Preparation and Antibacterial Activity of Some Spiramycin Derivatives

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In an effort to broaden the antibacterial activity of spiramycin, various derivatives of the antibiotic have been prepared.

The macrolide antibiotics, spiramycins, were isolated from *Streptomyces*[2,3] and their complete structures were reported by Kuehne.[4]

Pinnert-Sindico, *et al.*,[3] reported the protection, by spiramycin, of mice infected with virulent cultures of hemolytic streptococci, pneumococci, and staphylococci. Its high tissue affinity[5] and low toxicity made spiramycin a potentially attractive antibiotic.

The present work deals with an attempt to increase the spectrum of spiramycin. Modifications were made to evaluate such factors as hydrophilic character and efficiency of the functional groups. The preparation used as starting material was a mixture of spiramycin A, B, and C, which are, respectively, an alcohol, its acetate, and its propionate; thus the products are, of necessity, similar mixtures.

Table I lists the derivatives of spiramycin which were prepared for microbiological evaluation against *Escherichia coli*, a gram-negative, and *Staphylococcus albus*, a gram-positive organism. In Table II are given the yields, melting points, and microanalytical data of the substances studied.

TABLE I

ANTIBACTERIAL ACTIVITY OF SPIRAMYCIN DERIVATIVES

No.	Compd	MIC,[a] μg/ml *S. albus*	MIC,[a] μg/ml *E. coli*
1	Spiramycin dimethiodide	62.5	150.0
2	Spiramycin oxime	250.0	500.0
3	Spiramycin thiosemicarbazone	25.0	500.0
4	Spiramycin di-N-oxide	>150.0	>150.0
5	Tetrahydrospiramycin di-N-oxide	>250.0	>500.0
6	Triacetylspiramycin	22.0	62.5
7	Dibenzoylspiramycin	100.0	250.0
8	Spiramycin maleate	11.2	62.5
9	Triacetyl tetrahydrospiramycin	25.0	250.0
10	Spiramycin hydrazone	18.75	>75.0
11	Spiramycin phenylhydrazone	>25.0	>75.0
12	Spiramycin-2-benzothiazolyl hydrazone	>25.0	>75.0
13	Spiramycin-2-quinolylhydrazone	>25.0	>75.0
14	Dihydrospiramycin	>25.0	>75.0
15	Neospiramycin dimethiodide	>250.0	100.0
16	Tetrahydroneospiramycin	12.0	21.5
17	Hexahydroneospiramycin	>25.0	20.0
18	Hexahydrospiramycin	6.1	22.5
19	Spiramycin	13.4	42.2
20	Tetrahydrospiramycin	5.48	25.0
21	Neospiramycin	6.2	19.6
22	Forocidin	>25.0	>75.0

[a] Minimum inhibitory concentration.

Experimental Section

The melting points were determined in a Hershberg apparatus and are corrected. The ultraviolet spectra were measured in methanol on a Cary recording spectrophotometer. Nmr spectra were determined at 60 Mc with a Varian Associates A-60 instrument with tetramethylsilane as an internal reference standard. The term spiramycin is used to designate the mixture described in the introduction.

Hydrogenation of spiramycin and its derivatives was carried out by the procedure of Wettstein and co-workers[6] in the hydrogenation of individual spiramycin components.

Neospiramycin (23) was prepared by the method of Paul.[7]

Spiramycin Dimethiodides (1 and 15).—To a stirred solution of 2 g of the appropriate spiramycin derivative in 20 ml of dry 2-propanol, was added 1 g (7 mmoles) of methyl iodide and 1 g of anhydrous Na_2CO_3. The suspension was heated to 70° for 2.5 hr and filtered hot. The clear filtrate was evaporated, and the oily residue was solidified by the addition of anhydrous ether. The diquaternary salts were purified by reprecipitation from isopropyl alcohol with anhydrous ether.

Spiramycin oxime (2) was prepared by refluxing 1 g (1 mmole) of spiramycin and 0.1 g (15 mmoles) of hydroxylamine hydrochloride in 20 ml of ethanol for 4 hr. The ethanol was removed under reduced pressure and the residue was purified from a $CHCl_3$–hexane mixture.

Spiramycin Dibenzoate (7).—To 2 g (2 mmoles) of spiramycin and 1.2 g of anhydrous $NaHCO_3$ in 50 ml of anhydrous acetone was added 1 g (7 mmoles) of benzoyl chloride. The reaction mixture was stored for several days at 4° to separation of an oil, which was taken up in ether. The ester was precipitated by the addition of petroleum ether.

Spiramycin triacetates (6 and 9) were prepared from the appropriate spiramycin derivative by the method of Wagner and co-workers[8] in their acetylation of magnamycin.

Spiramycin Di-N-oxide (4).—To a solution of 2 g (4 mmoles) of spiramycin in 100 ml of methanol was added slowly 100 ml of a mixture of 3 vol of 30% H_2O_2 and 7 vol of methanol. The yellow reaction mixture was allowed to stand for 24 hr and evaporated. The residual oil was taken up in ethanol and precipitated with ether. This purification procedure was repeated three times. The ultraviolet spectrum showed the presence of a conjugated diene with a maximum at 231 mμ.

Tetrahydroxyspiramycin Di-N-oxide (5).—The hydroxylating agent was prepared according to Daniels and Fischer.[9] To a solution of 2 g (2 mmoles) of spiramycin in 10 ml of acetone and 3 ml of ether was added 2.6 ml of 30% H_2O_2, followed by the dropwise addition of 0.75 ml (1.5×10^{-5} mole of OsO_4) of the hydroxylating reagent. The reaction vessel was capped and kept at 30° for 16 hr. Upon the addition of 40 ml of ether, a white precipitate formed. It was collected and further purified by reprecipitation from an absolute ethanol–ether mixture. The ultraviolet spectrum failed to show the presence of a conjugated diene.

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TABLE II

ANALYTICAL DATA OF SPIRAMYCIN DERIVATIVES

Compd	% yield[a]	Mp, °C	Calcd,[b] % C	H	N	Found, % C	H	N
1	80.5	175–180	48.56	7.39	2.46	48.23	7.6	2.94
2	95.0	173–177[c]	54.8	8.19	4.38	54.44	8.56	4.07
3	83.0	140–145[d]	57.4	8.56	7.53	56.9	8.50	7.45
4	66.0	133–136[e]	54.49	8.66	2.86	53.55	8.41	3.11
5	21.8	163–165	54.8	8.27	3.2	55.02	8.11	2.99
6	71.5	125–128	60.92	8.27	2.8	61.27	8.72	2.59
7	57.5	130–133	64.75	7.92	2.57	64.36	7.57	2.25
8	37.8	143–145[f]	58.70	8.15	2.89	58.2	7.71	2.53
9	79.0	102–105	60.83	8.63	2.78	60.48	8.72	2.90
10	51.5	137–142[d]	60.0	8.83	6.30	59.65	8.79	6.15
11	33.2	135–137	62.55	8.40	5.78	62.51	8.60	5.79
12	33.1	148–151	59.90	8.16	6.73	59.66	7.85	6.60
13	49.0	125–128	62.71	8.33	6.87	62.20	8.54	5.95
14	26.8	127–130	60.91	8.99	3.19	60.45	9.07	3.35
15	28.8	173–176[f]	44.89	7.49	2.66	44.50	7.26	2.70
16	25.2	112–114	61.40	9.60	3.81	62.03	9.60	3.67
17	31.6	105–108	61.23	9.87	3.84	61.48	9.27	4.08

[a] No attempt was made to increase the yields. [b] The calculated values were obtained only after repeated elemental analyses of the starting material, which was reported to be of approximately equal parts of spiramycins A, B, and C, by Rhone-Poulenc of Paris (private communication). [c] As the dihydrochloride. [d] Prepared according to the method of Paul[7] in the synthesis of derivatives of individual spiramycins. [e] As the tetrahydrate. [f] As the monohydrate.

Spiramycin maleate (**8**) was synthesized by the method used by Stephens[10] for the preparation of erythromycin maleate. The ultraviolet spectrum showed a maximum at 232 mμ.

Dihydrospiramycin (**14**) was prepared according to the procedure used by Whaley[11] for the preparation of relomycin from tylosin.

Hydrazone Derivatives of Spiramycin (**11, 12, and 13**).—A solution of spiramycin in absolute ethanol was boiled for 4 hr with an equimolar amount of the appropriate hydrazine. The solvent was evaporated, and the residue was stirred overnight in 100 ml of cold water. The hydrazone derivative was dried and washed several times with petroleum ether (bp 30–60°).

Discussion

Taubeneck[12] has reported that erythromycin is 1000 times more active against L forms of *Proteus mirabilis* than against the gram-negative organism itself. Guze and Kalmanson[13] and Shockman and Lampen[14] with similar experiments, using streptococci and protoplasts, have shown no such difference in the activity of erythromycin. Improved penetration of the cell wall of gram-negative bacteria appears to be one means of broadening the spectrum of macrolide activity.

Agents such as dimethiodides **1** and **15** and di-N-oxides **4** and **5** of the spiramycins were tested with the expectation that they would act at the cell surface.[15] The agents were inactive in the test system used because the lack of surface activity precluded their absorption.

Since the cell walls of the gram-negative organisms are lipid in nature,[16] a more hydrophobic derivative should have better penetrating ability. Neospiramycin (**21**) prepared by mild, acid hydrolysis after Paul,[7] lacks one of the sugars, mycarose, and thus, would be expected to be a more hydrophobic agent. Indeed, the substance was twice as active as spiramycin against both test organisms. When more vigorous acid hydrolysis was carried out according to Corbaz and co-workers,[2] two sugars, forosamine and mycarose, were cleaved from the ring. The resulting lactone, called forocidin (**22**), had greatly reduced activity in the assay. The hydrophobic nature of forocidin would not differ greatly from neospiramycin, since forosamine, the second sugar which was lost, has little polar character. The lack of activity of forocidin might be ascribed to the loss of the dimethylamino group contained in the forosamine moiety.

Saturation of the conjugated diene system by hydrogenation using the procedure of Wettstein,[6] gave tetrahydrospiramycin (**20**), which was twice as active as spiramycin against the test organisms. Then neospiramycin was hydrogenated in an effort to combine the activity gains made by mild hydrolysis and by hydrogenation of the parent compound. Tetrahydroneospiramycin (**16**) was only half as active against *S. albus* as neospiramycin and tetrahydrospiramycin (**18**) and hexahydroneospiramycin (**17**). With all of the partially hydrogenated derivatives, the activity against gram-negative organisms remained constant at twice the activity of the parent compound. The results suggest that the diene system may be important for activity against *S. albus*. The presence of a conjugated diene or dienone system is common to many of the large-ring macrolides for which the activity ratio, gram-negative:gram-positive, is low, *i.e.*, magnamycin, magnamycin B, and oleandomycin. Oleandomycin, while not having an α,β-unsaturated carbonyl, has an exocyclic epoxide, adjacent to a ketone which, according to Wolff and co-workers,[17] may act as an unaltered sp^2 system. The regions of sp^2 character could

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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.

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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.

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