

Streptonigrin and Related Compounds III. Synthesis and Microbiological Activity of Destrioxyphenylstreptonigrin and Analogues

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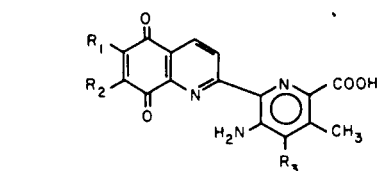
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Synthesis of three tricyclic analogues of streptonigrin, based on the 2-(2'-pyridyl)quinoline-5,8-dione system and with the characteristic substitution pattern of rings A and C of streptonigrin is described. The C-ring precursor, in the form of a substituted 2-acetylpyridine was condensed with either 3-hydroxy-5-methoxy-2-nitrobenzaldehyde or 3-hydroxy-4-methoxy-2-nitrobenzaldehyde, followed by reductive cyclization and oxidation to the corresponding quinones **12** and **29**. The 7-amino substitution was introduced in **12** via bromination and azidation. The 6-amino substitution was introduced through direct reaction of **29** with sodium azide. Destrioxyphenylstreptonigrin **2** was twice as active and the 6-amino-7-methoxy analogue **4** was as active as streptonigrin in a microbiological assay. A 4'-bromo analogue of **2** was 60% as active as streptonigrin.

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The importance of the antitumor antibiotic, streptonigrin (**1**) with its unique tetracyclic structure and the general approach for the planned synthesis of tricyclic analogues with the 2-(2'-pyridyl)quinoline system through the application of a modified Friedlander quinoline synthesis has been described (1). A similar approach, based on this general synthesis, has been published recently by Hibino and Weinreb (2). In this paper, is reported the synthesis and microbiological activity of three tricyclic analogues with the 7-amino-6-methoxy and the 6-amino-7-methoxyquinoline-5,8-dione systems (**2**, **3**, and **4**).

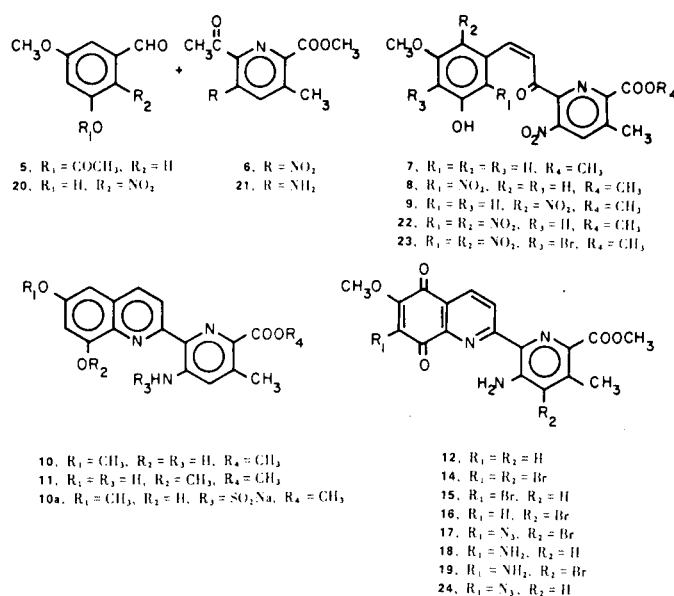


1. $R_1 = \text{OCH}_3$, $R_2 = \text{NH}_2$, $R_3 =$, $R_4 = \text{H}$
 2. $R_1 = \text{OCH}_3$, $R_2 = \text{NH}_2$, $R_3 = \text{H}$, $R_4 = \text{H}$
 3. $R_1 = \text{OCH}_3$, $R_2 = \text{NH}_2$, $R_3 = \text{Br}$, $R_4 = \text{H}$
 4. $R_1 = \text{NH}_2$, $R_2 = \text{OCH}_3$, $R_3 = \text{H}$, $R_4 = \text{H}$

The scheme employed for the synthesis of the 7-amino-6-methoxyquinoline-5,8-dione system is given below.

Base-catalyzed condensation of 3-acetoxy-5-methoxybenzaldehyde (**5**) (1) and 6-carbomethoxy-5-methyl-3-nitro-2-acetylpyridine (**6**) (3), followed by careful esterification with diazomethane (4) gave the azachalcone **7**. Nitration of **7** with nitric acid/acetic acid gave a 1:1 mixture of the 2-nitro (**8**) and the 6-nitro (**9**) derivatives which were separated by chromatography. Since **8** was of more direct application to the scheme, its preferential

Scheme 1



formation was studied and achieved by the use of nitronium tetrafluoroborate. When heated with aqueous methanolic sodium dithionite, **8** and **9** underwent reductive cyclization in a near quantitative yield to the respective isomeric 2-(2'-pyridyl)quinolines (**5**) **10** and **11**. Of these, only **10** formed a complex with Fe^{3+} as would be expected for the 8-hydroxyquinoline structure.

Oxidation of **10** by Fremy's salt or, preferably, periodic acid, gave the 5,8-dione **12** as a maroon crystalline solid. Similar oxidation of **11** yielded the 5,6-dione **13**. Michael addition of azide to the quinone system of **12** was studied under a variety of conditions, but only traces of the

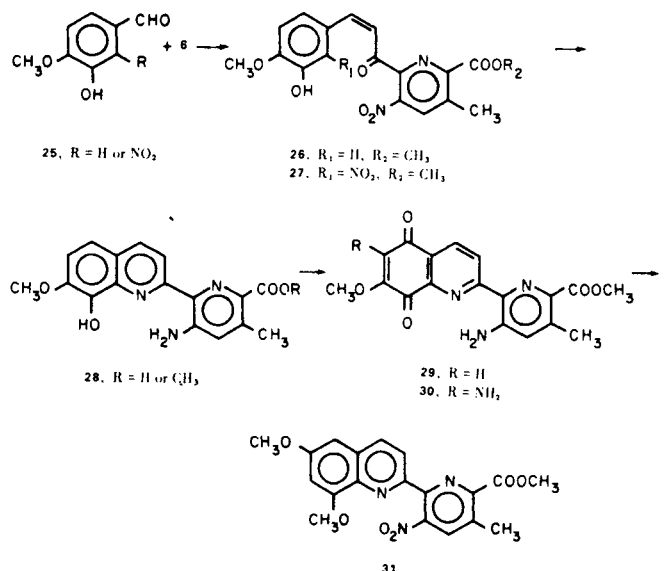
desired azidoquinone could be obtained. Bromination of **12** gave the 7,4'-dibromoquinone **14** together with two monobromo derivatives **15** and **16** as minor products. The 7-azido-4'-bromoquinone **17**, prepared from **14**, on hydrogenolysis in aqueous dioxane gave the 7-aminoquinone **18** from which destrioxypyphenylstreptonigrin **2** was obtained by careful saponification. Action of sodium dithionite on **17** gave the 7-amino-4'-bromoquinone **19**, from which **3**, the bromo analogue of **2** was prepared.

Because **6** was accessible only through a multi-step synthesis, some improvements in the above scheme with potentially fewer steps would be desirable, especially in the formation of the pyridylquinoline system and the introduction of the 7-amino substituent. Condensation of **6** and 3-hydroxy-5-methoxy-2-nitrobenzaldehyde in the presence of alkaline dithionite gave **10** ($R_4 = H$), although only in a low yield. This was found to be due to the instability of the aldehyde component (2-amino-3-hydroxy-5-methoxybenzaldehyde) and the low nucleophilicity of the amino ketone **21** which remained mostly unreacted. In the absence of the dithionite, the nitro-chalcone **8** ($R_4 = H$) was obtained in 50-60% yield and, from it, the pyridylquinoline **10** ($R_4 = H$) was readily prepared. This modification made **12** more readily accessible.

Since bromination of **12** gave a mixture of products, it would be desirable to introduce this substituent at an earlier step. Neither the aldehyde **20**, nor the nitro-chalcone **8** would be suitable because of the possibility of formation of a number of alternatives. Conversion of **7** to the 2,6-dinitro derivative **22**, followed by bromination in pyridine gave the 4-bromo compound **23** selectively in a 45% overall yield. After cyclization, the product was directly oxidized by Fremy's salt (or periodic acid) to yield a mixture of two quinones in a ratio 3:1. The major component was found to be identical with **15**, formed during the bromination of **12**. This not only established the structures of both, but also provided a better method for the preparation of **15**. The second quinone was the expected 5,6-quinone. Conversion of **15** to the 7-azido

derivative and hydrogenation gave **18**. This modification gave a more practical method for destrioxypyphenylstreptonigrin.

For the synthesis of the 6-amino-7-methoxy analogue **4**, a similar scheme, shown below was used. In spite of the similarity, there were significant differences in the reactivity of intermediates.



Although the desired chalcone **26** ($R_1 = R_2 = H$) could be readily obtained by the condensation of **6** with iso-vanillin, because of lack of selectivity in nitration, 2-nitroisovanillin (**6**) was found to be a more suitable intermediate for the condensation. After cyclization of **27** ($R = H$) to the pyridylquinoline **28** ($R = H$), oxidation by Fremy's salt to the corresponding quinone was not possible unless the carboxyl function was esterified. Also, periodic acid was not suitable for this oxidation, because it gave polymeric products. In contrast to **12**, the quinone **29** readily yielded the 6-amino derivative directly, on reaction with sodium azide in dimethyl formamide. Thus, the 6-amino-7-methoxy analogue **4** was obtained in a 30% overall yield, starting from **6**.

The uv spectra of the two analogues **2** and **4** resemble that of streptonigrin very closely. The spectrum is characteristic of the system: 2-(3'-amino-2'-pyridyl)quinoline (Fig. 1) and is only slightly affected by either oxidation of ring A to a quinone or by the presence of hydroxy or amine substituents in ring A or the presence of a trioxyphenyl substituent in ring C.

Some comments on the Friedlander quinoline synthesis for the formation of 2-(2'-pyridyl)quinoline system may be made, based on these and earlier studies (1). Condensation of an aminobenzaldehyde and a substituted 2-acetylpyridine proceeds to give a 30-60% yield of the 2-(2'-pyridyl)quinoline, provided that phenolic groups are absent, *ortho* or *para* to the amine function. These

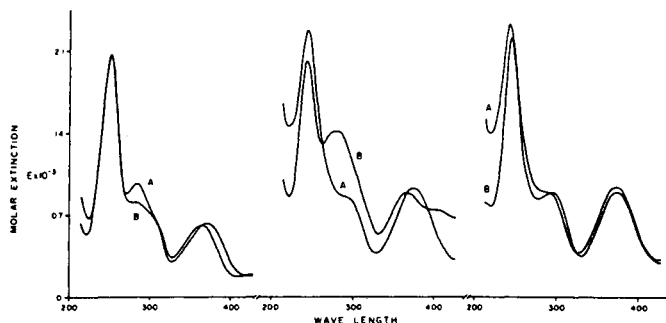


Fig. 1. Ultraviolet absorption spectra of tricyclic analogues of streptonigrin. Left: A, **12**; B, **29**; Center: A, streptonigrin; B, **4**; Right: A, streptonigrin, B, **2**.

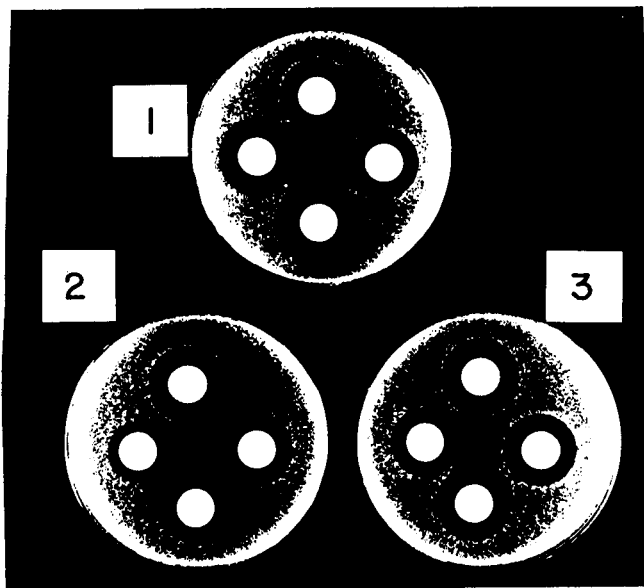


Fig. 2. Antimicrobial activity of tricyclic analogues of streptonigrin. The first two zones (clockwise) on each plate are those due to the streptonigrin standard: 25 and 12.5 $\mu\text{g./ml.}$ The other two in each plate are due to the analogues at the same concentrations. 1, destrioxypheylstreptonigrin 2; 2, 4' bromodestrioxypheylstreptonigrin 3; 3, isodestrioxypheylstreptonigrin 4.

contribute to the destruction of the aromatic ring under the alkaline conditions employed for the condensation. For instance, 2-amino-3,5-dimethoxybenzaldehyde and **6** gave a 45% yield of the desired product **31** while 2-amino-3-hydroxy-5-methoxybenzaldehyde gave less than 10% of the product **10**. In the presence of phenolic functions, the nitroaldehyde is best condensed with the 2-acetylpyridine (50-70%) and then cyclized (90%) to the quinoline for a better overall yield. The intermediate chalcone also serves for the introduction of other substituents. In the selection of the ketone component, it was observed that 2-acetylpyridine and **6** (with a 3-nitro group) both gave good yields while **21** (with a 3-amino function) showed little reactivity. Similar differences in activity are also observed with other substituents (these will appear in a later publication).

In a recent publication, Liao, Wittek and Cheng (7) reported the synthesis of the C-D ring moiety of streptonigrin and this paper describes the synthesis of the A-B-C ring system of streptonigrin.

All three analogues **2**, **3**, and **4** showed marked antimicrobial activity when tested against *B. subtilis* by the disc-plate method. In comparison with streptonigrin, destrioxypheylstreptonigrin (**2**) was twice as active and the 6-amino-7-methoxy analogue **4** was equally as active. The 4'-bromo analogue **3** was approximately 60% as active as streptonigrin (Fig. 2). These are currently being tested for their antitumor activity.

EXPERIMENTAL

Melting points were determined on a Fisher-Johns apparatus and are uncorrected. The following instruments were used for the spectra recorded: Beckman 25 (uv), Beckman-Acculab 3 (ir), Varian T-60 (nmr) with tetramethylsilane as internal standard, DuPont 490 chemical ionization (mass spectra) and Hitachi-Perkin Elmer RMU-6E (high resolution mass spectra). Thin-layer chromatography was carried out using silica gel Merck HF 254 + 366. Column chromatography was performed using a 1:1 mixture of silicic acid (Mallinckrodt + 275-325 mesh) and cellulose (tlc grade, Brown and Co).

6-Carbomethoxy-3'-hydroxy-5'-methoxy-5-methyl-3-nitro-1-azachalcone (**7**).

To a mixture of **5** (2 g.) (1) and **6** (2 g.) (2) in tetrahydrofuran (8 ml.) cooled to -5° was added precooled 2*N* potassium hydroxide (50 ml.). After stirring for 1 hour at -10 to -5° , the mixture was acidified and extracted with ethyl acetate. The extract was diluted with an equal volume of hexane and shaken with aqueous sodium bicarbonate. The aqueous layer was acidified and extracted with ethyl acetate. Concentration of the extract gave a solid which was crystallized from methanol, yield, based on **6**, 2.2 g. (73%), m.p. 191-193 $^{\circ}$.

Anal. Calcd. for $\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}_7$: C, 56.98; H, 3.94; N, 7.82. Found: C, 57.12; H, 4.08; N, 7.64.

The above acid was dissolved in a mixture of methanol and chloroform (1:1, 100 ml.) and treated with diazomethane, added in small portions until the acid was absent. Care was taken not to leave the mixture with diazomethane during the testing for completion of reaction. Concentration and crystallization from methanol gave a pale yellow crystalline solid **7**, yield, 2.1 g. (90%), m.p. 174-175 $^{\circ}$; nmr (deuteriochloroform): τ 1.78, s, 1H; τ , 2.57, 2.60, d, 2H; τ , 3.43, m, 3H; τ , 6.00, s, 3H; τ , 6.25, s, 3H; τ , 7.33, s, 3H; M^+ 372; uv (ethanol): λ max 327, 250 (sh) nm.

Anal. Calcd. for $\text{C}_{18}\text{H}_{16}\text{N}_2\text{O}_7$: C, 58.06; H, 4.33; N, 7.52. Found: C, 58.25; H, 4.31; N, 7.81.

In the presence of a slight excess of diazomethane, a mixture was obtained which was separated by chromatography. The first band was **7** and the second band, the cyclic addition product, was a pale yellow crystalline solid, m.p. 206-208 $^{\circ}$.

Anal. Calcd. for $\text{C}_{19}\text{H}_{18}\text{N}_4\text{O}_7$: C, 55.07; H, 4.38; N, 13.52. Found: C, 54.87; H, 4.62; N, 13.21.

6-Carbomethoxy-3'-hydroxy-5'-methoxy-5-methyl-3,2'-(and 6')-nitro-1-azachalcone **8** and **9**.

To a suspension of **7** (1.1 g.) in acetic acid (10 ml.) was added concentrated nitric acid (0.35 ml.) with stirring at room temperature until a clear reddish orange solution resulted. After 3 minutes, it was diluted with ice, saturated with salt and the solid filtered. It was taken up in benzene and applied to a column of silicic acid and cellulose (40 g., 1:1) in benzene. The first band, eluted with benzene gave **8** as a pale yellow crystalline solid, yield, 0.41 g. (30%), m.p. 139-140 $^{\circ}$; nmr (deuteriochloroform): τ , 1.80, s, 1H; τ , 1.90, 2.13, 2.79, 3.03, AB-q, $J = 15$ Hz, 2H; τ , 3.30, 3.35, 3.40, 3.45, AB-q, $J = 2$ Hz, 2H; τ , 6.00, s, 3H; τ , 6.10, s, 3H and τ , 7.32, s, 3H; uv (ethanol): λ max 260, 310 (sh) nm.

Anal. Calcd. for $\text{C}_{18}\text{H}_{15}\text{N}_3\text{O}_9$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.59; H, 3.89; N, 10.04.

The second band, eluted with 5% acetone/benzene gave **9** as a crystalline solid, yield, 0.38 g. (29%), m.p. 197-198 $^{\circ}$; nmr (deuteriochloroform): τ , 1.60, s, 1H; τ , 2.49, s, 2H; τ , 3.09, 3.14, 3.25, 3.30, AB-q, $J = 3$ Hz, 2H; τ , 6.04, s, 3H; τ , 6.12, s, 3H and τ , 7.30, s, 3H; uv (ethanol): λ max 328, 255 (sh) nm.

Anal. Calcd. for $C_{18}H_{15}N_3O_9$: C, 51.80; H, 3.62; N, 10.07. Found: C, 52.01; H, 3.75; N, 10.11.

In an improved procedure, a solution of **7** (1.1 g.) in nitromethane (30 ml.) and dichloromethane (30 ml.) at -40° was treated with nitronium tetrafluoroborate (0.5 g.). Stirring was continued until it showed the absence of starting material (10-15 minutes). Ice was added, the solvent layer separated and washed with aqueous bicarbonate. Concentration of the solvent layer gave a solid **8**, crystallized from benzene. The filtrate gave an additional quantity of **8** together with the amount of **9** present. The total yield of **8** was 0.85 g. (70%) and that of **9** was 0.18 g. (15%).

6-Carbomethoxy-3'-hydroxy-5'-methoxy-5-methyl-3,2',6'-trinitro-1-azachalcone (**22**).

The preceding reaction was carried out with an excess of the reagent (1.2 g.) for 2 hours at -40° . The mixture was processed as above and a crystalline solid was obtained which was recrystallized from a mixture of chloroform and benzene (1:1), yield, 0.85 g. (65%), m.p. 210-212°; nmr (deuteriochloroform): τ , 1.60, s, 1H; τ , 2.25, s, (broad), 1H; τ , 2.50, s, 1H; τ , 2.40, s, 1H; τ , 3.06, s, 1H; τ , 5.95, s, 6H; τ , 7.30, s, 3H; uv (ethanol): λ sh 258 nm.

Anal. Calcd. for $C_{18}H_{14}N_4O_{11}$: C, 46.19; H, 3.27; N, 11.52. Found: C, 46.45; H, 3.05; N, 11.82.

8-Hydroxy-6-methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline (**10**).

A mixture of **8** (0.42 g.), sodium dithionite (1.3 g.) in tetrahydrofuran (5 ml.), water (5 ml.) and methanol (3 ml.) was boiled under reflux for 30 minutes. The initial dark red color changed to pale yellow. When an aliquot was partitioned between phosphate buffer (0.2M, pH 7) and ethyl acetate, spectral analysis (260 nm) gave a distribution coefficient (solvent/aqueous) of 0.5. To the reaction mixture were added 12N sulfuric acid (2 ml.) and methanol (10 ml.) and the heating continued for 3-5 minutes more. Spectral analysis of an aliquot gave a distribution coefficient of 8-10. The cooled, diluted reaction mixture was neutralized with sodium bicarbonate and extracted with ethyl acetate and the extract concentrated to dryness. The solid was crystallized from methanol-chloroform (5:1), yield, 0.3 g. (90%), m.p. 267-268°; nmr (deuteriochloroform + dimethyl sulfoxide- d_6): τ , 1.39, 1.52, 1.75, 1.90, AB-q, J = 8 Hz, 2H; τ , 2.99, s, 1H; τ , 3.20, s, 2H; τ , 6.0, s, 6H and τ , 7.54, s, 3H; M^+ 339; uv (ethanol): λ max 260, 360 nm. The compound gave a dark green color with ferric chloride.

Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.92; H, 5.25; N, 12.45.

When the reaction was stopped without the acid hydrolysis, it was cooled and extracted with ethyl acetate to remove **10**. The aqueous layer was concentrated, saturated with ethyl acetate and cooled. The crystalline solid **10a** was filtered and recrystallized from aqueous methanol, m.p. $> 300^\circ$. It was converted to **10** when heated in acid.

Anal. Calcd. for $C_{18}H_{16}N_3O_6SNa$: C, 50.82; H, 3.79; N, 9.88. Found: C, 51.01; H, 3.95; N, 10.12.

6-Hydroxy-8-methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline (**11**).

The same procedure given under **10** was repeated with **9** and the product crystallized from methanol-chloroform (4:1), m.p. 257-259°; M^+ 339; uv (ethanol); λ max 260, 360 nm. It gave no characteristic color with ferric chloride.

Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.55; H, 5.12; N, 12.54.

6-Methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (**12**).

To a solution of **10** (0.68 g.) in dimethylformamide (10 ml.) and acetic acid (3 ml.) was added potassium nitrosodisulfonate (1 g.) in water (20 ml.) and the mixture stirred for 4-6 hours. Two more portions of the reagent (0.25 g. each) were added during this period. When the reaction was complete as shown by tlc (and absence of ferric chloride test) the mixture was diluted with saturated salt and the solid filtered. It was crystallized from chloroform-methanol (1:1); yield, 0.45 g. (64%), m.p. 275-276°; M^+ 353; uv (ethanol): λ max 370, 285, 255 nm.

Anal. Calcd. for $C_{18}H_{15}N_3O_5$: C, 59.71; H, 4.45; N, 11.61. Found: C, 59.66; H, 4.38; N, 11.58.

In an alternative method, a solution of **10** (0.68 g.) in methanol (10 ml.) and chloroform (5 ml.) was treated with sodium periodate (0.86 g.) in 4 ml. of LV sulfuric acid. After 20 minutes, it was diluted with phosphate buffer (1M, pH 7, 25 ml.) and extracted with chloroform. The solvent layer was concentrated and the maroon solid crystallized as before, yield 0.52 g. (73%).

8-Methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,6-dione (**13**).

Oxidation of **11** (0.68 g.) was carried out with periodic acid as described under **12**. The product was crystallized from methanol-chloroform (1:1), yield, 0.5 g. (70%), m.p. 267-268°; M^+ 353.

Anal. Calcd. for $C_{18}H_{15}N_3O_5$: C, 59.71; H, 4.45; N, 11.61. Found: C, 59.88; H, 4.30; N, 11.86.

7-Bromo-6-methoxy-2-(3'-amino-4'-bromo-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (**14**).

Bromine (0.2 ml.) was added to a solution of **12** (0.25 g.) in chloroform (50 ml.). After 4 hours tlc showed a mixture of one major and two minor products. The reaction mixture was shaken with aqueous ascorbic acid (0.5 g. in 10 ml.) to remove the bromine. Concentration of the solvent layer and purification by chromatography on silicic acid/cellulose in benzene gave the major product **14** as a reddish brown crystalline solid, yield, 0.21 g. (50%), m.p. 245-247°; M^+ 509.

Anal. Calcd. for $C_{18}H_{13}Br_2N_3O_5$: C, 42.29; H, 2.56; Br, 31.27; N, 8.22. Found: C, 42.45; H, 2.72; Br, 30.98; N, 8.45.

The first minor band **15** was crystallized from dimethylformamide and methanol (1:5), m.p. $> 300^\circ$; M^+ 431.

Anal. Calcd. for $C_{18}H_{14}BrN_3O_5$: C, 50.01; H, 3.26; Br, 18.48; N, 9.72. Found: C, 49.83; H, 3.10; Br, 18.21; N, 9.88.

The second minor band **16** was crystallized from methanol-chloroform, m.p. 258-260; M^+ 431.

Anal. Calcd. for $C_{18}H_{14}BrN_3O_5$: C, 50.01; H, 3.26; Br, 18.48; N, 9.72. Found: C, 50.12; H, 3.04; Br, 18.71; N, 9.81.

7-Azido-6-methoxy-2-(3'-amino-4'-bromo-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (**17**).

A solution of **14** (0.25 g.) in dimethylformamide (10 ml.) was stirred with sodium azide (50 mg.) for 30 minutes at room temperature. It was diluted with saturated salt solution and the solid filtered. It was crystallized from dioxane, yield, 0.2 g. (90%), m.p. 205-207°. It showed an intense band at 2120 cm^{-1} .

Anal. Calcd. for $C_{18}H_{13}BrN_6O_5$: C, 45.68; H, 2.77; Br, 16.89; N, 17.75. Found: C, 45.22; H, 2.48; Br, 16.01; N, 17.39.

7-Amino-6-methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (**18**).

In a Parr hydrogenation vessel, **17** (0.25 g.) was dissolved in tetrahydrofuran (10 ml.) and water (2 ml.) and hydrogenated at

50 psi in the presence of palladium-carbon. After 1 hour, 0.2 ml. of 2*N* potassium hydroxide was added and the hydrogenation continued for 6 more hours when tlc showed two products in a ratio of 9:1. Dilution with water, removal of catalyst, extraction with ethyl acetate and concentration of the extract gave a dark brown crystalline solid, recrystallized from methanol-chloroform (1:1), yield, 0.15 g. (80%) m.p. 266-268°.

Anal. Calcd. for C₁₈H₁₆N₄O₅: C, 58.69; H, 4.38; N, 15.21; M⁺ 368.1120. Found: C, 58.42; H, 4.15; N, 15.33; M⁺ 368.1092.

Destrioxyphenylstreptonigrin (2).

A sample of **18** (0.2 g.) in 1:1 tetrahydrofuran/water (10 ml.) was heated with sodium dithionite (0.1 g.) in a nitrogen atmosphere. After a pale yellow clear solution was obtained, 2*N* potassium hydroxide (2 ml.) was added and the mixture heated at 50° for 30 minutes.

It was cooled, acidified with 1*M* potassium dihydrogen phosphate while the nitrogen was bubbled through and extracted with ethyl acetate. Concentration of the extract gave a deep brown crystalline solid recrystallized from chloroform methanol (1:1), yield, 0.12 g. (62%), m.p. 285-287°.

Anal. Calcd. for C₁₇H₁₄N₄O₅: C, 57.62; H, 3.98; N, 15.81. Found: C, 57.83; H, 4.27; N, 15.56.

7-Amino-6-methoxy-2-(3'-amino-4'-bromo-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (19).

A mixture of **17** (0.1 g.) and sodium dithionite (0.2 g.) in 1:1 methanol-water (10 ml.) was boiled for 2 minutes until a pale yellow solution resulted. Cooling, acidification (pH 4), extraction with ethyl acetate and concentration of the extract gave a dark brown crystalline solid, recrystallized from tetrahydrofuran, yield, 0.06 g. (67%) m.p. 270-272°; M⁺ 446.

Anal. Calcd. for C₁₈H₁₅BrN₄O₅: C, 48.34; H, 3.31; Br, 17.87; N, 12.53. Found: C, 48.69; H, 3.33; Br, 17.56; N, 12.37.

Bromodestrioxyphenylstreptonigrin (3).

Conversion of **19** (0.1 g.) to **3** was carried out as described under **2**. The product was a dark brown crystalline solid, yield, 0.06 g., m.p. 260-262°.

Anal. Calcd. for C₁₇H₁₃BrN₄O₅: C, 47.14; H, 3.02; Br, 18.44; N, 12.93. Found: C, 47.39; H, 3.33; Br, 18.12; N, 12.69.

4'-Bromo-6-carbomethoxy-3'-hydroxy-5'-methoxy-5-methyl-3,2',6'-trinitro-1-azachalcone (23).

A solution of **22** (0.4 g.) in pyridine (10 ml.) was treated with bromine (0.1 ml.) in pyridine (2 ml.). After 10 minutes, it was diluted with ice and acidified (pH 2). Ascorbic acid (0.5 g.) was added to decompose the excess bromine and the yellow solid filtered. It was crystallized from methanol, yield, 0.45 g. (84%), m.p. 162-164°; nmr (dimethylsulfoxide-d₆): τ, 1.77, s, 1H; τ, 2.57, s, 1H; τ, 2.60, s, 1H; τ, 5.93, s, 6H; τ, 7.30, s, 3H; uv (ethanol): λ sh 260 nm.

Anal. Calcd. for C₁₈H₁₃BrN₄O₁₁: C, 39.94; H, 2.42; Br, 14.77; N, 10.35. Found: C, 39.88; H, 2.63; Br, 15.02; N, 9.98.

7-Bromo-6-methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (15).

Reductive cyclization of **23** (0.5 g.) was carried out as described under **10**. The ethyl acetate extract containing the product was concentrated to dryness and taken up directly into a mixture of dimethylformamide (10 ml.) and acetic acid (4 ml.). A solution of Fremy's salt (1.2 g.) in 50 ml. of water was added in

three portions over three hours to the stirred reaction mixture. The mixture was saturated with salt and the reddish brown solid filtered. It was recrystallized from a mixture of dimethylformamide and methanol (1:4), yield, 0.25 g. (60%), m.p. > 300°; M⁺ 431.

Anal. Calcd. for C₁₈H₁₄BrN₃O₅: C, 50.01; H, 3.26; Br, 18.48; N, 9.72. Found: C, 49.79; H, 3.51; Br, 18.17; N, 9.36.

7-Azido-6-methoxy-2-(3'-amino-6'-carbomethoxy-5'-methyl-2'-pyridyl)quinoline-5,8-dione (24).

Conversion of **15** (0.2 g.) to **24** was carried out as described under **17**. The product, a dark brown crystalline solid was recrystallized from dimethylformamide-benzene (1:1), yield 0.15 g. (68%), m.p. 158-160°.

Anal. Calcd. for C₁₈H₁₄N₆O₅: C, 54.82; H, 3.52; N, 21.32. Found: C, 54.51; H, 3.63; N, 20.98.

3-Hydroxy-5-methoxy-2-nitrobenzaldehyde (20).

A solution of 3-hydroxy-5-methoxybenzaldehyde (1.5 g.) (8) in dichloromethane (50 ml.) and nitromethane (25 ml.) was cooled to -40° and treated with nitronium tetrafluoroborate (1.6 g.). After tlc showed that the starting material was absent (10-15 minutes), ice was added to the bluish-brown reaction mixture and the solvent layer separated. It was washed with 0.2 *M* phosphate buffer (pH 7, 40 ml.) and the concentrated extract purified by chromatography. The major product **20** from the benzene eluate was crystallized from 2:1 ether-hexane m.p. 157-159°, yield, 1.1 g. (55%); nmr (deuteriochloroform): τ -0.05, s, 1H; τ 3.07, broad, 1H; τ 3.25, s, 2H; τ 6.05, s, 3H; uv: 345 and 270 nm.

Anal. Calcd. for C₈H₇NO₅: C, 48.74; H, 3.58; N, 7.11. Found: C, 48.95; H, 3.51; N, 7.38.

Direct Formation of 10.

A solution of **20** and **6** (0.24 g. each) in 1:1 methanol/water (6 ml.) was heated with sodium dithionite (1.2 g.) at 100° for 5 minutes. It was cooled to 0° and stirred with 2*N* potassium hydroxide (12 ml.) for 6 hours at 0°. It was acidified (pH 4), extracted with ethyl acetate and the extract was treated with diazomethane. Separation by chromatography gave the first band **21** as a crystalline solid, m.p. 142-143°, yield 0.14 g., nmr (deuteriochloroform): τ 3.20, s, 1H; τ 3.27, broad, 2H; τ 6.04, s, 3H; τ 7.24, s, 3H; τ 7.44, s, 3H; uv (ethanol): 350, 285, 225 nm.

Anal. Calcd. for C₁₀H₁₂N₂O₃: C, 57.68; H, 5.81; N, 13.46. Found: C, 57.42; H, 5.68; N, 13.70.

Elution with 5% acetone/benzene, gave **10**, yield, 0.03 g. 6-Carboxymethoxy-5-methyl-3,2'-dinitro-3'-hydroxy-5'-methoxy-1-azachalcone (**8**, R₄ = H).

The procedure was similar to that given under **7**, with **20** and **6** (0.2 g. each). The product was crystallized from methanol, m.p. 187-188°, yield 0.13 g. (55%).

Anal. Calcd. for C₁₇H₁₃N₃O₉: C, 50.63; H, 3.25; N, 10.42. Found: C, 50.89; H, 3.47; N, 10.19.

3-Hydroxy-4-methoxy-2-nitrobenzaldehyde (25, R = NO₂).

The following is a more selective method for the preparation of the 2-nitro derivative than was described (6).

The reaction was carried out as given under **20**, using isovanillin (6 g.). The extract was washed with 0.2*M* phosphate buffer (pH 7, 100 ml.) to remove any 2,6-dinitro derivative, concentrated and partitioned between benzene and 0.2*M* phosphate buffer (pH 7) using four separatory funnels. The aqueous

layers on acidification gave the 2-nitro derivative which was crystallized from benzene, m.p. 148-149°, yield 4.5 g. (57%); nmr (deuteriochloroform): τ 0.20, s, 1H; τ 2.50, 2.64, 2.87, 3.00, AB-q, J = 8 Hz, 2H; τ 4.25, broad, 1H; τ 6.0, s, 3H; uv (ethanol): 315, 275 nm.

Anal. Calcd. for $C_8H_7NO_5$: C, 48.74; H, 3.58; N, 7.11. Found: C, 48.51; H, 3.78; N, 7.32.

The solvent layers, (after 4-8 washes with the buffer) were extracted with 5% aqueous sodium bicarbonate to recover the 6-nitro compound, crystallized from benzene, m.p. 188-189°, yield, 1.3 g. (17%); nmr (deuteriochloroform): τ -0.30, s, 1H; τ 2.40, s, 1H; τ 2.70, s, 1H; τ 5.34, broad, 1H; τ 6.00, s, 3H; uv (ethanol): 310, 266 nm.

Anal. Calcd. for $C_8H_7NO_5$: C, 48.74; H, 3.58; N, 7.11. Found: C, 49.02; H, 3.89; N, 7.42.

6-Carbomethoxy-5-methyl-3-nitro-3'-hydroxy-4'-methoxy-1-azachalcone (**26**).

The procedure was similar to that described under **7**, with isovanillan (1.8 g.) and **6** (2.38 g.). The product, which was the acid corresponding to **26** was obtained as a yellow crystalline solid, m.p. 214-216°, yield, 2.4 g. (65%).

Anal. Calcd. for $C_{17}H_{14}N_2O_7$: C, 56.98; H, 3.94; N, 7.82. Found: C, 56.86; H, 3.98; N, 7.87.

The acid was treated with diazomethane as given under **7** until the esterification was complete. Concentration to dryness and crystallization from 1:9 chloroform and methanol gave **26** as a yellow crystalline solid, m.p. 171-173°, yield 2.4 g. (90%); nmr (deuteriochloroform): τ 1.18, s, 1H; τ 2.47, 2.60, d, 2H; τ 2.75, 2.88, 3.08, 3.21, AB-q, J = 8 Hz, 2H; τ 2.88, s, 1H; τ 6.08, s, 6H; τ 7.34, s, 3H.

Anal. Calcd. for $C_{18}H_{16}N_2O_7$: C, 57.85; H, 4.80; N, 7.35. Found: C, 58.06; H, 4.53; N, 7.52.

Nitration of **26**.

The procedure was the same as that described under **8** with 0.92 g. of **26** and 0.35 g. of nitronium tetrafluoroborate. The dark reaction product was purified by chromatography. The two isomers had somewhat close Rf values. Small amounts of the two were separated for characterization. For the 2-nitro derivative, see below. The 6-nitrochalcone was obtained as a very pale yellow crystalline solid, m.p. 185-187°, yield, 0.35 g. (33%).

Anal. Calcd. for $C_{18}H_{15}N_3O_9$: C, 51.80; H, 3.62; N, 10.07. Found: C, 52.05; H, 3.83; N, 9.73.

6-Carbomethoxy-5-methyl-3,2'-dinitro-3'-hydroxy-4'-methoxy-1-azachalcone (**27**).

A solution of **25** ($R_1 = NO_2$, 0.7 g.) and **6** (0.72 g.) in tetrahydrofuran (5 ml.) was treated with 2N potassium hydroxide and the mixture stirred at -10 to -5° for 1 hour. The mixture was acidified and extracted twice with ethyl acetate. The solvent layer was extracted four times with 0.2 M phosphate buffer (pH 7). The combined aqueous layer was acidified and extracted with ethyl acetate. Concentration of the extract gave the chalcone acid as a yellow crystalline solid, m.p. 212-214°, yield 0.85 g. (75%).

Anal. Calcd. for $C_{17}H_{13}N_3O_9$: C, 50.63; H, 3.25; N, 10.42. Found: C, 50.91; H, 3.55; N, 10.21.

The acid was esterified by stirring in 0.5% (v/v) sulfuric acid in methanol (100 ml.) for 24 hours. Dilution with water, neutralization, extraction with chloroform and concentration gave **27** as a yellow crystalline solid, m.p. 216-217°, yield, 0.8 g. (90%); nmr (deuteriochloroform): τ 1.67, s, 1H; τ 2.44, 2.57, d, 2H; τ 2.70, 2.84, 2.94, 3.07, AB-q, J = 8 Hz, 2H; τ 6.04, s, 6H; τ 7.34, s, 3H.

Anal. Calcd. for $C_{18}H_{15}N_3O_9$: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.80; H, 3.44; N, 9.78.

8-Hydroxy-7-methoxy-2-(3'-amino-5'-methyl-6'-carbomethoxy-2'-pyridyl)quinoline (**28**).

The procedure was the same as that described under **10**; from 0.42 g. of **27**, yield, 0.31 g. (90%), m.p. 278-280°; M^+ 339; uv (ethanol): 260 and 360 nm. It gave a dark green color with ferric chloride.

Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.61; H, 5.19; N, 12.35.

6-Hydroxy-7-methoxy-2-(3'-amino-5'-methyl-6'-carbomethoxy-2'-pyridyl)quinoline.

The 6-nitrochalcone (by-product in the nitration of **26**) was converted to the quinoline as given under **10**. The product was a cream colored crystalline solid, m.p. 268-270°.

Anal. Calcd. for $C_{18}H_{17}N_3O_4$: C, 63.71; H, 5.05; N, 12.38. Found: C, 63.92; H, 5.32; N, 12.04.

7-Methoxy-2-(3'-amino-5'-methyl-6'-carbomethoxy-2'-pyridyl)quinoline-5,8-dione (**29**).

A procedure similar to that given under **12** was used with 0.34 g. of **28**. The quinone (0.3 g., 86%) was obtained as a red crystalline solid from chloroform or a nearly black crystalline solid from chloroform-methanol, m.p. 275-277°; M^+ 353; uv (ethanol): 365, 280 (sh), 250 nm.

Anal. Calcd. for $C_{18}H_{15}N_3O_5$: C, 59.71; H, 4.45; N, 11.61. Found: C, 59.79; H, 4.54; N, 11.44.

6-Amino-7-methoxy-2-(3'-amino-5'-methyl-6'-carbomethoxy-2'-pyridyl)quinoline-5,8-dione (**30**).

A solution of the quinone **29** (0.35 g.) in dimethylformamide (45 ml.) was stirred at 50° with sodium azide (0.1 g.) and acetic acid (1 ml.). After 10 hours, additional quantities (0.1 g. and 1 ml., respectively) were added and stirring continued for another 16-20 hours. The reaction mixture was diluted with chloroform (150 ml.) and washed three times with water (250 ml. each). The solvent layer was concentrated to dryness and the solid crystallized from chloroform-methanol (1:4). The product was a dark brown crystalline solid, m.p. >300°; yield, 0.23 g.; M^+ 368; uv (ethanol): 375, 275, 245 nm.

Anal. Calcd. for $C_{18}H_{16}N_4O_5$: C, 58.69; H, 4.38; N, 15.21. Found: C, 58.32; H, 4.33; N, 15.49.

Isodextrioxyphenylstreptonigrin (**4**).

Conversion of **30** (0.2 g.) to **4** was carried out as given under **2**. It separated as a dark brown crystalline solid from a mixture of dimethylformamide and methanol (1:2), m.p. >300°; yield, 0.12 g.

Anal. Calcd. for $C_{17}H_{14}N_4O_5$: C, 57.89; H, 4.24; N, 13.37. Found: C, 57.62; H, 3.98; N, 13.70.

3,5-Dimethoxy-2-nitrobenzaldehyde.

To a solution of 3,5-dimethoxybenzaldehyde (Aldrich, 5 g.) in acetic acid (12 ml.) was added a mixture of concentrated nitric acid (30 ml.) and water (25 ml.). The mixture slowly darkened and temperature rose to 60° with fuming in about an hour. After this, it was cooled, diluted with water (200 ml.) and extracted with chloroform. The extract was washed with sodium bicarbonate and concentrated to dryness. The solid was crystallized from benzene, m.p. 109-111°, yield, 2 g. (33%); nmr (deuteriochloroform): τ 0.09, s, 1H; τ 3.07, 3.12, 3.22, 3.27 AB-q, J = 3 Hz, 2H; τ 6.10 s, 3H.

Anal. Calcd. for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.32; H, 4.55; N, 6.89.

5,7-Dimethoxy-2-(6'-carbomethoxy-5'-methyl-3'-nitro-2'-pyridyl)-quinoline (**31**).

A mixture of 3,5-dimethoxy-2-nitrobenzaldehyde (0.4 g.) and sodium dithionite (1 g.) in 1:1 methanol-water (5 ml.) was heated under reflux for 5 minutes. When it showed completion of reduction, 6*N* hydrochloric acid was added to lower the pH to 2 to decompose any excess dithionite. To this was added a solution of **6** (0.36 g.) in tetrahydrofuran (2 ml.), followed by 2*N* potassium hydroxide (10 ml.) and the mixture stirred at 0° for 3 hours. After acidification and extraction with ethyl acetate, the solvent layer was washed with aqueous bicarbonate. The aqueous layer was acidified, extracted with ethyl acetate and the extract treated with diazomethane until esterification was complete. Concentration gave a crystalline solid, recrystallized from methanol, m.p. 175-176°, yield 0.19 g. (50%); M^+ , 383; nmr (deuteriochloroform): τ 1.87, 1.90, d, 3H; τ 2.14, s, 1H; τ 3.37, s, 2H; τ 6.04, s, 6H; τ 6.15, s, 3H; τ 7.40, s, 3H.

Anal. Calcd. for $C_{19}H_{17}N_3O_6$: C, 59.53; H, 4.47; N, 10.96. Found: C, 59.28; H, 4.58; N, 11.22.

Antibacterial Activity.

The samples were dissolved in dimethylsulfoxide, diluted with 1% aqueous sodium bicarbonate and concentrations in the range 100 μ g. - 3 μ g./ml. were prepared. These were tested by the standard disc-plate assay using *B. subtilis* grown on Difco streptomycin agar. Typical results are shown in Fig. 2.

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- (4) The azachalcone **7** reacts with diazomethane to form a cyclic addition product which is not useful for the scheme. Acid-catalyzed esterification gave deeply colored by-products, difficult to separate from **7**.
- (5) The reaction actually gave a variable mixture of **10** (or **11**) and a water soluble component, isolated and characterized as the sodium salt of the aminosulfonic acid **10a**. Gentle acid hydrolysis of the mixture prior to work up was necessary for the maximum yield of **10**.
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