function by forming a π complex with a receptor site. Recently, Fried[18] has shown along these lines that fusidic acid and related steroidal derivatives, which have antibacterial activity, require at least one anionic site close to an oxygen function or to the sp^2 electrons of a double bond.

The aldehyde group was found to be essential for maximum activity. Replacement of the carbonyl by a small hydrazone derivative resulted in compounds which still had relatively small, minimum inhibitory concentrations against *S. albus*. The hydrazone (**10**) and thiosemicarbazone (**3**) derivatives had about half the activity of spiramycin, while bulkier hydrazone derivatives were inactive. Reduction of the aldehyde to the alcohol, dihydrospiramycin (**14**), reduced the activity at least twofold. This is seen also in the hexahydroneospiramycin, which is only half as active as the tetrahydro derivative. The electronic character of the group appears to be important for maximum activity but can be masked by bulky substituents resulting in the loss of activity.

(18) I. Fried, G. W. Krakower, D. Rosenthal, and H. Basch, *J. Med. Chem.*, **8**, 279 (1965).

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Anticoccidial Activity in a Series of Alkyl 6,7-Dialkoxy-4-hydroxy-3-quinolinecarboxylates

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The preparation and anticoccidial activity of a number of 6,7-dialkoxy-4-hydroxy-3-quinolinecarboxylates are reported. A number of highly active compounds were obtained, and the relationships of structure and activity are discussed. One of the most active compounds, ethyl 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate, is undergoing extensive evaluation.

Our interest in the synthesis and biological evaluation of quinoline compounds[1] led to the screening of these compounds for anticoccidial properties. Several 6,7-dialkoxy-4-hydroxy-3-quinolinecarboxylates, which are useful intermediates in the synthesis of many types of 4-substituted quinolines, were found to possess desirable activity. Accordingly, a more extensive program was initiated to study the effect of certain structural changes on the anticoccidial activity of this type of quinoline compound.

Chemistry.—Most of the compounds in Table I were prepared by known procedures.[2] The appropriately substituted 1,2-dialkoxybenzenes were obtained by alkylation of catechol with alkyl halides or alkyl sulfates in ethanol or dimethylformamide in the presence of a base such as sodium methoxide, sodium hydroxide, or potassium carbonate. Nitration of the ethers with dilute nitric acid readily produced the 1,2-dialkoxy-4-nitrobenzenes which were then reduced catalytically. The substituted anilines were condensed with diethyl ethoxymethylenemalonate or dimethyl methoxymethylenemalonate, and cyclization of the anilinomethylenemalonic esters was effected in boiling Dowtherm A®[3] after the procedure of Price, *et al.*,[2d] to give the 4-hydroxy-3-quinolinecarboxylates.

Esters other than ethyl or methyl were prepared by two general procedures. The methyl or ethyl quinolinecarboxylates were either heated with the appropriate alcohol in the presence of *p*-toluenesulfonic acid or were saponified and treated with thionyl chloride and then with the appropriate alcohol.

Compounds **4** and **13** (Table I) were prepared by acetylation of the corresponding hydroxy derivatives with a mixture of acetic anhydride and sodium acetate.

Biological Methods and Results.—White Rock Cross chicks (Cobb's strain) 7–14 days of age were used in the anticoccidial evaluations. The chicks were maintained in wire-floored battery cages, 20 chicks/cage, equipped with outside feeders and waterers.

An open formula, custom mix, chick starter mash composed of natural feedstuffs and supplemented with minerals and vitamins but containing no other feed additives was used as the basal ration. The compounds under test were mixed in desired amounts with 6.8 kg of mash. The mixing was done in a stainless steel twin-shell mixer. The medicated feeds were placed in the feeding troughs immediately after the inoculation of the chicks with sporulated oocysts of *Eimeria tenella*.

The chicks were inoculated by gavage with a 1-ml tuberculin syringe. The inoculum was standardized to provide 250,000 sporulated oocysts suspended in 1 ml of 0.75% carboxymethylcellulose. The tests were terminated 10 days after inoculation.

In all experiments, two groups (20 chicks/group) of nonmedicated, inoculated chicks were maintained for controls.

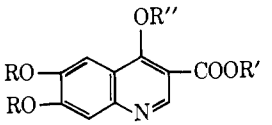
(1) (a) R. K. Bickerton, R. F. Dailey, W. T. Rockhold, and R. H. Butler, *J. Pharmacol. Exptl. Therap.*, **144**, 218 (1964); (b) G. R. Petitt and A. B. Neill, *Can. J. Chem.*, **42**, 1764 (1964); (c) British Patent 942,524 (1963).

(2) (a) M. Conrad and L. Limpach, *Ber.*, **20**, 944 (1887); (b) R. G. Gould and W. A. Jacobs, *J. Am. Chem. Soc.*, **61**, 2890 (1939); (c) B. Riegel, G. R. Lappin, B. H. Adelson, R. I. Jackson, C. J. Albisetti, Jr., R. M. Dodson, and R. B. Baker, *J. Am. Chem. Soc.*, **68**, 1264 (1946); (d) C. C. Price and R. M. Roberts, *ibid.*, **68**, 1204 (1946).

(3) A commercial heat-transfer liquid, which is a eutectic of 26.5% biphenyl and 73.5% diphenyl ether.

TABLE I

ALKYL 6,7-DIALKOXY-4-HYDROXY-3-QUINOLINECARBOXYLATES



No.[a]	R	R'	Mp, °C[c]	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	Min[e] effective dose, % in feed
1	CH_3	C_2H_5	272–273[d]	...	...	...	...	...	...	...	>0.1
2	C_2H_5	CH_3	277–278	$C_{15}H_{17}NO_5$	61.85	5.88	4.81	61.73	5.96	4.95	0.00825
3	C_2H_5	C_2H_5	285–286	$C_{16}H_{19}NO_5$	62.94	6.27	4.59	62.79	6.36	4.57	0.0165
4[b]	C_2H_5	C_2H_5	168–170	$C_{18}H_{21}NO_6$	62.24	6.10	4.03	62.19	6.08	4.05	0.0055
5	C_2H_5	C_3H_7	261–264	$C_{17}H_{21}NO_5$	63.93	6.63	4.39	63.85	6.48	4.38	0.03
6	C_2H_5	$CH(CH_3)_2$	254–256	$C_{17}H_{21}NO_5$	63.93	6.63	4.39	63.78	6.59	4.54	0.022
7	C_2H_5	$CH_2CH{=}CH_2$	255–257	$C_{17}H_{19}NO_5$	64.34	6.04	4.41	64.10	6.12	4.48	0.011
8	C_2H_5	$CH_2CH(CH_3)_2$	262–265	$C_{18}H_{23}NO_5$	64.85	6.95	4.20	64.97	6.94	4.41	0.033
9	C_3H_7	CH_3	262–264	$C_{17}H_{21}NO_5$	63.93	6.63	4.39	63.87	6.64	4.49	0.011
10	C_3H_7	C_2H_5	271–273	$C_{18}H_{23}NO_5$	64.85	6.95	4.20	64.70	6.80	4.41	0.00825
11	C_3H_7	$CH(CH_3)_2$	244–246	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.65	7.20	4.11	0.022
12	$CH(CH_3)_2$	CH_3	255–257	$C_{17}H_{21}NO_5$	63.93	6.63	4.39	64.03	6.52	4.53	0.00275
13[b]	$CH(CH_3)_2$	CH_3	103–105	$C_{19}H_{23}NO_6$	63.14	6.42	3.88	63.03	6.44	4.00	0.00275
14	$CH(CH_3)_2$	C_2H_5	234–235	$C_{18}H_{23}NO_5$	64.85	6.95	4.20	64.53	6.94	4.37	0.0055
15	$CH(CH_3)_2$	C_3H_7	233–236	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.76	7.30	3.79	0.011
16	$CH(CH_3)_2$	$CH(CH_3)_2$	222–225	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.39	7.24	3.90	0.0055
17	$CH(CH_3)_2$	$CH_2CH{=}CH_2$	238–240	$C_{19}H_{23}NO_5$	66.07	6.71	4.06	66.02	6.66	4.12	0.00825
18	C_4H_9	C_2H_5	279–284	$C_{20}H_{27}NO_5$	66.46	7.53	3.88	66.36	7.42	3.87	0.1
19	$CH_2CH(CH_3)_2$	CH_3	280–282	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.75	7.18	4.16	0.00275
20	$CH_2CH(CH_3)_2$	C_2H_5	288–291	$C_{20}H_{27}NO_5$	66.46	7.53	3.88	66.43	7.52	4.02	<0.0007
21	$CH_2CH(CH_3)_2$	C_3H_7	229–230	$C_{21}H_{29}NO_5$	67.18	7.79	3.73	67.37	7.68	4.16	0.011
22	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	245–248	$C_{21}H_{29}NO_5$	67.18	7.79	3.73	67.26	7.72	3.90	0.011
23	$CH_2CH(CH_3)_2$	$CH_2CH{=}CH_2$	234–235	$C_{21}H_{27}NO_5$	67.54	7.29	3.75	67.59	7.15	3.95	0.00275
24	$CH(CH_3)CH_2CH_3$	CH_3	246–248	$C_{19}H_{25}NO_5$	65.69	7.25	4.03	65.77	7.10	4.01	0.0055
25	$CH(CH_3)CH_2CH_3$	C_2H_5	216–217	$C_{20}H_{27}NO_5$	66.46	7.53	3.88	66.51	7.50	3.97	0.055
26	$CH_2CH(CH_3)CH_2CH_3$	CH_3	265–266	$C_{21}H_{29}NO_5$	67.18	7.79	3.73	67.42	7.67	3.79	0.00825
27	$C_{10}H_{21}$	CH_3	234–240	$C_{31}H_{49}NO_5$	72.19	9.58	2.72	72.15	9.64	2.66	0.033

[a] R' = H unless otherwise noted. [b] R'' = $COCH_3$. [c] Melting points are corrected. [d] See ref 2c. [e] See text.

At the end of the experimental period, the number of surviving birds in each group was recorded. For each group the percentage of deaths was calculated from the ratio of the number of deaths to the total number (20) of birds used. A compound is considered active or effective if the number of deaths in the untreated control group is statistically significantly greater than that of the treated group.

From the biological data in Table I it is apparent that optimal anticoccidial activity is obtained when the ether groups (RO) are isobutoxy (**20**). Branched groups at these positions confer greater activity than normal alkyl groups; the dimethoxy compound (**1**) is totally inactive. Within the isobutoxy series modifications of the ester group result in the following order of activity for R^1: ethyl > methyl = allyl > propyl and isopropyl. This order of activity varies somewhat in the other alkoxy series. Acetylation of the 4-hydroxy group results in increased activity in the diethoxy compound (compare **3** and **4**), and in the same activity for the diisopropoxy compounds (**12** and **13**).

One of the most active compounds, ethyl 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (**20**),[4] is undergoing extensive evaluation. This compound at 0.00825% in feed compares very favorably with amprolium[5] at 0.0125% against *E. tenella* infections.

(4) Generic name, buquinolate.

(5) Amprol®.

Experimental Section

The experimental procedures described below are typical for the preparation of the compounds listed in Table I.

Ethyl 4-Hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (20).—To a solution of 197 g (1.8 moles) of catechol in 730 ml of absolute ethanol was added 143 g (3.6 moles) of NaOH pellets in a nitrogen atmosphere. When salt formation was complete, 490 g (3.6 moles) of isobutyl bromide was added dropwise at 55–65°, and the mixture refluxed for 17 hr without nitrogen. After filtration, the filtrate was concentrated and the residue was taken up in water and extracted with ether. The combined extracts were washed with 10% NaOH solution and then with water. After drying the ether solution was concentrated to give crude *o*-diisobutoxybenzene, 125 g (33%).

At a temperature below 25°, 105 g (0.47 mole) of *o*-diisobutoxybenzene was added dropwise to a 1:1 solution of water and concentrated HNO_3 over a period of 30 min. Stirring was continued with occasional cooling until the reaction was no longer exothermic. The mixture was poured into ice–water and the resulting solid was filtered, washed well with water, dissolved in warm methanol, and filtered. A little water was added to start crystallization of the 3,4-diisobutoxynitrobenzene, yield 49 g (39%).

Hydrogenation of 16 g (0.06 mole) of the nitro compound in 200 ml of absolute ethanol over 2 g of 5% Pd–C catalyst (50% water) at 2.8 kg/cm² was complete in 20 min. After filtration, 13 g (0.06 mole) of diethyl ethoxymethylenemalonate[6] was added, and the solution was heated under reflux for 1 hr. The white crystalline product which separated upon cooling was filtered, washed with ethanol, and air-dried, giving 18 g (75%) of the anilinomethylenemalonate.

A 4.8-g portion of this malonate was added to 48 ml of boiling Dowtherm A. After boiling for 12 min, the solution was allowed

(6) Kay-Fries Chemicals, Inc.

to cool to room temperature. The product was filtered, washed with a little Dowtherm, then with acetone, and dried at 100° to yield 3.9 g (92%) of **20**.

Methyl 4-Hydroxy-6,7-bis(2-methylbutoxy)-3-quinolinecarboxylate (26).—To a solution of 110 g (1 mole) of catechol in 3800 ml of dimethylformamide (DMF) in a nitrogen atmosphere was added 108 g (2 moles) of sodium methoxide powder with stirring. The reaction mixture was stirred at 40–45° for 1 additional hr and then 302 g (2 moles) of 1-bromo-2-methylbutane was added in about 45 min. A clear solution was obtained about 1.5 hr after the addition, kept under nitrogen overnight, and then poured into water. This mixture was acidified to pH 5 and extracted with benzene. The combined extracts were washed with 10% NaOH solution followed by water. After drying, the benzene was stripped *in vacuo*, yielding 98 g (39%) of *o*-bis(2-methylbutoxy)benzene.

To a solution of 200 ml of concentrated HNO_3 and 200 ml of water was added gradually 86 g (0.344 mole) of *o*-bis(2-methylbutoxy)benzene with stirring, so that the temperature never rose above 30°. Addition was completed in 1.5 hr and the reaction mixture was further stirred for 2.5 hr and then poured over cracked ice. The gummy solid was dissolved in ether and washed well with water until the washings were almost neutral. After drying, the ether was distilled to yield 87 g (86%) of dark, liquid 3,4-bis(2-methylbutoxy)nitrobenzene.

Hydrogenation of 30 g (0.1 mole) of the crude nitro compound in 200 ml of absolute ethanol over 8 g of 5% Pd-C catalyst (50% water) at 2.8 kg/cm² initial pressure was complete in 1 hr. After filtration, the filtrate was heated under reflux with 17 g (0.1 mole) of dimethyl methoxymethylenemalonate[6] for 2 hr. The solution was stripped *in vacuo* to yield 40 g of residue. A 120-g sample of the anilinomethylenemalonate prepared in this way was added to 500 ml of boiling Dowtherm A. After boiling for 10 min, the solution was allowed to cool to room temperature, the solid product was filtered, and the filtrate was again heated to boiling as rapidly as possible. After four such heating periods of 8–10 min each, 46 g (41%) of crude product was obtained. Recrystallization from 2 l. of DMF with charcoal gave 37 g of white product.

Propyl 6,7-Diethoxy-4-hydroxy-3-quinolinecarboxylate (5).—A mixture of 61 g (0.2 mole) of ethyl 6,7-diethoxy-4-hydroxy-3-quinolinecarboxylate (**3**), 1600 ml of propanol, and 2 g of *p*-toluenesulfonic acid was heated under reflux for 6 days. The mixture was filtered hot, and the solid was washed with ether and dried to give 42 g of product. A second crop of 15 g separated from the filtrate, giving a total of 57 g, 189°, of the propyl ester. Recrystallization of 55 g from DMF with charcoal gave 50 g of pure material.

Propyl 4-Hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (21). A mixture of 37 g (0.1 mole) of ethyl 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (**20**) and 300 ml of 10% NaOH solution was heated under reflux for 4 hr. After filtration the hot solution was acidified with HCl and allowed to cool. The precipitate was filtered, washed well with water, and dried. A suspension of 19 g of this solid in 600 ml of benzene was heated under reflux with a Dean–Stark trap to remove any water still present. Then 5 ml of $SOCl_2$ was added and the mixture was heated under reflux for 10 hr and allowed to cool overnight. The crude acid chloride (18 g) was filtered, washed with benzene and ether, and dried at 100°.

A mixture of 6.0 g (0.017 mole) of the acid chloride in 300 ml of propanol was refluxed for 1.75 hr. The solution was filtered and cooled, and a small amount of NH_4OH was added to adjust the solution to pH 7–8. The precipitate was triturated with water, filtered, and dried at 100°. The yield was 4.8 g (76%) of the propyl ester. Recrystallization of 1 g from 100 ml of absolute ethanol gave 0.85 g of white needles.

Ethyl 4-Acetoxy-6,7-diethoxy-3-quinolinecarboxylate (4).—A mixture of 50 g (0.16 mole) of ethyl 6,7-diethoxy-4-hydroxy-3-quinolinecarboxylate (**3**) and 30 g of anhydrous sodium acetate in 1250 ml of acetic anhydride was heated under reflux for 2.25 hr and then allowed to cool. The solid was collected and washed with cold water and dried at 100°; yield, 49 g (86%) of the 4-acetoxy compound. Recrystallization from ethanol gave an analytical sample.

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Preparation of Substituted Phenethyl Alcohols and a Study of Their Bacteriostatic Action in *Escherichia coli* B[1]

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Fifteen analogs of phenethyl alcohol have been prepared to explore the effect of ring substituents, and to a lesser degree solubility, on bacteriostatic activity. All of the monomethoxy- and dimethoxy-, all of the monohydroxy-, and three dihydroxyphenethyl alcohols have been prepared by the utilization of a variety of procedures and subjected to biological evaluation. The bacteriostatic concentration of each of the phenethyl alcohols for *Escherichia coli* B was determined and the reversibility of the bacteriostasis established. The influence of *p*-methoxy-, 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohol in the synthesis of DNA, RNA, and protein by *E. coli* B was studied. Each of these compounds, as well as the parent compound, caused 100% inhibition of the synthesis of DNA, RNA, and protein when present at its bacteriostatic concentration. When the inhibitor was removed, these biosynthetic processes, as well as cell division, were reinitiated and resumed, rates parallel to those characteristic of the control, uninhibited culture.

In 1953, Lilley and Brewer[3] reported that phenethyl alcohol exhibited a marked inhibitory effect on gram-negative bacteria while inhibiting gram-positive bacteria only slightly if at all. They found that the incorporation of 2.05×10^{-5} moles/ml of phenethyl alcohol in a trypticase soy agar preparation produced a medium which could be used to isolate selectively the gram-positive bacteria from a mixed bacterial flora.

Phenethyl alcohol appeared to be one of the few compounds which is more inhibitory to gram-negative than to gram-positive bacteria. This unusual property of the material prompted Berrah and Konetzka[4] to

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(2) This report constitutes part of the thesis submitted by E. Z. Khafagy for the Ph.D. degree, University of Nebraska, 1966.

(3) E. D. Lilley and J. H. Brewer, *J. Am. Pharm. Assoc.*, **42**, 6 (1953).

(4) G. Berrah and W. Konetzka, *J. Bacteriol.*, **83**, 738 (1962).