,9-Dihydroxybenzo[g]-quinoline-5,10-dione.—A mixture of 5.0 Gm. (0.030 mole) quinolinic acid and 33.5 Gm. phosphoryl chloride was heated under reflux for 45 minutes. At the end of this time, 1.5 Gm. (0.012 mole) hydroquinone monomethyl ether and 3.0 Gm. phosphorus pentoxide were added, and the mixture again refluxed for 3 hours. The cooled reaction mixture was poured on crushed ice to decompose the phosphorus pentoxide and phosphoryl chloride. The dark brown precipitate was removed by suction filtration and recrystallized from absolute methanol, yielding 1.7 Gm. (58.4%) dark purple leaflets, m.p. 308–310° (uncorrected).

Anal.—Calcd. for $C_{13}H_7NO_4$: C, 64.73; H, 2.92; N, 5.81. Found: C, 64.65; H, 2.95; N, 5.85.

Alternate Method.—A mixture of 8.0 Gm. (0.048 mole) quinolinic acid, 6.0 Gm. (0.043 mole) hydroquinone dimethyl ether, 10 Gm. phosphorus pentoxide and 42.0 Gm. phosphoryl chloride was refluxed for 5 hours. After the isolation and purification reported above, the yield was 7.4 Gm. (70.6%) of 6,9-dihydroxybenzo[g]-quinoline-5,10-dione, identical with that obtained above as established by analysis and mixed melting point.

6 - Hydroxy - 9 - chlorobenzo[g]quinoline - 5,10 - dione.—To a mixture of 42.0 Gm. phosphoryl chloride and 10 Gm. phosphorus pentoxide were added 5.0 Gm. (0.030 mole) quinolinic acid and 3.5 Gm. (0.027 mole) *p*-chlorophenol. After refluxing for 6 hours, the mixture was cooled and poured onto chipped ice. The dark orange precipitate was removed by filtration. Recrystallized from absolute methanol, the orange crystals melted at 318–320° (uncorrected). The yield was 6.8 Gm. (70.9%).

Anal.—Calcd. for $C_{13}H_6NO_3Cl$: C, 60.13; H, 2.33; N, 5.40; Cl, 13.66. Found: C, 60.02; H, 2.38; N, 5.45; Cl, 13.75.

6,9 - Dichlorobenzo[g]quinoline - 5,10 - dione.—A mixture of 8.0 Gm. (0.048 mole) quinolinic acid, 5.0 Gm. (0.034 mole) *p*-dichlorobenzene, 10.0 Gm. phosphorus pentoxide and 50.0 Gm. phosphoryl chloride was refluxed for 6 hours, cooled, then poured on chipped ice.

The reddish-brown tar-like mass was dissolved in acetone and reprecipitated by the addition of water. The resulting brown powder was recrystallized twice from 95% ethanol to give fine dark yellow crystals (4.6 Gm. = 48.4%), m.p. 316–317° (uncorrected).

Anal.—Calcd. for $C_{13}H_5NO_2Cl_2$: C, 56.14; H, 1.81; N, 5.04; Cl, 25.50. Found: C, 55.80; H, 1.85; N, 4.95; Cl, 25.12.

Bacteriological Screening

A weighed sample of each of the compounds was dissolved in absolute methanol, and the volume adjusted so that the final solution contained 0.300 mg./ml. Filter paper disks were impregnated by placing them on a glass plate and allowing 0.2 ml. of a solution of one of the compounds to flow slowly onto the surface of the disk. The solvent was then allowed to evaporate, leaving each of the disks impregnated with 60 mcg. of one of the compounds under test. For comparison of activity, disks containing 40 mcg. of tetracycline were used.

The impregnated disks were placed on tryptose-glucose-agar plates inoculated with one of the following organisms: β-hemolytic *Strep. pyogenes*, *Proteus vulgaris*, *Escherichia coli*, *Staph. aureus*, *Salmonella enteritidis*, *Bacillus cereus* var. *terminalis*. The disks were gently pressed directly into the surface of the agar with the aid of a sterile glass rod. After covering, the cultures were incubated at 37° for 24 hours, then examined for inhibition of growth of the organisms.

The results obtained are shown in Table I.

SUMMARY

The following hitherto unreported compounds were synthesized and screened for bacteriostatic

TABLE I.—INHIBITION OF GROWTH OF TEST ORGANISMS[a]

Test Compound	*Strep. pyogenes*, β-hemolytic	*P. vulgaris*	*E. coli*	*Staph. aureus*	*Salmonella enteritidis*	*B. cereus* var. *terminalis*
1-Hydroxy-4-aminoanthraquinone	−	−	−	−	±	++
1-Hydroxy-4-dimethylaminoanthraquinone	−	+	+	−	−	−
6,9-Dihydroxybenzo[g]quinoline-5,10-dione	++	−	±	−	±	−
6-Hydroxy-9-chlorobenzo[g]quinoline-5,10-dione	−	−	−	−	+	−
6,9-Dichlorobenzo[g]quinoline-5,10-dione	−	−	−	−	−	−
Bis-(4-chlorophenyl)quinolinate	−	−	−	−	−	−
2-Hydroxybenzo[g]quinoline-5,10-dione-4-carboxylic acid	+	±	−	−	−	−
Tetracycline (40 mcg.)	+	−	++	++	−	+

[a] Radii of zones of inhibition represented by different symbols are: (−) No inhibition. (±) Less than 2 mm. (+) 2–10 mm. (++) Greater than 10 mm.

activity: 6,9-dihydroxybenzo[g]quinoline-5,10-dione(I), 6-hydroxy-9-chlorobenzo[g]quinoline-5,10-dione(II), 6,9-dichlorobenzo[g]quinoline-5,10-dione(III), bis-(4-chlorophenyl) quinolinate (IV), 1-hydroxy-4-dimethylaminoanthraquinone(V).

In addition, two previously reported compounds were prepared and tested: 2-hydroxybenzo[g] quinoline-5,10-dione-4-carboxylic acid(VI) and 1-hydroxy-4-aminoanthraquinone(VII).

While most of the compounds tested showed some degree of inhibition to the growth of some organisms, none were found to approach tetracycline in effectiveness.

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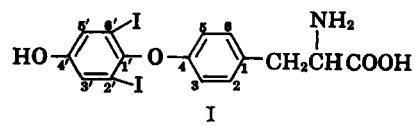
Thyroxine Analogs IX

4′-Deoxy-4′-β-L-alanyl-3,5-diiodo-L-thyronine and Related Stereoisomers as Thyroxine Antagonists

By EUGENE C. JORGENSEN and RICHARD CAVESTRI

On the basis of structural considerations and the activity of 2′,6′-diiodothyronine (I), 4′-deoxy-4′-β-L-alanyl-3,5-diiodo-L-thyronine (VIIb)[1] and a mixture of related stereoisomers have been prepared and tested for activity as thyroxine antagonists in the rat antigoiter assay. The L,L-isomer (VIIb) proved moderately effective as an antagonist at a molar ratio to L-thyroxine of 100:1. The mixed DL,DL-isomers (VIIa) as well as the L,L-isomer (VIIb) were ineffective when tested at a molar ratio to L-thyroxine of 200:1.

STUDIES directed at the preparation of peripheral antagonists to the thyroid hormones have had two principal objectives: (*a*) the development of an agent capable of rapidly reducing the undesirable effects due to high levels of circulating hormone in the various forms of hyperthyroidism, and (*b*) the gaining of a better understanding of the nature of interactions between the thyroid hormones and their biological receptor sites (1). 2′,6′-Diiodo-DL-thyronine (I) was the first compound containing the intact thyronine nucleus characteristic of thyroxine which was shown to possess thyroxine antagonistic properties. Among a number of halogenated thyronines prepared by Niemann and McCasland (2) and tested by Cortell (3),



I

only the 2′,6′-diiodo analog (I) showed thyroxine

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Paper VIII in this series is Reference 17.
[1] An alternate chemical name, 3,5-diiodo-4-(4′-L-phenylalanyloxy)-L-phenylalanine, is used in the *Experimental* section.