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SYNTHETIC APPROACHES TO STREPTONIGRIN AND BIOLOGICAL ACTIVITIES ON THE QUINOLINEQUINONE SYSTEMS

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The present review covers literatures on synthetic approaches to streptonigrin, metabolite of streptomyces flocculus. A summary of biological activities on the quinolinequinone systems is also presented. The contents of this review are as follows:

1. Introduction
2. Synthetic approaches to the quinoline-quinone systems
3. Synthetic approaches to the pyridine ring systems
4. Biological activities
5. Conclusion

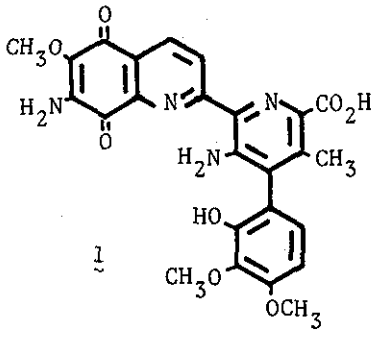
1. Introduction

Streptonigrin, isolated by Rao and Cullen[1] as a metabolite of streptomyces flocculus, is an antitumor antibiotic, which was shown by chemical and spectroscopic studies to have the structure 1 by Woodward et al.[2]. This structure was confirmed by ^{13}C-NMR[3]

and X-ray analysis[4]. Lipscomb et al. have reported that rings A, B, and C, including the carboxyl group on ring C, is very nearly coplanar, and that ring D is virtually perpendicular to ring C.

On the other hand, the antitumor and antibacterial activity of streptonigrin is similar to those of the mitomycin class which also has an aminoquinone fragment, a closely common structural feature related to several antitumor agents[5].

Several synthetic efforts to the quinolinequinone system (A-B or A-B-C ring) and the pyridine ring system (C-D or A-B-C-D) of streptonigrin are described in this review. The author also wishes to refer to biological activity on some quinolinequinone derivatives.



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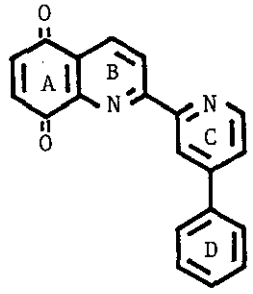
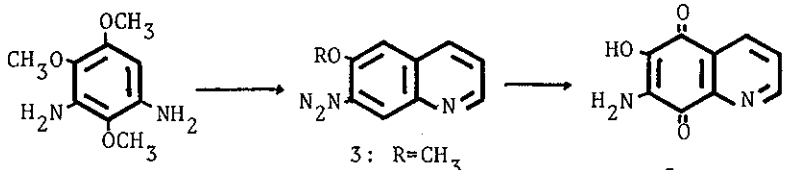


2. Synthetic approaches to the quinolinequinone systems

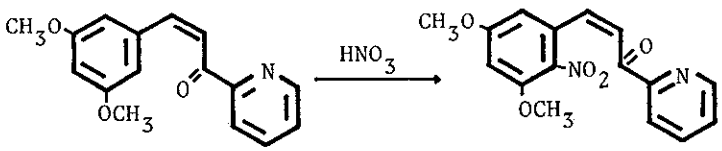
In 1965, Kametani et al.[6,7] have synthesized appropriately substituted quinoline portion 3 by the Skraup reaction of 2, in 50 % yield. Cleavage of the trimethyl ether 3 with 48 % hydrobromic acid gives the trihydroxyquinoline 4, in 96 % yield, which is converted to the 5,8-quinolinequinone 5 in 86 % yield by air

oxidation in alkaline solution.



2 3: R=CH_3 4: R=H 5

Rao[8] also attempted a synthetic study of 2,2-pyridyl-5,8-quinolinequinone system. 6,8-Dimethoxy-2,2-pyridylquinoline 8 is prepared by the Baeyer-Drewson quinoline synthesis[9]. The nitrochalcone 7, obtained by nitration of the chalcone 6 in 45 % yield, is subjected to reductive cyclization with sodium hydrosulfite to give the 2,2-pyridylquinoline 8 in 61 % yield. Nitration of 8 with a mixture of 1:1 sulfuric acid-nitric acid gives 6-hydroxy-7-nitro-2,2-pyridyl-5,8-quinolinequinone 9 in 40 % yield, which is reduced with sodium hydrosulfite to give 7-aminoquinolinequinone 10 in 75 % yield. Methylation of 10 with diazomethane gives 7-amino-6-methoxy-2,2-pyridyl-5,8-quinolinequinone 11 in 90 % yield.



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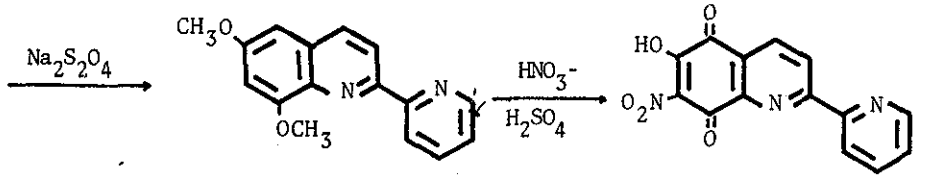

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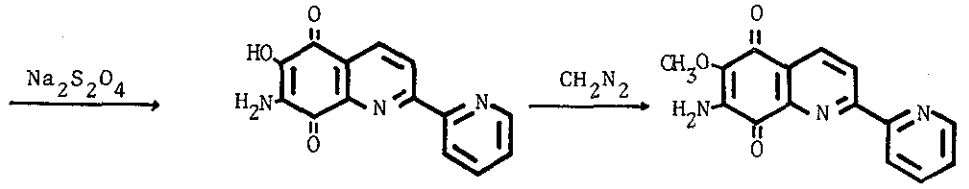
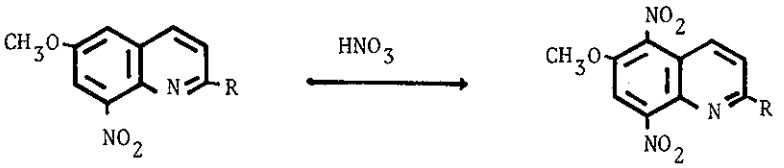


10 11

Liao et al. have reported a synthesis of the A-B ring portion in 1967[10] and 1976[11]. They prepared quinoline nucleus 12 and 13 by the Skraup method developed by Mihklina et al.[12]. Nitration of 6-methoxy-8-nitroquinoline 12 gives the 5,8-dinitro derivative 14 in quantitative yield. Reduction of 14, followed by oxidation of 16 as described by Pratt and Drakes[13], gives 6-methoxy-5,8-quinoline-quinone 18. Bromination of 18 with bromine in acetic acid in the presence of sodium acetate gives the 7-bromo derivative 20, in quantitative yield. Replacement of the bromine at the 7-position in 20 by azido group by the use of sodium azide in aqueous ethanol yields the 7-azido derivative 22, catalytic hydrogenation of which over platinum oxide gives 7-amino-6-methoxy-5,8-quinolinequinone 24 in 60 % yield. The corresponding 2-methylquinoline and quinoline-quinone analogs can be prepared through the methods reported by Wan et al.[14] and Tsizin et al.[15] and through a similar synthetic

route (13 ⟶ 15 ⟶ 17 ⟶ 19 ⟶ 21 ⟶ 23 ⟶ 25).

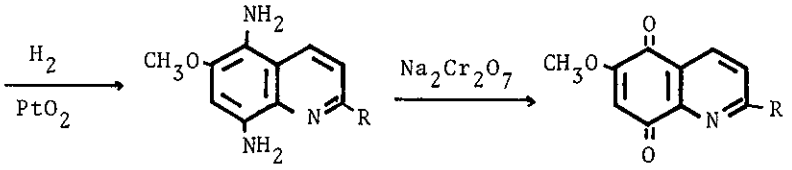


12: R=H

13: R=CH_3

14: R=H

15: R=CH_3

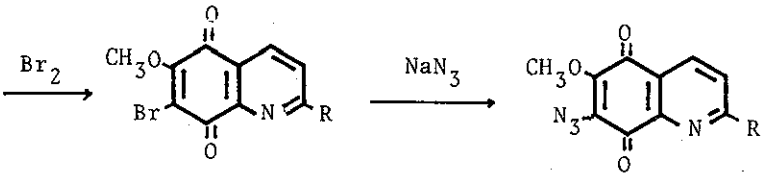


16: R=H

17: R=CH_3

18: R=H

19: R=CH_3

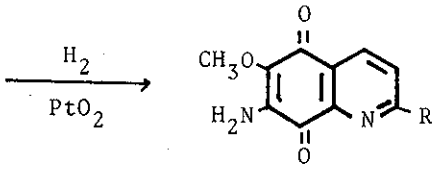


20: R=H

21: R=CH_3

22: R=H

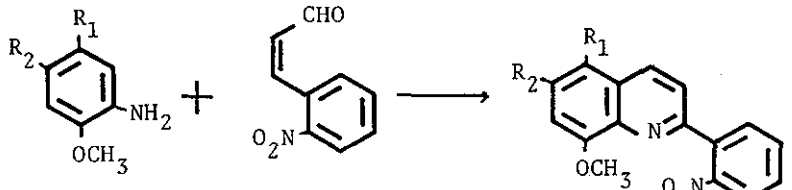
23 R=CH_3



24: R=H

25: R=CH_3

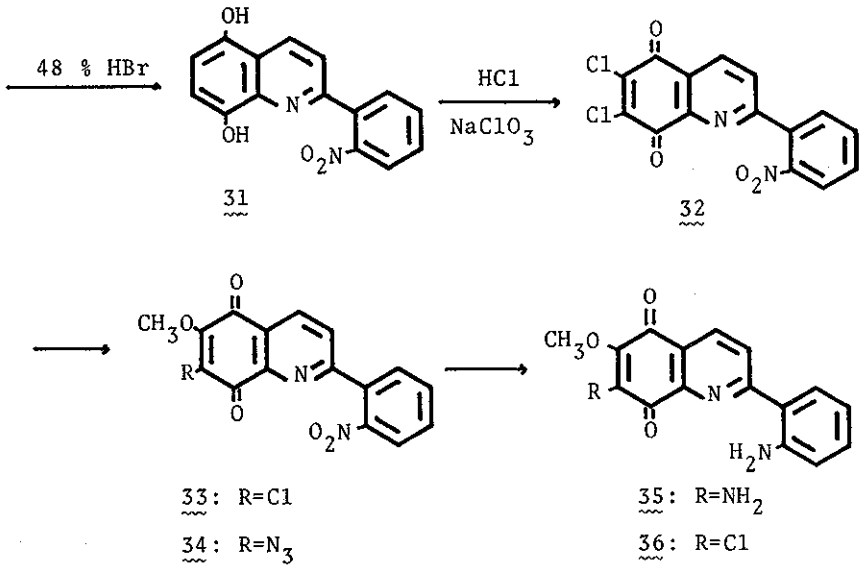
Lown and his co-workers[16] also synthesized the quinoline-quinone system possessing an aminophenyl group at the 2-position. 5,8-Dimethoxy-2-(o-nitrophenyl)quinoline 29 is prepared by the Skraup condensation of 2,5-dimethoxyaniline 26 with o-nitrocinnamaldehyde 28 in the presence of sodium m-nitrobenzenesulfonate in 15 % yield. 6,8-Dimethoxy-2-(o-nitrophenyl)quinoline 30 is also prepared from 2,4-dimethoxyaniline, in a similar fassion, in 13 % yield. Treatment of 5,8-dimethoxyquinoline 29 with 48 % hydrobromic acid affords 5,8-dihydroxyquinoline 31 in 40 % yield. Oxidative chlorination of 31 with hydrochloric acid and sodium chlorate affords the 6,7-dichloro-quinolinequinone derivative 32 in 77 % yield. Nucleophilic displacement of the chlorine at the 6-position by a methoxy group can be carried out by the use of sodium methoxide in methanol to give 7-chloro-6-methoxy-2-(o-nitrophenyl)-5,8-quinolinequinone 33 in 86 % yield. Reaction of 33 with sodium azide affords the corresponding azide 34 with recovery of 33. Hydrogenation of the mixture of 34 and 33, without separation, over platinum oxide gives the desired 7-amino-2-(o-aminophenyl)quinolinequinone 35 in 28 % yield with 36.



26: R_1=OCH_3, R_2=H
27: R_1=H, R_2=OCH_3

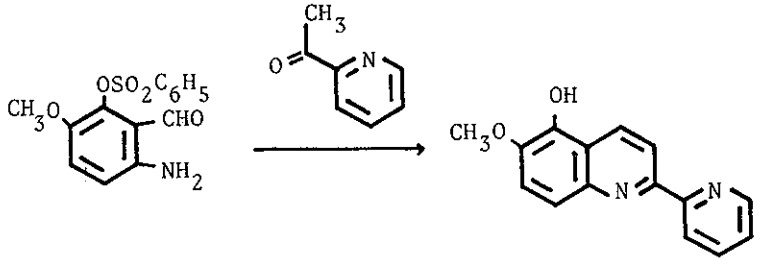
28

29: R_1=OCH_3, R_2=H
30: R_1=H, R_2=OCH_3

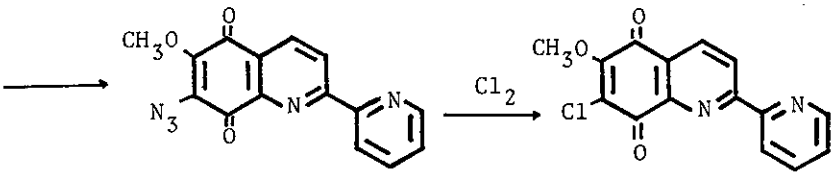


Weinreb et al.[17] recently reported synthetic effort to the quinolinequinone system possessing a nonsubstituted pyridine ring at the 2-position. For the preparation of quinoline nucleus, they employed the direct Friedlaender reaction. Condensation of o-aminobenzaldehyde 37, prepared from the corresponding o-nitrobenzaldehyde derivative, with 2-acetylpyridine using Triton B as a catalyst gives 5-hydroxy-6-methoxy-2,2-pyridylquinoline 38, in 33 % overall yield from o-nitrobenzaldehyde derivative, without purification of intermediates. 5-Hydroxyquinoline derivative 38 can be oxidized to the quinolinequinone 39 with Fremy's salt in 74 % yield. Salcomine-oxygen method[18] is also applied to the synthesis of 39 from 38, though in lower yield (39 %). Treatment of quinoline 39 with chlorine in chloroform at 0° provides the chloroquinone 40 in 78 % yield.

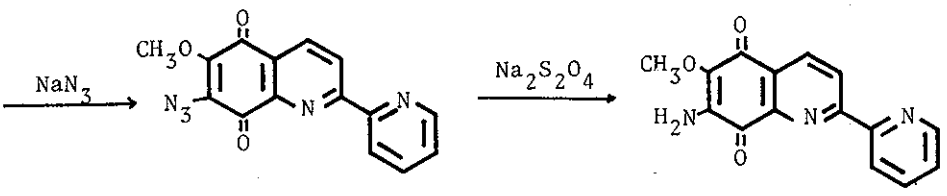
Replacement of the chlorine at the 7-position in 40 by azido group can be easily accomplished with sodium azide to give the azidoquinone 41 in 80 % yield. Reduction of 41 with sodium hydrosulfite leads to the purple aminoquinone 11 which had been previously prepared by Rao through the different route[8].



37 38



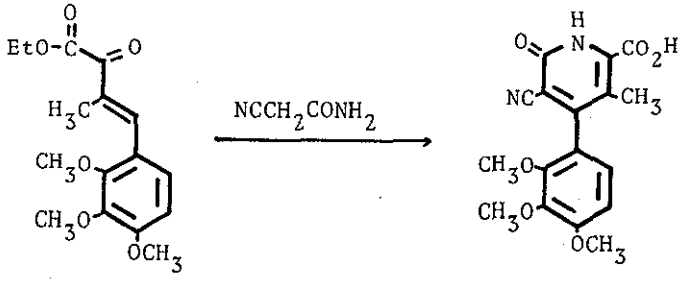
39 40



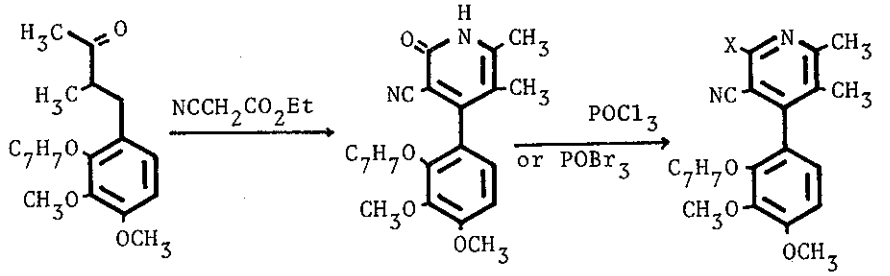
41 11

3. Synthetic approaches to the pyridine ring systems

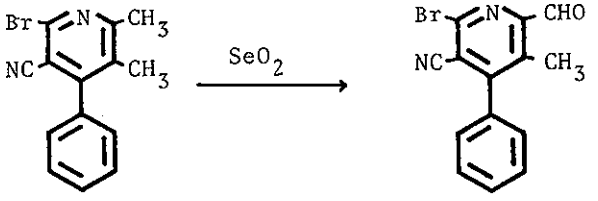
Kametani et al.[19-25,27] have reported many synthetic efforts regarding the pyridine ring systems. Namely, a condensation of ethyl 3-(2,3,4-trimethoxybenzylidene)-2-oxobutyrate 42 with cyanoacetamide in the presence of sodium ethoxide gives appropriately substituted pyridine derivative 43 in 88.5 % yield. Similarly, a condensation of 4-(2-benzyloxy-3,4-dimethoxyphenyl)-3-methyl-3-buten-2-one 44 with ethyl cyanoacetate in the presence of ammonium acetate also gives the pyridone derivative 45, which is treated with phosphoryl chloride to afford the chloropyridine 46. On treatment of 45 with phosphoryl bromide, the bromopyridine 47 is obtained. Selective oxidation of methyl group at the 6-position on the pyridine ring would be applicable to introduction of carboxyl function in C-D ring system. Oxidation of 48 with selenium dioxide leads to the formation of 49 by the difference of the reactivity of two methyl group.



42 43



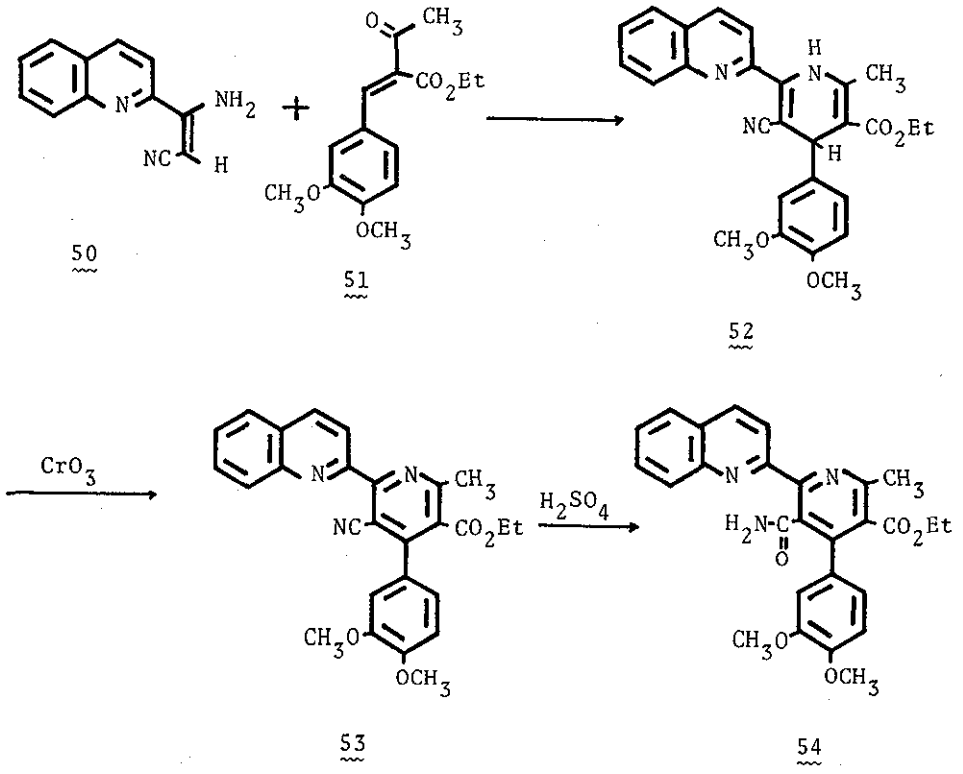
44 45 46: X=Cl 47: X=Br



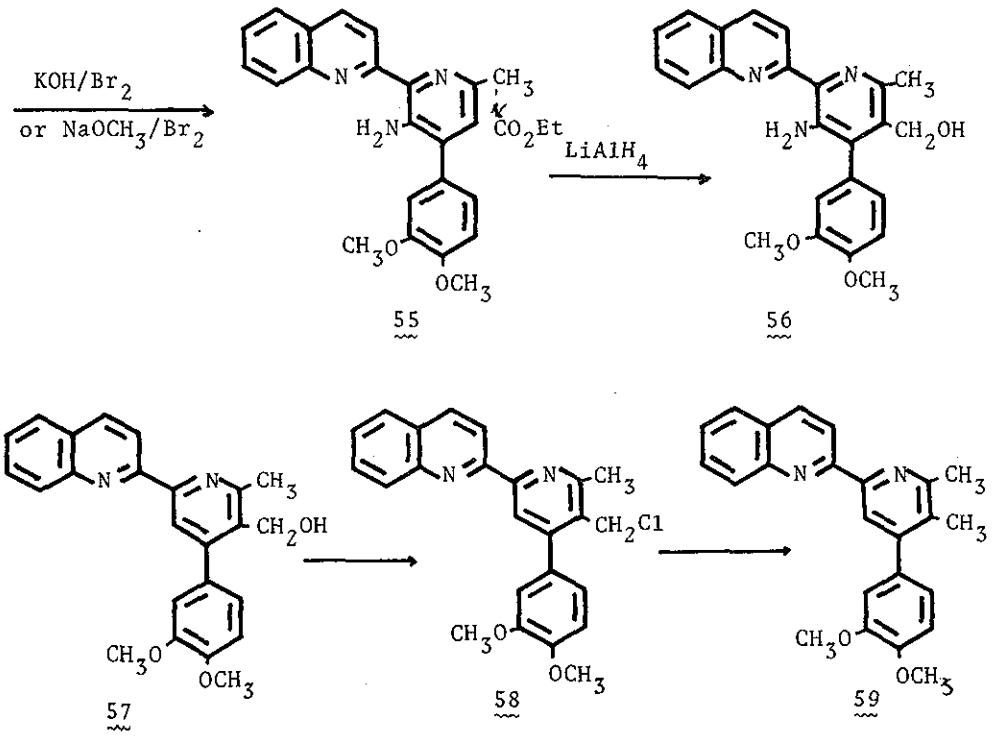
48 49

The attempts to synthesize the 4-phenylpyridine moiety possessing quinoline nucleus at the α-position have been reported. Condensation of the aminonitrile compound 50, prepared by the Thorpe reaction of 2-cyanoquinoline with acetonitrile[26], with ethyl α-acetylcinnamate 51 in xylene gives 1,4-dihydropyridine 52, in moderate yield, dehydrogenation of which with chromic anhydride affords the expected 2,2-quinolylpyridine 53, in 75 % yield. For the preparation of the aminopyridine derivative 56, transformation of cyano group to amino group is examined. Hydrolysis of the nitrile 53 with conc. sulfuric acid gives the amide 54 in 74 % yield. Hofmann reaction of 54 with potassium hydroxide and bromine gives the corres-

ponding amino derivative 55 in 40 % yield. The use of sodium methoxide and bromine results in improvement of this method and 55 is obtained in 80 % yield. Reduction of 55 with lithium aluminum hydride gives the alcohol 56, which might promise to afford the corresponding dimethyl derivative. On the other hand, the dimethylpyridine 59 is derived from the alcohol *via* the chloride 58.

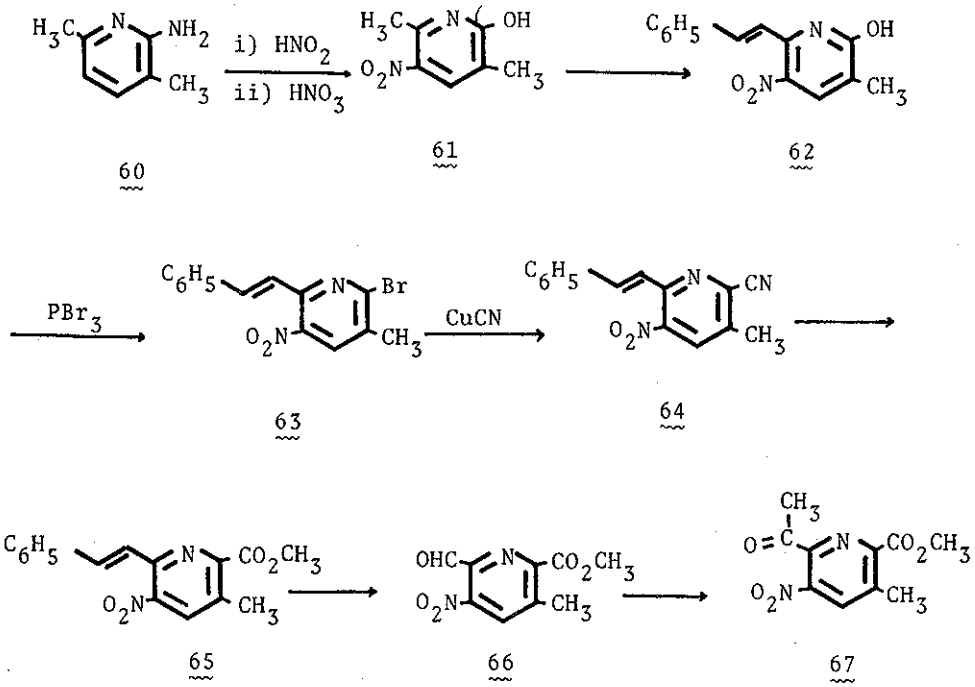


50 51 52 53 54



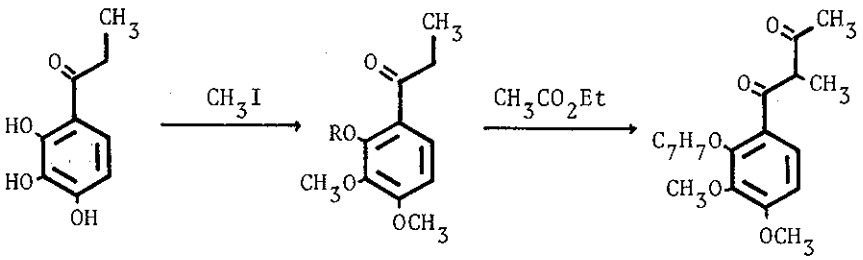
Rao et al.[28] have reported a synthesis of 2-acetylpyridine 67, which might be a useful precursor leading to desphenylstreptonigrin, from 6-amino-2,5-lutidine 60. Treatment of 60 with nitrous acid, followed by nitration of 6-hydroxypyridine gives the 6-hydroxy-3-nitro-2,5-lutidine 61, in 53 % yield. Bromination of 62, obtained by condensation of 61 with benzaldehyde, with phosphorous tribromide in nitrobenzene gives the 6-bromopyridine 63 in 70 % yield. Replacement of bromine by cyano group by using cuprous cyanide gives the 6-cyanopyridine 64 in 80 % yield. Hydrolysis of the nitrile 64 with methanolic sulfuric acid gives the methyl ester 65 in good yield. Reductive decomposition of the ozonide of 65 gives the pyridine-

aldehyde 66. On treatment of 66 with diazomethane, 2-acetylpyridine 67 is obtained accompanying with formation of the epoxide as a separable mixture in a ratio of 3:1.

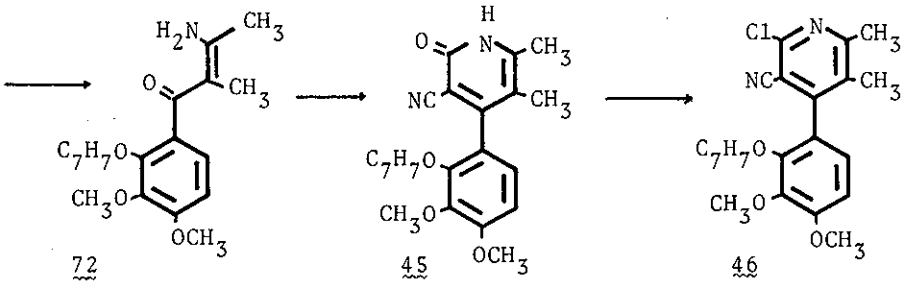


Liao et al.[29] have synthesized 5-amino-4-(2-hydroxy-3,4-dimethoxyphenyl)-3-methyl-2-pyridinecarboxylic acid, that is C-D ring moiety of streptonigrin as follows. Partial methylation of gallopropiophenone 68, obtained by the Friedel-Crafts reaction of pyrogallol with propionic anhydride, with methyl iodide and potassium carbonate in acetone gives 2-hydroxy-3,4-dimethoxypropiophenone 69, in quantitative yield. Benzylation of 69 with benzyl chloride, followed by base-catalyzed condensation of 70 with a large excess

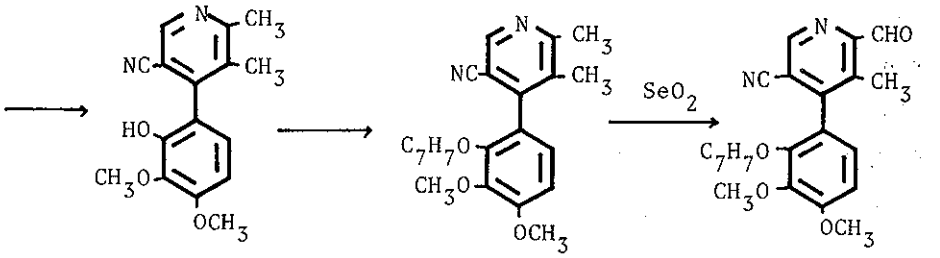
of ethyl acetate gives the 3-benzoyl-2-butanone derivative 71. Condensation of the enamido 72, obtained on treatment of 71 with ammonia in 61 % yield, with ethyl cyanoacetate in the presence of sodium ethoxide gives the 2-pyridone 45, in 68 % yield, which is identical with the synthetic material prepared by Kametani as described before. The 2-pyridone 45 is heated with dichlorophosphine oxide at 140-150° to give 46 in 75 % yield. Catalytic hydrogenation of 46 with either 5 % palladium-calcium carbonate or 10 % palladium-charcoal in the presence of aqueous ammonia results in formation of the nitrile 73 in 95 % yield, through simultanious loss of the protecting benzyl group. Oxidation of the O-benzyl derivative 74 with selenium dioxide in glacial acetic acid gives the pyridine-2-carboxaldehyde 75 in 80 % yield. Hydrolysis of the cyano group of the



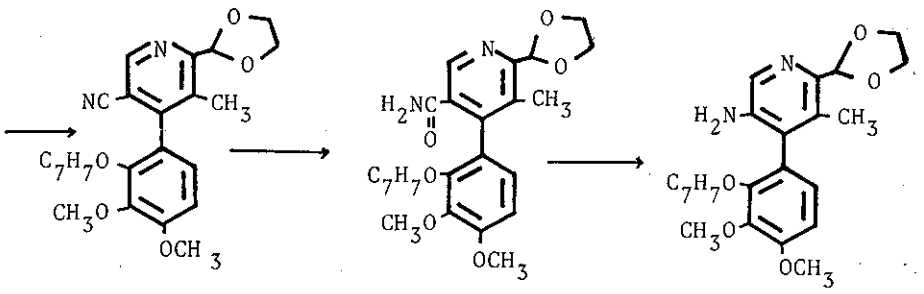
68 69: R=H 70: R=C_7H_7 71



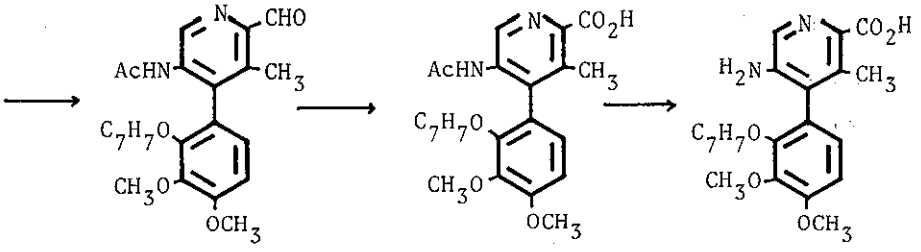
72 45 46



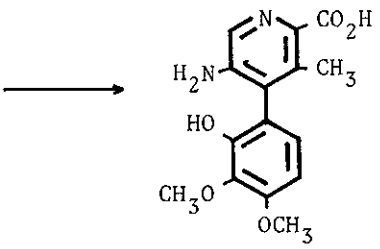
73 74 75



76 77 78



79 80 81



82

cyanopyridine 76, obtained from 75 by the usual method in 80 % yield, under basic condition gives the amide 77 in 66 % yield. Hofmann reaction of 77 by the use of sodium methoxide and bromine affords the methyl carbamate 78 in 67 % yield. Hydrolysis of 78, followed by treatment of 79 with silver oxide in a dilute basic solution gives the 2-pyridinecarboxylic acid 80, which possesses all the required functionality of C-D ring. Catalytic hydrogenolysis of the amino compound 81, obtained by hydrolysis of the carbamate 80 under basic condition, yields 82, that is C-D ring portion of streptonigrin.

4. Biological activities

Streptonigrin is of interest as a potential chemotherapeutic agent since it exhibits a broad spectrum of inhibition of various tumor e.g. carcinoma 755, sarcoma 180, lewis lung carcinoma, Walker carcinosarcoma 256, and certain types of lymphomas[30-32]. The mode of the action of streptonigrin has not yet been fully elucidated. Evidence to date indicates that streptonigrin exerts its antitumor action by two distinct mechanism:

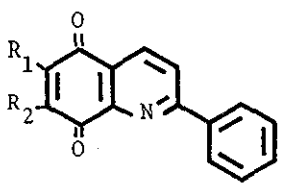
i) interferences with the normal respiratory mechanism of the cell.

ii) disruption of the replicative mechanism of the cell[32].

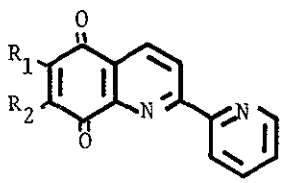
Lown et al.[33] have examined some aspects of streptonigrin as they relate to these effects including the findings that streptonigrin cleaves DNA by generating superoxide and hydroxyl radicals. The quinolinequinone moiety of streptonigrin has been indicated to be essential for antineoplastic activity through chemical modification[30]. Other functional groups which gave an influence to the

degree of antitumor activity have been found[30,35]. For example, esterification of the carboxyl function reduces the Walker carcinosarcoma 256 activity[35]. Replacement of the amino function on the quinoline ring by a hydroxyl or a methoxyl group leads to loss of activity[30], and conversion of a methoxyl on the quinoline ring to a hydroxyl group greatly reduces both activity and toxicity[36]. A p-quinone moiety is evidently necessary since the corresponding dimethoxy-o-quinone has considerably reduced the activity[29].

In a earlier work, the quinolinequinone system has been synthesized as potential antimalarial agents[14,37] and also has been synthesized for examination on their amebecidal activity and parasiticides[13].



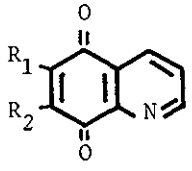
83: R_1=NH_2, R_2=OCH_3
84: R_1=OCH_3, R_2=NH_2
85: R_1=OH, R_2=NH_2
86: R_1=OH, R_2=NO_2



9: R_1=OH, R_2=NO_2
10: R_1=OH, R_2=NH_2
11: R_1=OCH_3, R_2=NH_2
87: R_1=NH_2, R_2=OCH_3

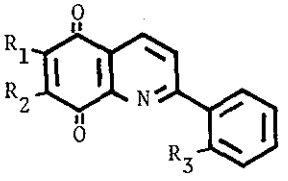
In a model study on streptonigrin toward antibacterial activity, Rao[8] has reported that various amino-methoxyquinolinequinones and aminoquinolinequinones have shown significant antibacterial activity on examination by a simple disc-plate procedure against Bacillus subtilis in the concentration range 25-100 μg/ml. In comparison with streptonigrin, 83, 11, and 84 are approximately 25 % as active and 87 is 10 % as active in this system. 7-Amino-6-hydroxyquinolinequinone 10 and 85 and 6-hydroxy-7-nitroquinolinequinone 9 and 86 are inactive.

A model study of streptonigrin toward antineoplastic activity has also been reported. Lown et al.[38] have shown that a series of substituted 5,8-quinolinequinones, in the presence of NADH, cleave PM2 cccDNA (covalently-closed circular-DNA) at different rates and percentage tumor weight inhibition of Walker carcinosarcoma 256



18: R_1=OCH_3, R_2=H
20: R_1=OCH_3, R_2=Br
22: R_1=OCH_3, R_2=N_3
24: R_1=OCH_3, R_2=NH_2
88: R_1=R_2=Br
89: R_1=R_2=Cl
90: R_1=R_2=NH_2
91: R_1=R_2=H
92: R_1=OCH_3, R_2=Cl
93: R_1=NH_2, R_2=H
94: R_1=NH_2, R_2=Br
95: R_1=NH_2, R_2=Cl
96: R_1=R_2=OCH_3
97: R_1=NH_2, R_2=OCH_3
98: R_1=$NHCOCH_3$, R_2=Cl
99: R_1=$N(CH_3)_2$, R_2=Cl
100: R_1=R_2=NH_2

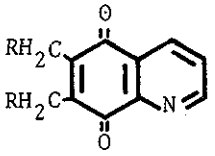
induced by the basic 5,8-quinolinequinones (18, 20, 22, 24, and 88-100).



32: $R_1=R_2=Cl$, $R_3=NO_2$
33: $R_1=OCH_3$, $R_2=Cl$, $R_3=NO_2$
36: $R_1=OCH_3$, $R_2=R_3=NH_2$
37: $R_1=OCH_3$, $R_2=Cl$, $R_3=NH_2$
101: $R_1=R_2=Br$, $R_3=NO_2$
102: $R_1=NH_2$, $R_2=Br$, $R_3=NO_2$
103: $R_1=OCH_3$, $R_2=Br$, $R_3=NO_2$
104: $R_1=H$, $R_2=NH_2$, $R_3=NO_2$
105: $R_1=H$, $R_2=R_3=NH_2$

Furthermore, the rate of single strand cleavage of PM2 ccc-DNA induced by compounds (32, 33, 36, and 101-105) has been compared regarding the antitumor activity. The 2-(o-nitrophenyl) derivatives (32, 33, and 101-104) give consistently more rapid DNA cleavage than the 2-(o-aminophenyl) compounds (36, 37, and 105). However, 2-(o-aminophenyl) compounds which are most closely similar to the substituent pattern of streptonigrin is close to the rate of scission of DNA of the latter in the presence of NADPH[16].

On the other hand, Sartorelli et al.[39] have reported on the different system that 6,7-bis-bromomethyl-5,8-quinolinequinone 107 has a moderate antitumor activity at the optimal daily dosage level of 15 mg/kg, prolonging the life span of sarcoma 180 bearing mice from 13.5 days for untreated tumor bearing animals about 22 days, and that 6,7-dimethyl-5,8-quinolinequinones 106 is inactive.



106: R=H

107: R=Br

5. Conclusion

The structure of streptonigrin has elucidated completely[4]. Although the total syntheis of streptonigrin has not yet been reported, at present, the synthetic routes to A-B ring system have been almost attempted and achieved by many reseach groups. Furthermore, C-D ring has been also synthesized[29]. The formation of 2,2-quinolylpyridines, that is A-B-C-D ring system, has been attempted by Kametani et al.[20,23,24]. A synthesis of 2,2-pyridylquinoline-quinone system has been achieved through two different routes[8,17]. 2-Acetylpyridine derivatives possessing appropriate substituents might be useful intermediates for the total synthesis of streptonigrin as Weinreb suggested[17].

The mechanism of an action of streptonigrin seems to involve extensive degradation of DNA. Recent work by Lown[16] has shown that a single strand scission of PM2 ccc-DNA by 5,8-quinolinequinones, including streptonigrin, correlates well with inhibition of Walker carcinoma. A large nearly coplanar region, rings A-B and C of streptonigrin, in a molecule which interacts with DNA produces a theory based upon intercalation, for example of the type shown in elegant study of 1:2 complex of actinomycin D with deoxyguanosin[40,41]. However, it seems that intercalation of streptonigrin into DNA

structure originates a local reactivity in its molecule, most probably in the aminoquinone region[2,4].

Streptonigrin will be prepared and the problem of its asymmetry because of twisting of ring D from the plane described by rings A, B, and C will be solved.

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