SYNTHESIS OF LAVENDAMYCIN

A THESIS

SUBMITTED TO THE

UNIVERSITY OF POONA

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

(IN CHEMISTRY)

BY

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SEPTEMBER 1985

DEDICATED TO MY PARENTS

Certified that the work incorporated in the thesis "SYNTHESIS OF LAVENDAMYCIN" submitted by Mr.S.P.Chavan was carried out by the candidate under my supervision. Such material as has been obtained from other sources has been duly acknowledged in the thesis.

(A. V. RAMA RAO)
Supervisor

C O N T E N T S

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September 1985

ABSTRACT

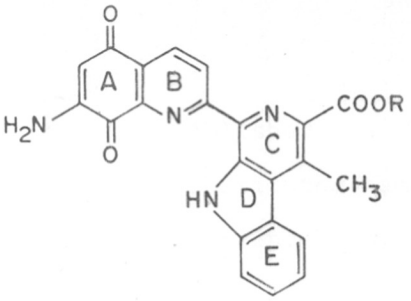
Lavendamycin (1), a dark red pentacyclic quinone and an antitumour antibiotic isolated from fermentation broths of Streptomyces lavendulae by Doyle et al., was shown to have a fully substituted β -carboline skeleton. Lavendamycin bears a striking similarity with that of a tetracyclic antitumour antibiotic, streptonigrin (3) and is believed to have same biogenetic origin.

The strategy of synthesis of (1) revolves around Bischler-Napieralski reaction and concerned with the preparation of two key intermediates, namely β -methyltryptophan 8 and quinaldic acid with suitable substituents and both in turn could be obtained from easily accessible indole 4 and 8-hydroxyquinoline 9 respectively.

In order to test the efficacy of the synthetic plan as described above, some model studies with moderately functionalised intermediates 10 and 7 were first carried out.

8-Methoxyquinaldic acid (10) was prepared from 8-hydroxyquinoline (9), by oxidising it to its N-oxide followed by treatment with dimethylsulphate, sodium cyanide, methylation followed by saponification.

The condensation of 8-methoxyquinaldic acid (10) with tryptophan 7 was effected using methylchloroformate through mixed anhydride. The amide 29 on treatment with $POCl_3$ underwent smooth cyclisation to the corresponding β -carboline 35.



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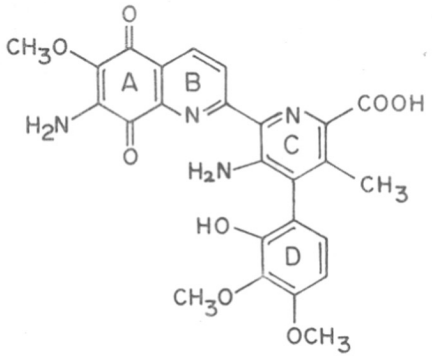
2 : R = CH_3



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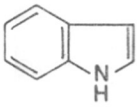

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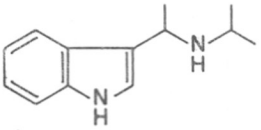

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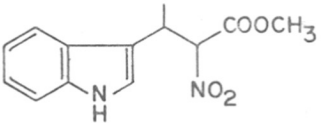

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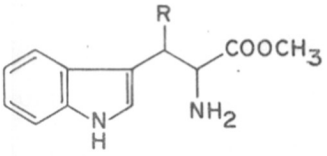

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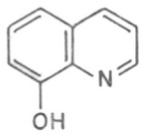
8 : R = CH_3



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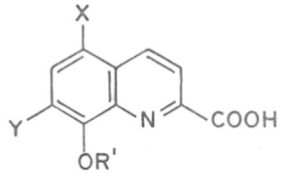


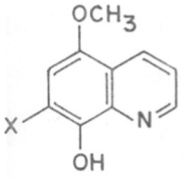
10 : R' = CH_3 , X = Y = H

11 : R' = $CH_2\phi$, X = Y = H

12 : R' = CH_3 , X = Br , Y = H

13 : R' = CH_3 , X = NH_2 , Y = Br

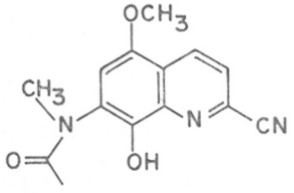
14 : R' = CH_3 , X = OCH_3 , Y = Br



15 : X = H

16 : X = $N_2\phi$

17 : X = NHAc



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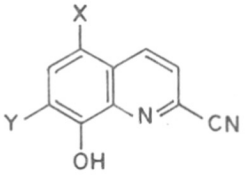


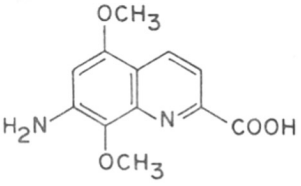
22 : X = Y = H

23 : X = NO , Y = H

24 : X = NHAc , Y = H

25 : X = NHAc , Y = Br

26 : X = OH , Y = Br



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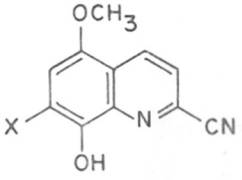


20 : X = H

21 : X = $N_2\phi$

Having established the strategy with tryptophan, the properly substituted namely β -methyltryptophan 8 was prepared from indole by three high yielding sequential steps namely, (i) Mannich reaction 5 (ii) condensation with methylnitroacetate 6 (iii) the reduction of the nitro group to amine.

Condensation of β -methyltryptophan with 8-methoxy-quinaldic acid (10) employing methylchloroformate furnished the corresponding amide 30. Cyclisation of the amide 30 afforded the desired β -carboline 36. Demethylation of 36 followed by esterification yielded the phenol 37. The β -carboline 38 was also prepared by cyclisation of the amide 31 which in turn was prepared from the acid 11 and β -methyltryptophan 8. Attempted functionalisation (Ring A) of 37 taking advantage of phenol by nitrosation was unsuccessful.

The failure to functionalise 37 (ring A) to lavendamycin led to prepare properly substituted quinaldic acid. Following the same set of reactions with moderately functionalised quinaldic acid 12, the corresponding β -carboline 39 was obtained to ensure the generality of the approach.

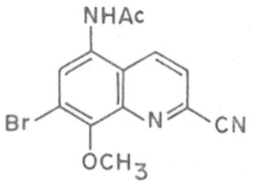
Efforts were then directed towards the synthesis of 7-aminoquinaldic acid 19. Selective monomethylation of 5,8-dihydroxyquinoline (obtained from 8-hydroxyquinoline) afforded 5-methoxy-8-hydroxyquinoline (15). Coupling of 15 with benzene diazonium chloride furnished the azo compound 16.

Reduction of the azo compound 16, N-oxidation, followed by treatment with dimethylsulphate and sodium cyanide, led to the formation of undesired N-methyl nitrile 18. Introduction of amine by condensation of phenol 20with phenyldiazonium chloride via 21 was also unsuccessful.

Since the direct introduction of amine at C-7 posed some unforeseen problems, introduction of bromine at C-7 position as a latent amine was considered mandatory.

Attention was focussed towards the synthesis of 8-methoxyquinaldic acid having substituents masked for quinone and amine so that these functionalities could be derived at the later stages. This was effected as follows. 8-Hydroxy-quinaldonitrile (22) was subjected to nitrosation reaction to give 5-nitroso-8-hydroxyquinaldonitrile (23) which was further reduced to the corresponding amine and isolated as the amide 24. Methylation of the bromophenol 25, obtained from 24 using NBS, furnished 27. Hydrolysis of the nitrile 27 then yielded the aminoquinaldic acid 13 whose condensation with β -methyltryptophan 8 afforded the amide 33. Various attempts to cyclise amide 33 using reagents such as $POCl_3$, PPE, PPA, P_2O_5-$POCl_3$ etc. failed to yield the β -carboline 40. This was apparently due to the amido function interfering in the reaction.

Since the anomalous behaviour of the amide 33 towards cyclisation by various dehydrating agents was thought to arise



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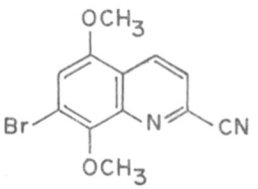

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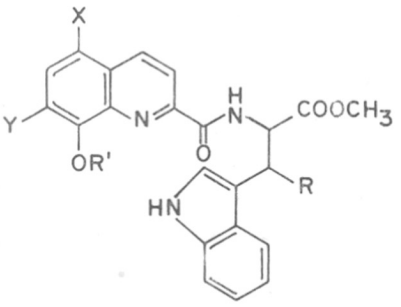
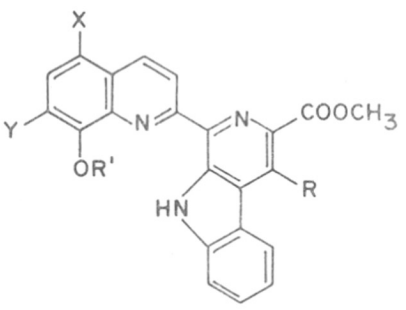




29 : R = H , R' = CH_3 , X = Y = H

30 : R = CH_3 , R' = CH_3 , X = Y = H

31 : R = CH_3 , R' = $CH_2\phi$, X = Y = H

32 : R = CH_3 , R' = CH_3 , X = Br , Y = H

33 : R = CH_3 , R' = CH_3 , X = $NHCOOCH_3$, Y = Br

34 : R = CH_3 , R' = CH_3 , X = OCH_3 , Y = Br

35 : R = H , R' = CH_3 , X = Y = H

36 : R = CH_3 , R' = CH_3 , X = Y = H

37 : R = CH_3 , R' = H , X = Y = H

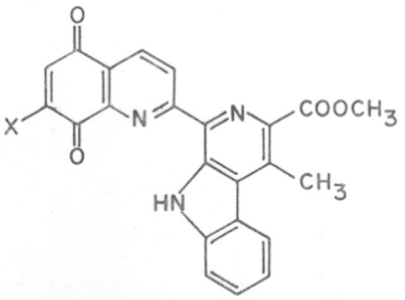
38 : R = CH_3 , R' = $CH_2\phi$, X = Y = H

39 : R = CH_3 , R' = CH_3 , X = Br , Y = H

40 : R = CH_3 , R' = CH_3 , X = $NHCOOCH_3$, Y = Br

41 : R = CH_3 , R' = CH_3 , X = OCH_3 , Y = Br

42 : R = CH_3 , R' = H , X = OH , Y = Br



43 : X = Br

44 : X = N_3

from the urethane moiety in 33, it was thought worthwhile to replace it by a non-interfering group such as OCH_3. Accordingly, acetamido bromophenol 25 was oxidixed to 2-cyano-7-bromo-5,8-quinolinequinone which was reduced to the corresponding hydroquinone which on conventional methylation of 28 followed by hydrolysis furnished the dimethoxy bromoacid 14.

Condensation of the acid 14 with β-methyltryptophan 8 furnished the amide 34 which underwent smooth cyclisation to the corresponding β-carboline derivative 41. Direct oxidation of the cyclised product 41 to the bromoquinone 43 was initially found to be unsatisfactory. However, this problem was later circumvented as follows. Exhaustive demethylation using aqueous HBr followed by esterification yielded the corresponding hydroquinone ester 42. Oxidation of 42 to bromoquinone 43 was effected using cerric ammonium nitrate. The compound 43 was found to have identical properties with the reported data by Kende *et al.* Since the transformation of bromoquinone 43 to lavendamycin methyl ester (2) *via* azidoquinone 44 has already been reported by Kende *et al.* this route for the β-carboline 43 would constitute a formal total synthesis of lavendamycin methyl ester (2).

This part of the thesis briefly deals with isolation and characterisation of lavendamycin. Biological activity and biosynthesis of streptonigrin is also dealt with because of the fact that lavendamycin has remarkable similarity with streptonigrin and is believed to be originated by same precursors. In order to clear the confusion in the nomenclature of carbolines, a few lines are also devoted to its nomenclature and is then followed by a brief outline about the general methods of synthesis of β-carbolines.

Dreadful diseases such as cancer have plagued the mankind for years. The need to combat diseases by chemotherapy apart from radiation and surgery or in combination has led to the isolation of an array of biologically active compounds. Untiring efforts of the natural product chemists have led to isolation and characterisation of compounds which have proved to be extremely potent drugs. These biologically active compounds are of diverse nature which range from aliphatic, aromatic, heterocyclic, carbohydrates and exhibit activity against variety of diseases like tuberculosis, leprosy, cancer etc.

A large number of anticancer drugs are now being used in medical practice which have been approved by National Cancer Institute (USA) while many are undergoing clinical trials. All these drugs can be broadly classified into four categories namely, (1) alkylating agents (2) antimetabolites (3) antibiotics and (4) miscellaneous compounds.

Among the various compounds which are promising as antitumour agents, natural products, either of plant or of microbial origin, are showing much specificity in the anticancer properties.

Streptonigrin

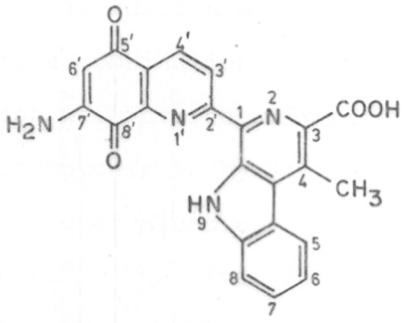
The isolation of a potent antitumour antibiotic compound streptonigrin (2) (Chart 1) from the culture of *Streptomyces flocculus* (ATCC 13852) in 1959 (US)[1] and subsequently from

other actinomycetes such as Actinomyces albus var. bruneomycini (USSR)[2], S. echinatus and S. rufochromogenes (France)[3] being named as bruneomycin and rufochromomycin respectively, aroused interest in this heterocyclic compound. Streptonigrin is active against a variety of gram-positive and gram-negative bacteria, and has pronounced activity against numerous experimental tumours. In late sixties testing progressed to a phase III clinical trial for the treatment of lymphoproliferative diseases; the antibiotic appeared to be as effective as chlorambucil but exhibited somewhat greater degree of toxicity[4]. Therefore, there was a decline in the interest of streptonigrin in US. However, promising results from France and Soviet Union, where streptonigrin has been used in combination therapies, have indicated a need to reassess the potential of this antibiotic.

The lethality of streptonigrin is apparently due to a redox process, involving the quinone portion of the molecule, that leads to uncoupling of oxidative phosphorylation and depletion of cellular ATP. As a secondary effect, superoxide produced in the redox reaction reacts with its dismutation product (hydrogen peroxide) to yield hydroxy radicals that cause single-strand breaks in DNA.

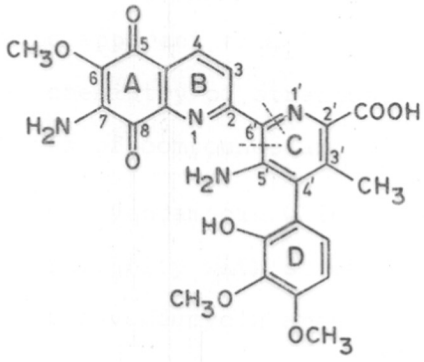
A few simple derivatives of streptonigrin have been prepared by methylation of the carboxylic acid or modification of the quinone portion and from such studies a minimum structural

CHART 1



1

LAVENDAMYCIN (1981)



2

STREPTONIGRIN (1959)

requirement has been suggested for the activity[5]. Although the isolation of this molecule was done way back in 1959, the structure elucidation was brilliantly done by Woodward in 1963 using a combination of spectral and degradative methods[6] which was later confirmed by X-ray in 1975[7]. Model studies of streptonigrin by Kametani, Rao, Liao and their coworkers provided valuable information on the construction of the subunits[8]. The first construction of the complete quinone carbon framework was secured in 1978[9], and the first total synthesis of streptonigrin was itself achieved in 1980 by the Weinreb group[10]. This was then followed by Kende's synthesis[11] in 1981 and Boger's synthesis[12] in 1983.

It is apparent from the above discussions of the history of chemistry of streptonigrin that alkaloids belonging to this class of compounds are biologically important.

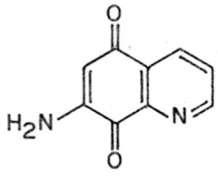
Since lavendamycin which belongs to the above class has close similarity with streptonigrin, interest in the chemistry of lavendamycin arose.

Isolation and characterisation of lavendamycin

Investigation of the fermentation broths of Streptomyces lavendulae by a group of Bristol laboratories in 1981 led to the isolation of a novel antitumour antibiotic, which bears a striking similarity with streptonigrin (2) and named as lavendamycin (1)[13] (Chart 1).

It was obtained as a dark red solid. m.p.> 300°(dec.). The limited solubility of lavendamycin in organic solvents precluded the efforts to grow crystals for X-ray analysis. However, the structure determination was done by careful analysis of the spectral data of the parent acid as well as its methyl ester[14]. The task of determination of the position of the amino group on the quinone was based on the structural analogy of 1 with streptonigrin 2 coupled with the comparison of ^{13}C-NMR spectrum with that of the model compounds (Chart 2), 7-amino-5,8-quinolinequinone (3) and 6-amino-5,8-quinolinequinone (4). The ^{13}C-NMR spectrum of lavendamycin closely resembled the ^{13}C-NMR spectrum of the model 3 which led to the establishment of the position of the amino group at C-7' of quinolinequinone. In addition a simple β -carboline model (5) also aided the structural elucidation of lavendamycin as 2.

CHART 2



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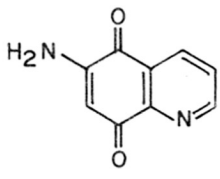

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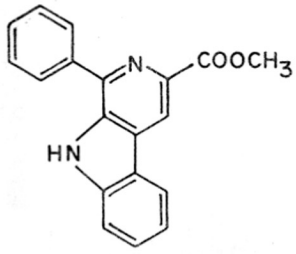


5

Biological activity

The activity of lavendamycin 1 was investigated by Balitz et al.[14] and was found to exhibit interesting biological activity as antitumour antibiotic. Its microbial activity was found to be similar to streptonigrin 2. The minimum inhibitory concentration of lavendamycin against Streptomyces pneumoniae, S. pyogenes, Staphylococcus aureus, Streptococcus faecalis, E. coli etc. showed inhibition pattern similar to streptonigrin but is less potent. The only exception is with fungi Trichophyton rubrum, T. mentagrophytes and Microsporum canis where lavendamycin is found to be more potent.

Lavendamycin was tested for its ability to induce bacteriophage production in the lysogenic strain of Escherichia col W. 1709 (λ). The minimum inducing concentration of 0.003 μg/ml is comparable to that of streptonigrin, 0.008 μg/ml, thus indicating a dissociation between inducing activity and other biological properties in comparing the two antibiotics.

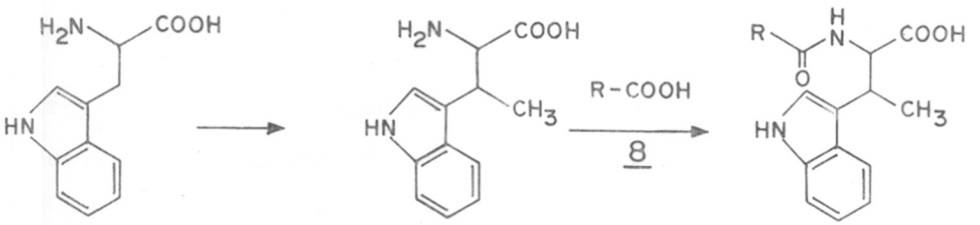
Lavendamycin exhibited antitumour activity against P-388-J leukemia but no inhibitory effect on standard P-388 or L-1210 leukemias.

Biosynthesis

Biosynthetic pathway for lavendamycin 1 has not been reported so far. However, it is believed that lavendamycin is a biosynthetic precursor for streptonigrin 2 and therefore it

SCHEME 1

BIOSYNTHESIS



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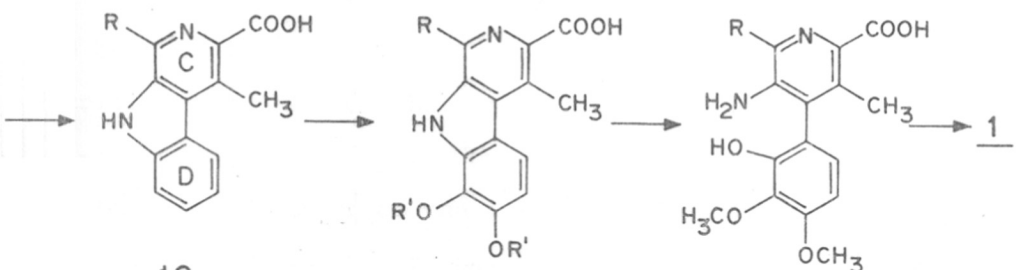
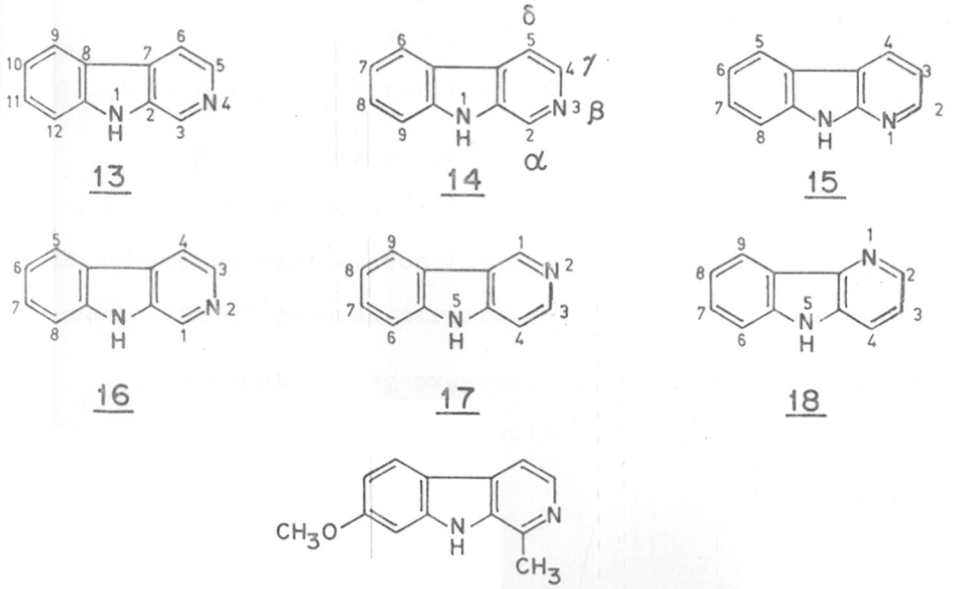


10 11 12

CHART 3



13 14 15

16 17 18

19 HARMINE

is worthwhile discussing a few lines regarding the biosynthetic pathway of streptonigrin.

The biosynthesis of the tetracyclic antitumour quinone streptonigrin 2 has been brilliantly explored in recent studies by Gould and his collaborators[15,16]. The biosynthesis of 2 is now known in considerable detail and the antibiotic can be viewed as being constructed *via* a convergent pathway involving the two major units shown by the dotted line in the structure 2.

Although some of the details of the process remains to be elucidated, it is well established that the aryl pyridine C-D rings of this antibiotic originate from β-methyltryptophan (7). Thus, a critical examination led to the establishment of the intermediacy of β-methyltryptophan (7) which provides a rational origin for the methyl group at C-3' of 2. Tryptophan (6) was shown to undergo C-methylation (rarely encountered in amino acids) to β-methyltryptophan (7) at an early stage in the biosynthesis of streptonigrin and was isolated for the first time as a free amino acid.

The β-methyltryptophan then condenses with an acid 8 or a precursor thereof, to furnish an amide 9. The amide 9 then undergoes cyclisation followed by aromatisation to a β-carboline of general structure 10.

Subsequent D-ring oxygenation and unprecedented ring cleavage of the putative β-carboline intermediate 10 affords

4-phenylpicolinic acid 12 grouping with C-5' amine derived from the original indole nitrogen. The exact details of the oxidative cleavage (N-C bond) of D-ring has not been demonstrated. Such a cleavage reaction of an indole or β-carboline, either chemically or biochemically is unprecedented.

It was recognised that lavendamycin might be the β-carboline intermediate of the Gould biosynthetic scheme, but the actual intermediacy of lavendamycin in the biosynthesis of streptonigrin by Streptomyces flocculus has not been demonstrated.

The biosynthetic studies thus led to the establishment of previously unknown pathways involved in the formation of both quinoline and pyridine portions of 2.

Preamble of carbolines

The total synthesis of natural products containing the pyridine ring has occupied the heterocyclic chemists since the 19th century as it embraces a wide variety of molecules whose number and structural type has grown enormously. The trend is bound to continue in view of the continuous development in fermentation, isolation and structural elucidation techniques.

A carboline ring system may be regarded as a carbazole in which one of the -CH= groups of a benzene ring

has been replaced by -N=. Carbolines, which constitute a sub-set of heterocyclic compounds, have interested synthetic chemists. It is interesting to mention here that on β-carbolines hundreds of papers have been published during past decades, which is twice as much as appeared during preceding seventy years. The renewed interest in the chemistry of carbolines was due to the developments in the chemistry and pharmacology of these alkaloids.

a) Nomenclature

The nomenclature of β-carbolines (Chart 3) has been repeatedly modified, and the compounds have been numbered in an astonishing variety of ways since Perkin and Robinson[17] introduced the name carboline for the ring system for harmala alkaloids. In the first version of carboline nomenclature, the parent compound of the series, whose trivial name was norharman was referred to as 4-carboline and numbered as in 13 (harmine = 11-methoxy-3-methyl-4-carboline). The numbering of carboline system was later modified[18] to that shown in 14 and the position of the basic nitrogen in the pyridine ring was designated by a Greek letter. The Greek letters have also been used in conjunction with the original system of numbering (harmine = 11-methoxy-3-methyl-β-carboline)[19]. According to "Ring Index"[20] the system is classified as pyrido-indole and numbered as in 16 (harmine = 7-methoxy-1-methyl)-9H pyrido[3,4-b]indole). This is the nomenclature adopted by

Chemical abstracts, according to which α-carboline (15) is 9H-pyrido[2,3-b]indole, β-carboline (16) is 9H-pyrido [3,4-b]indole, γ-carboline (17) is 5H-pyrido[4,3-b]indole and δ-carboline (18) is 5H-pyrido[3,2-b]indole. The numbering used in 16 was introduced also in conjunction with the carboline nomenclature[21] (harmine = 7-methoxy-1-methyl-β-carboline). This is the system which, at the present time, appears to be most widely adopted[20]. The same numbering has been used without the Greek letter convention (harmine = 7-methoxy-1-methyl)-2-carboline). In this thesis, the carboline rather than pyridoindole nomenclature is adopted with the numbering for α-, β-, γ- and δ-carbolines as shown in structures 15 - 18 respectively, as recommended by the editor of Journal of Chemical Society in his report on the nomenclature, 1952[22], and by the definitive IUPAC 1957, rules of organic nomenclature.

b) General Methods of Synthesis (β-Carbolines)

The methods available for the synthesis of β-carbolines are several and are discussed below (Chart 4 and 5). Out of these methods 3 & 4 are most extensively applied. The different methods are summarised as follows.

1. In this procedure Graebe-Ullmann carbazole synthesis[23] has been utilized for e.g. 3-(2'-aminoanilino)pyridine (22) (prepared from 3-bromo-pyridine (20) and O-phenylenediamine (21) on nitrous acid treatment furnished the corresponding benztriazole 23

Heating of the compound 23 in presence of lewis acid catalyst at elevated temperature furnished a mixture of β (16) and δ (18) carbolines from which β-carboline could be isolated in very low yields. This method suffered from a drawback as the preparation of 3-(2'-aminoanilino) pyridine 22 is tedious and the reaction offers a mixture of β and δ carbolines in low yields along with 3-anilinopyridine (24) as a side product.

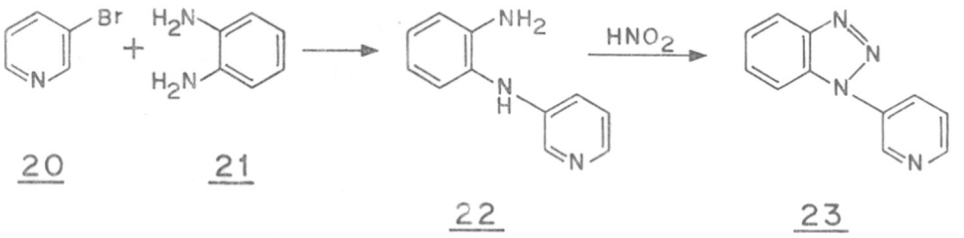
2. 2-Indolylcarboxamidodialkyl acetals 25 (of general formula), which could be easily prepared from the corresponding indole acid and the amine, was subjected to acid treatment, led to the formation of the corresponding 1-keto-1,2-dihydro-β-carboline 26[24]. This method has a limited utility as it leads to different products depending on the substitution pattern in 25.

3. This method which involves the cyclisation of a suitable amide 29 in the presence of reagents such as $POCl_3$, P_2O_5, PPE etc. is most commonly used, to prepare 3,4-dihydro-β-carbolines 30[25]. This method is truly analogous to the method used for the isoquinoline synthesis by Bischler-Napieralski reaction. The advantage of this method lies in the fact that a desired substituent could be easily introduced at 1-position of β-carbolines by making use of the desired acid chloride. In addition the procedure offers β-carbolines as exclusive products without the formation of any undesirable products.

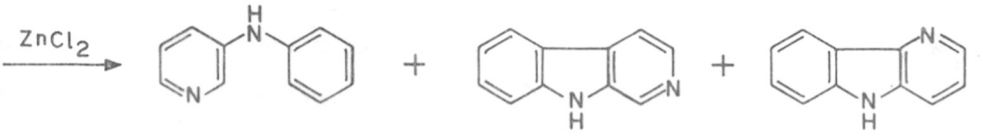
CHART 4

GENERAL METHODS OF SYNTHESES OF β-CARBOLINES

1. GRAEBE - ULLMANN

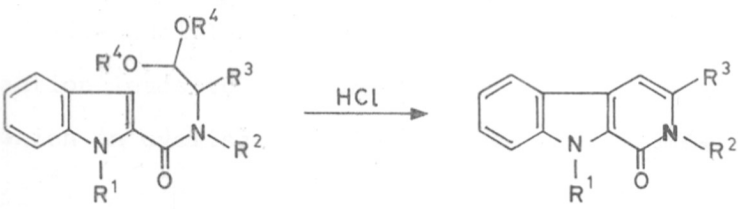


20 21 22 23



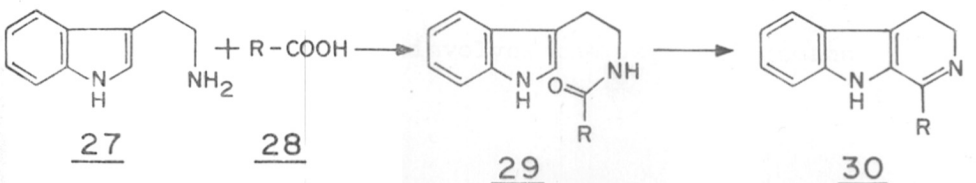
24 16 18

2.



25 26

3. BISCHLER NAPIERALSKI REACTION



27 28 29 30

4. The salient features of this method[25] for the synthesis of β -carbolines are (a) formation of Schiff's base from tryptamine and suitable aldehyde and (b) cyclisation of the resulting Schiff's base to form 1,2,3,4-tetrahydro- β-carbolines. This method also enables to introduce a desired substituent at 1-position depending upon the aldehyde being used. This procedure is an adaptation of the Pictet-Spengler isoquinoline synthesis.

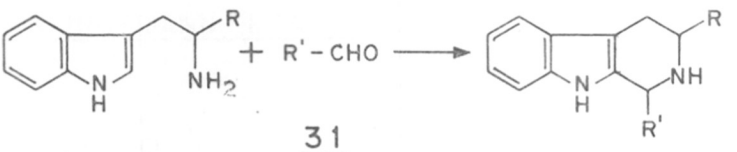
5. This method is similar to the one reported earlier (4). In this case, tryptophan 6 is being used instead of tryptamine and on reaction with aldehyde 31 followed by cyclisation, 3-carboxylic acid derivatives of 1,2,3,4-tetrahydro- β -carboline 33 is obtained[27]. Therefore this could be utilised for functionalisation of the 3-position.

6. In this synthesis Curtius reaction is being explored. For instance, β -(3-indolyl)propionylazide (34), when subjected to Curtius reaction leads to the formation of the intermediate carbimide 35 which on cyclisation gives 1-keto, 1,2,3,4 tetrahydro- β -carboline 36[28].

7. When 2-carboethoxy-3-aldehyde 37 is condensed with hippuric acid 38, the corresponding azlactone 39 is obtained. Hydrolysis of the azlactone with a base followed by cyclisation gives 1-keto-1,2-dihydro- β -carboline-3-carboxylic acid 41[29]. This method is suitable for the functionalisation of C-3 position of β -carboline.

8. Another synthetic route involves the Japp-Klingemann

4.& 5. PICTET-SPENGLER REACTION



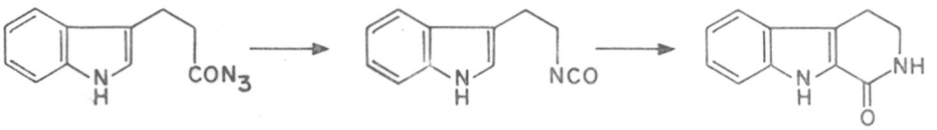
31

27 : R = H
6 : R = COOH

32 : R = H
33 : R = COOH

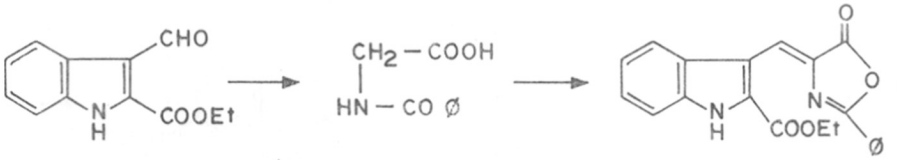
CHART 5

6.

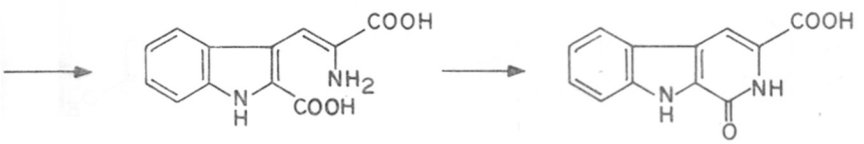


34 35 36

7.

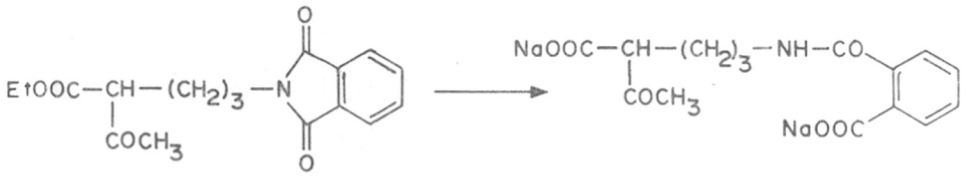


37 38 39

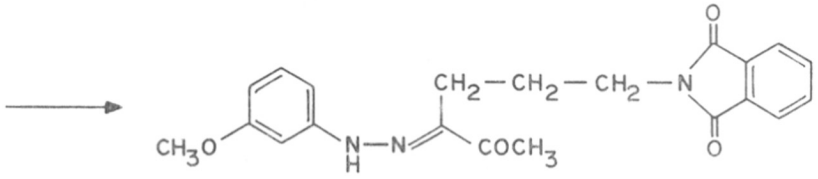


40 41

8. JAPP KLINGEMANN REACTION



42 43



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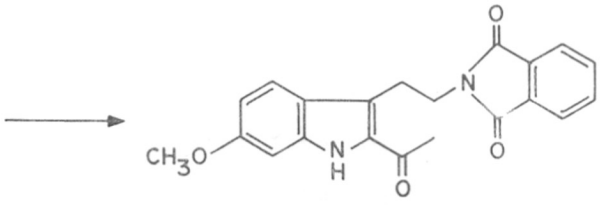

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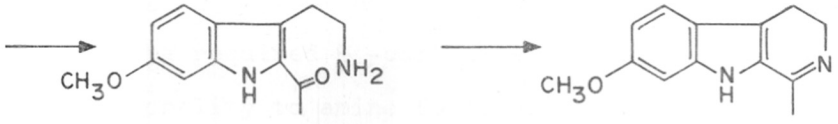


46 47

reaction[30] for the synthesis of indole derivatives. This method involves 42 which is converted into the aryl hydrazone 44 and then cyclised to give indole derivative 45. Removal of the phthalimido group followed by cyclisation offers 1-methyl-3,4 dihydro-β-carboline 47.

Reported Syntheses of Lavendamycin

While the present work was in progress and some of the model studies had already been done, Kende and Ebetino reported the first regiospecific synthesis of lavendamycin. Later (after completion of present work) one more synthesis of lavendamycin by Hibino appeared.

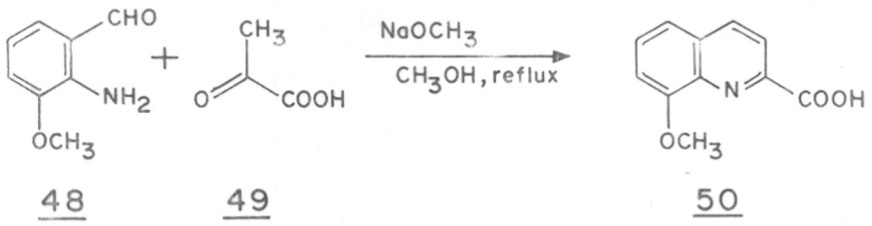
In addition to this, significant amount of work has been carried out in order to build lavendamycin skeleton as well as part of it. It is pertinent to include all of them in the umbrella of this chapter, because the chemistry involved in these studies is directly or indirectly related to the present work.

Kende's approach[31]

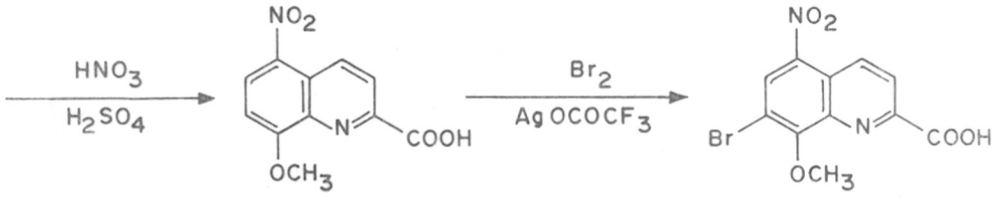
Synthesis of lavendamycin by Kende and Ebetino[31] (Scheme 2) was based on Bischler-Napieralski reaction. For example, 5-nitro-7-bromo-8-methoxyquinaldic acid (52) was prepared from 8-methoxyquinaldic acid (50) by nitration and bromination. This was condensed with β-methyltryptophan (54) using carbodiimide 53 as condensing agent and the resultant amide 55 was cyclised with PPE to get the required β-carboline derivative 56. Reduction of nitro functionality to amine followed by oxidation with $K_2Cr_2O_7$ then

SCHEME 2

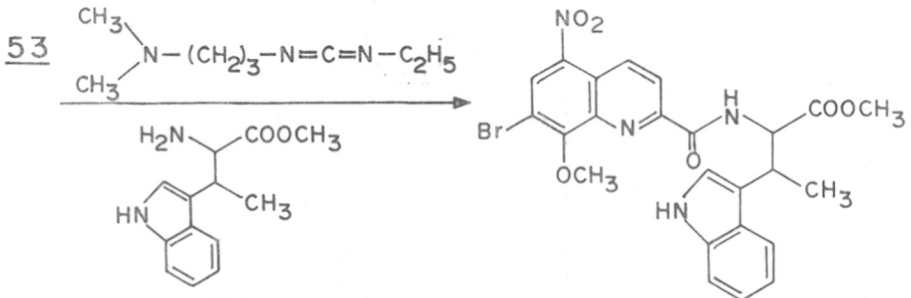
KENDE'S APPROACH (1984)



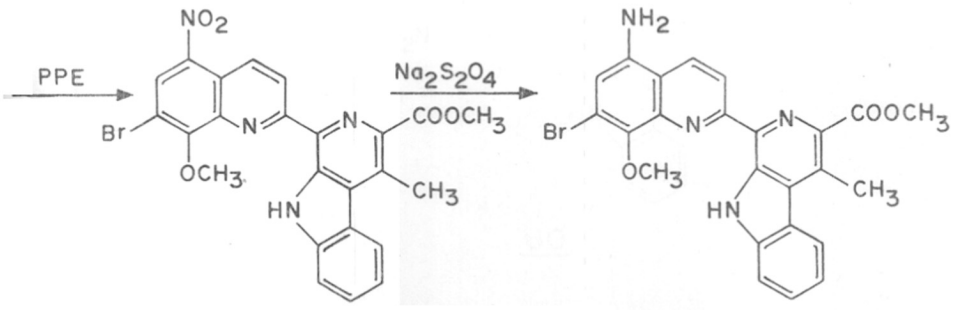
48 49 50



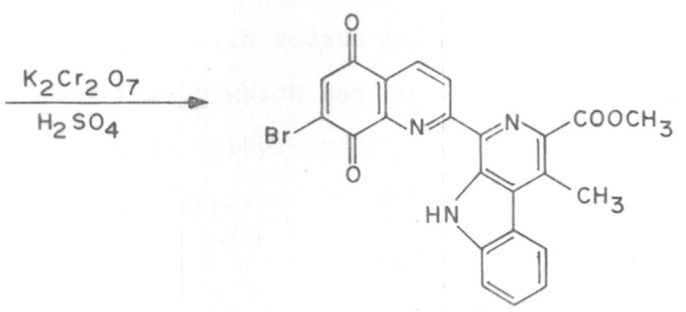
51 52



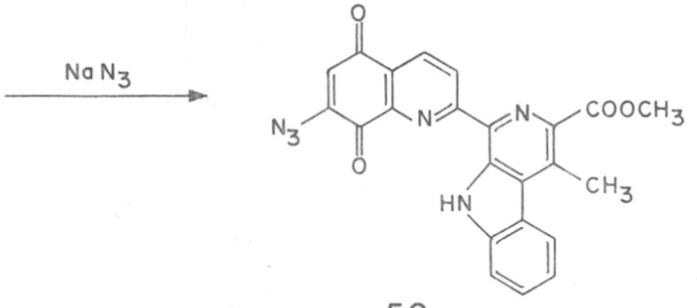
54 55



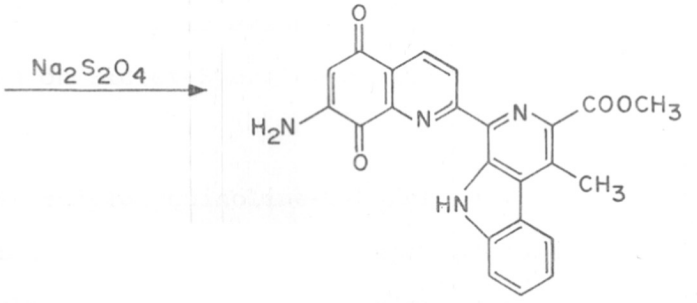
56 57


K2Cr2O7
H2SO4
O
Br
N
O
N
COOCH3
CH3
HN

58


NaN3
O
N3
N
O
N
COOCH3
CH3
HN

59


Na2S2O4
O
H2N
N
O
N
COOCH3
CH3
HN

60

furnished the 7'-bromoquinone 58. Nucleophilic displacement of the bromoquinone with sodium azide furnished the 7'-azido-quinone derivative 59 which was reduced to lavendamycin methyl ester 60. The same methyl ester was obtained from natural lavendamycin by esterification and then compared with the synthetic sample.

Boger's approach to CDE Rings of lavendamycin[32]

This approach (Scheme 3) was based on two novel reactions. The 4-arylpicolinic acid derivative (CE rings) 69 was obtained by Diels-Alder reaction of α-aryl enamine 64 with 1,2,4 triazine derivative 65 and then the transformation of ester function at C-3 to amine derivative 69 was effected.

The second novel step was the palladium assisted cyclisation to build the β-carboline derivative 70 (CDE rings) of lavendamycin[32].

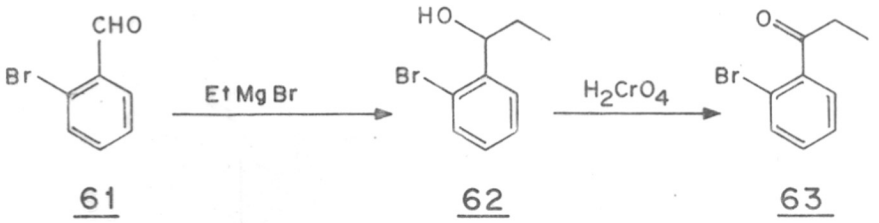
Hibino's approach[33,34]

Initially Hibino studied the synthesis of demethyl lavendamycin[33] in which methyl group at C-4 position was lacking. Later similar approach (Scheme 4) was exploited for the total synthesis of lavendamycin[34]. The strategy of the synthesis was based on Pictet-Spengler cyclisation to furnish β-carboline derivative.

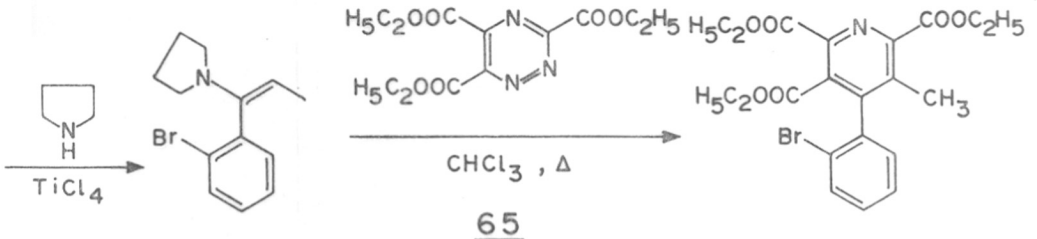
8-Benzyloxyquinoline-2-aldehyde (71) was prepared from 8-benzyloxyquinoline in four steps which was then condensed with β-methyltryptophan 72 in refluxing benzene to yield the

SCHEME 3

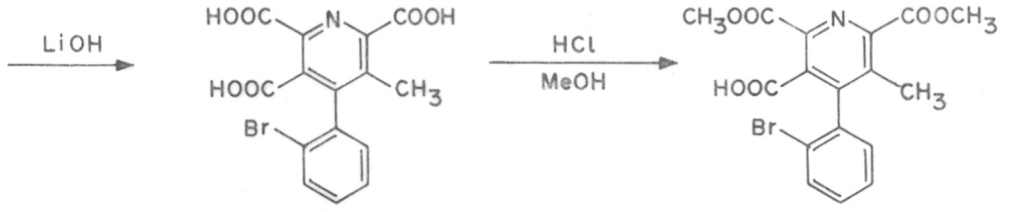
BOGER'S APPROACH (1984)



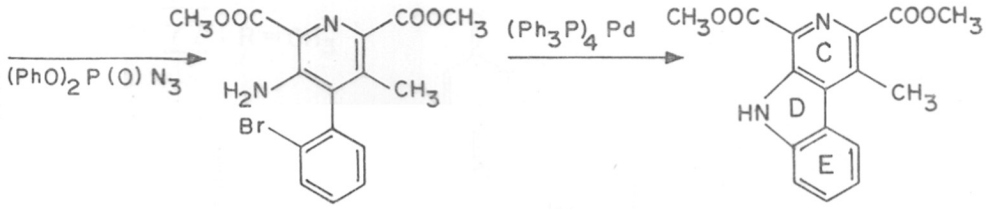
61 62 63



64 65 66



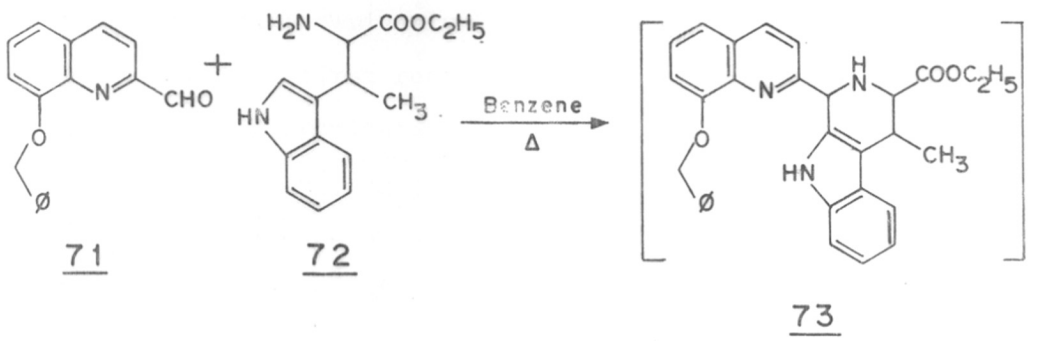
67 68



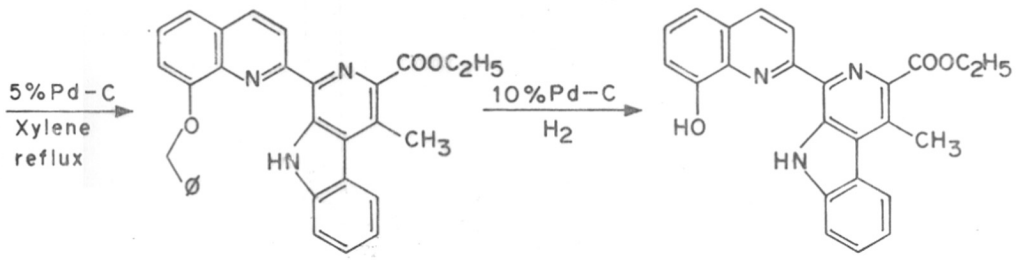
69 70

SCHEME 4

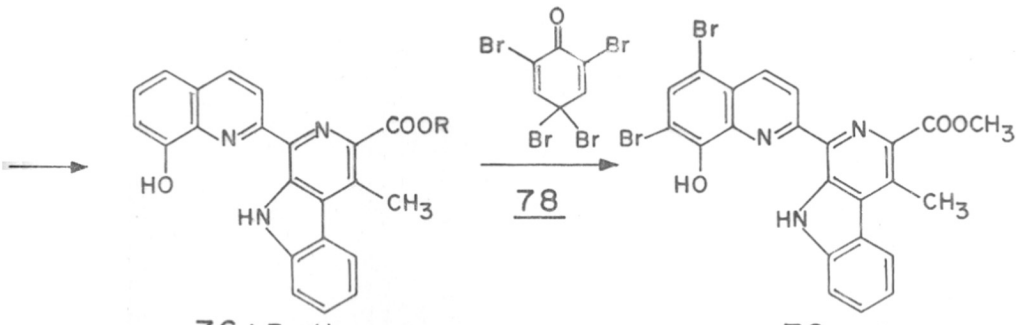
HIBINO'S APPROACH (1985)



71 72 73



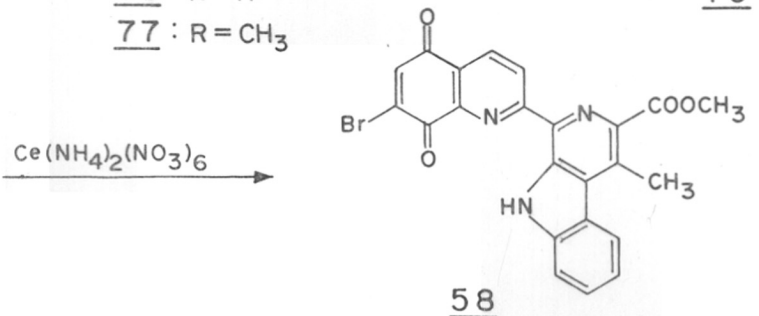
74 75



76 : R = H
77 : R = CH_3

78

79



58

corresponding tetrahydro-β-carboline 73 which was aromatised to the β-carboline derivative 74. Functionalisation of ring A was carried out by first removing the benzyl group and then careful selective brominating 77 to obtain 5',7'-dibromo-8'-hydroxy derivative 79. Oxidation of 79 with CAN gave the bromoquinone 58 which had already been converted into lavendamycin methyl ester 60 by Kende's group.

The work on the synthesis of lavendamycin was initiated in these laboratories in late 1982. This part of the thesis concerns with the successful synthesis of lavendamycin. The strategy revolves around Bischler-Napieralski cyclisation to form the β-carboline system. Apart from the unforeseen problems which were encountered during the synthesis and which were eventually circumvented, these heterocyclic compounds also posed solubility problems, thus rendering the characterisation and progress of the reaction difficult. The synthesis of lavendamycin has attracted the attention of scientists from all over the world and this could be judged by the appearance of four synthesis towards lavendamycin during a short span after it was isolated.

PRESENT WORK

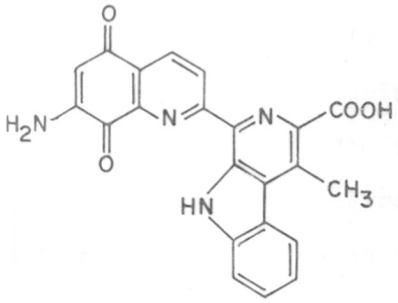
The isolation of lavendamycin (1), a novel antitumour antibiotic from the fermentation broths of Streptomyces lavendulae has triggered off another synthetic puzzle to organic chemists. It was shown to possess a β-carboline skeleton accompanied by varied functional groups.

Lavendamycin is closely related to streptonigrin 2 (Chart 1). It was demonstrated by biosynthetic studies that streptonigrin was originated from β-methyltryptophan via the formation of β-carboline skeleton and since lavendamycin also possesses a β-carboline system, it might have a biosynthetic link between β-methyltryptophan and streptonigrin.

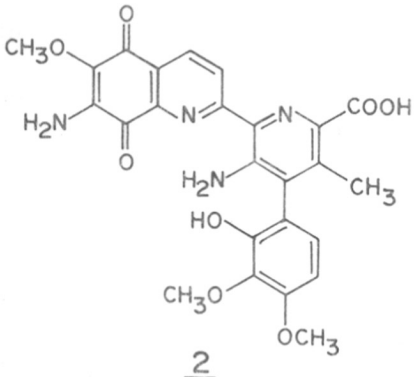
Fascinating structural features coupled with interesting biological activity of lavendamycin led to undertake the synthesis of this complex molecule 1 and its analogues with a view to find better activity than the parent compound. In addition when this work was initiated in these laboratories, no synthesis of 1 was reported.

The retrosynthesis (Scheme 1) of 1 called for judicial planning and revealed that the compound 4 was the important key intermediate because of the fact that cyclisation of the amide 4 could be effected with rather ease (Bischler-Napieralski reaction) and moreover, the suitable functional groups X and Y could be transformed into

CHART 1



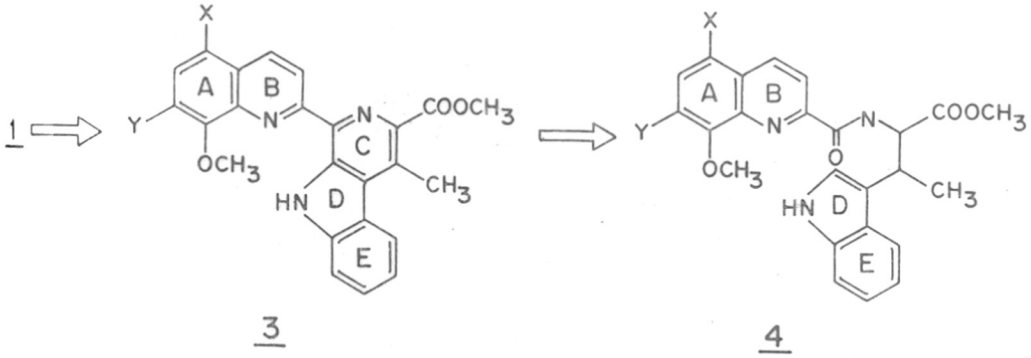
1



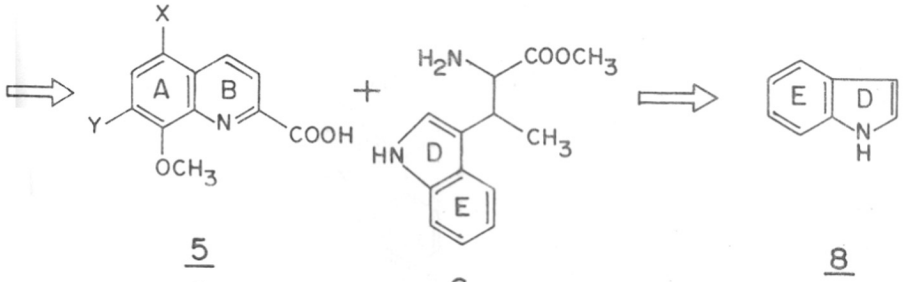
2

SCHEME : 1

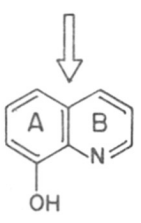
RETROSYNTHESIS



3 4



5 6 8



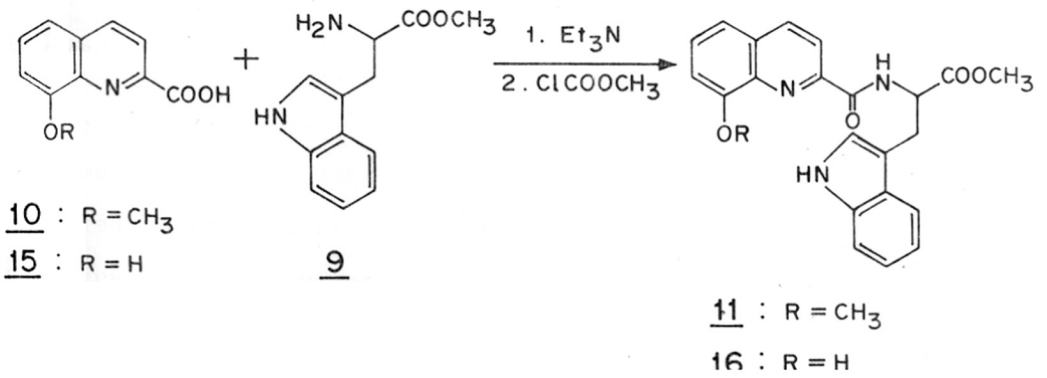
7

amino-quinone skeleton. Such type of cyclisations have often been used during the synthesis of β-carboline derivatives[25]. The intermediate 4 in turn could be produced by simple coupling reaction of quinaldic acid 5 and β-methyltryptophan 6. It was also envisaged that 5 and 6 could be prepared from 8-hydroxyquinoline (7) and indole (8) respectively, the latter two reactants being commercially available.

Thus, it became clear from the above discussion that synthetic strategy would comprise of constructing AB and DE rings first followed by the C ring at the later stages.

In order to test the efficacy of the synthetic planning as described above, it was felt necessary to do some model studies with moderately functionalised intermediates. For these model studies, simple 8-methoxyquinaldic acid (10) (AB rings) and tryptophan 9 (DE rings) were chosen as intermediates (Scheme 2).

SCHEME 2



10 : R = CH_3

15 : R = H

9

11 : R = CH_3

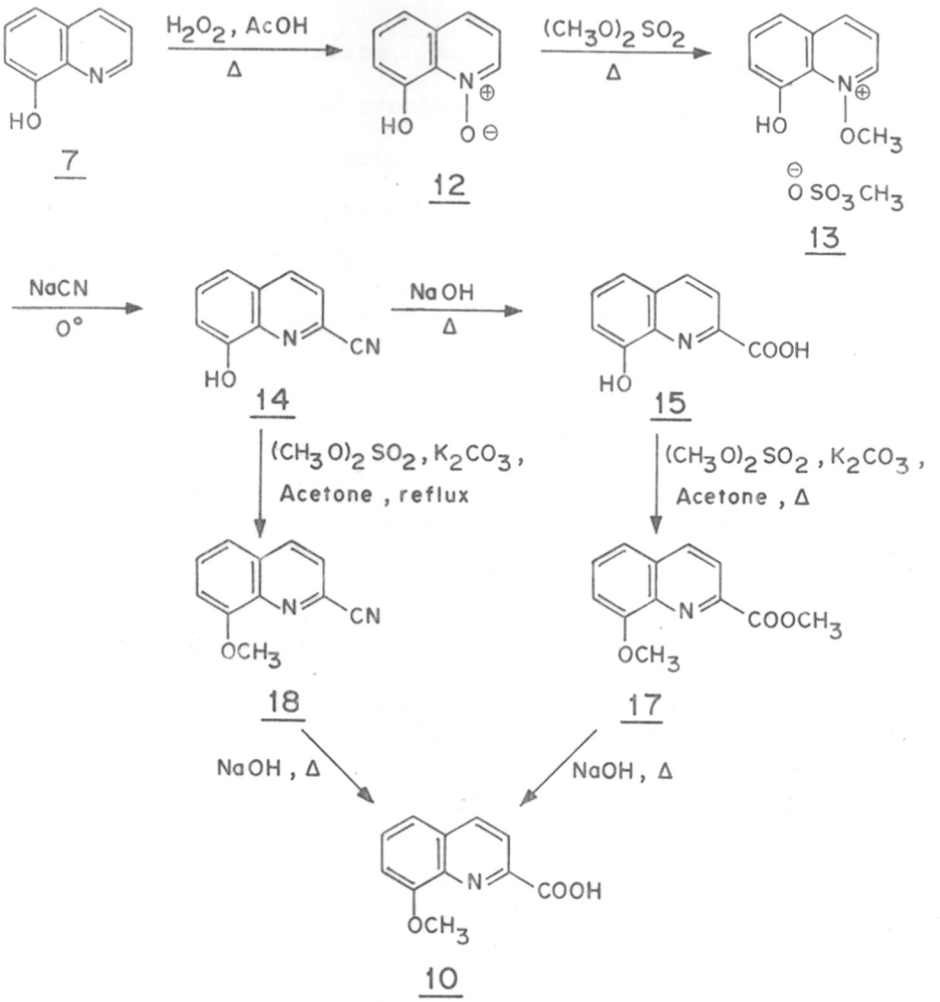
16 : R = H

In order to prepare 8-methoxyquinaldic acid (10), 8-hydroxyquinoline (7) was the obvious choice. In addition to being easily accessible it has two functionalities present viz. OH and C=N which could be capitalised for functionalisation at C-2, C-5 and C-7 positions of the molecule. For example, 8-OH group was particularly suited for introducing substituents at C-5 and C-7 positions while C=N could act as carbonyl equivalent for carrying out nucleophilic substitution at C-2.

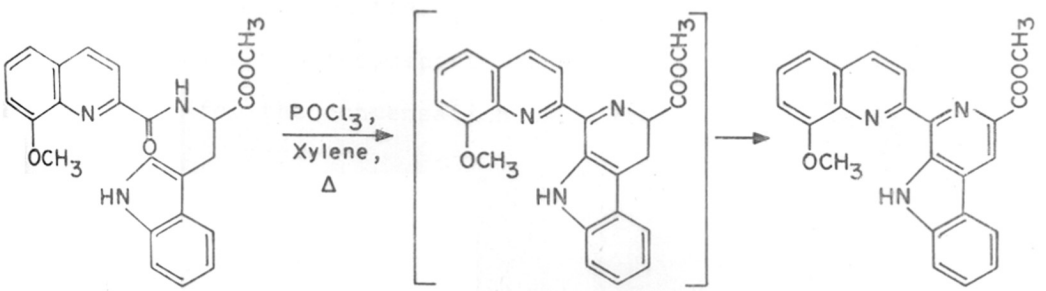
Accordingly (Scheme 3), 8-hydroxyquinoline (7) was converted into the N-oxide 12 (50-70%) by treatment with hydrogenperoxide in hot acetic acid (70-80°) employing a modified literature procedure[35]. Alternately, the same conversion was conducted efficiently by employing m-CPBA as the oxidising agent in multigram quantities. Treatment of the N-oxide 12 with dimethylsulphate at water bath temperature furnished the salt 13 which was directly converted into 8-hydroxyquinaldonitrile[36] (14) by treatment of sodium cyanide in 72% yield. The 8-hydroxyquinaldonitrile (14) was transformed into the 8-hydroxyquinaldic acid 15 by a reported procedure[37] in 72% yield.

Condensation of this hydroxyacid 15 with tryptophan 9 using methylchloroformate as the condensing agent was attempted but failed to yield the desired amide 16. It was therefore, felt to protect the OH group as methyl ether and

SCHEME 3



SCHEME 4



then attempt condensation. Accordingly, the hydroxyacid 15 was converted to 17 by treatment with dimethylsulphate in presence of anhydrous potassium carbonate in boiling acetone. Saponification of the ester 17 with alkali furnished the 8-methoxyquinaldic acid 10. The methoxyacid 10 was also prepared from 2-cyano derivative 14 as follows. Methylation of 14 with dimethylsulphate in the presence of anhydrous potassium carbonate in boiling acetone for 1 hour afforded 8-methoxyquinaldonitrile 18 in 95% yield. The ^{1}H-NMR spectrum of this compound 18 showed a singlet at 4.1 ppm for methoxyl group while rest of the protons appeared at the expected chemical shifts. IR spectrum showed the absorption at 2240 cm^{-1} (C≡N). The absence of absorption due to OH group in the region of 3500 cm^{-1} was also observed. Further confirmation was gleaned from its mass spectrum, where the molecular ion peak appeared at m/z 184. The nitrile 18 was then hydrolysed by refluxing with aqueous sodium hydroxide for 4 hours to afford 8-methoxyquinaldic acid (10) in 60% yield. The properties of this compound were found to be identical with the reported data[38].

Having achieved the synthesis of 8-methoxyquinaldic acid (10) attention was focussed towards establishing suitable conditions for the condensation of tryptophan 9 and quinaldic acid 10.

Tryptophan was esterified to its methyl ester using standard procedure[39] for esterification of aminoacids and was isolated as its hydrochloride.

Although there were several methods available for the condensation of an acid with an amine to form an amide, a few were explored. For example, with DCC as condensing agent, the acid 10 and the amine 9 failed to provide the desired amide 11. In addition, attempts to prepare the acid chloride of quinaldic acid 10 using conventional reagents such as $SOCl_2$, oxalyl chloride met with failure. Therefore, it was decided to make use of mixed anhydride procedure.

Accordingly, the acid 10 was treated with one equivalent of triethylamine and toluene-p-sulphonyl chloride at 0° to furnish a mixed anhydride which was subsequently treated with methyltryptophan hydrochloride. It was gratifying to note that the desired amide 11 was obtained in 50% yield. The ^{1}H-NMR (Fig.1) of the amide 11 indicated a doublet resonating at 3.44 ppm (J = 6.3 Hz) integrating for two protons assigned to $C\underline{H}_2CH$, while the appearance of a multiplet at 4.9-5.2 ppm integrating for one proton was attributed to NH $C\underline{H}$ $COOCH_3$ while two singlets at 3.62 ppm and 3.98 ppm were assigned to two methoxyls. The rest of the protons resonated at the expected chemical shifts. IR spectrum showed the presence of absorption at 3330 cm^{-1} assignable for NH, 1760 cm^{-1} for $COOCH_3$ and 1670 cm^{-1} for CONH, thus clearly indicating the formation of the amide 11. Molecular ion peak in the mass spectrum was seen at m/z 403.

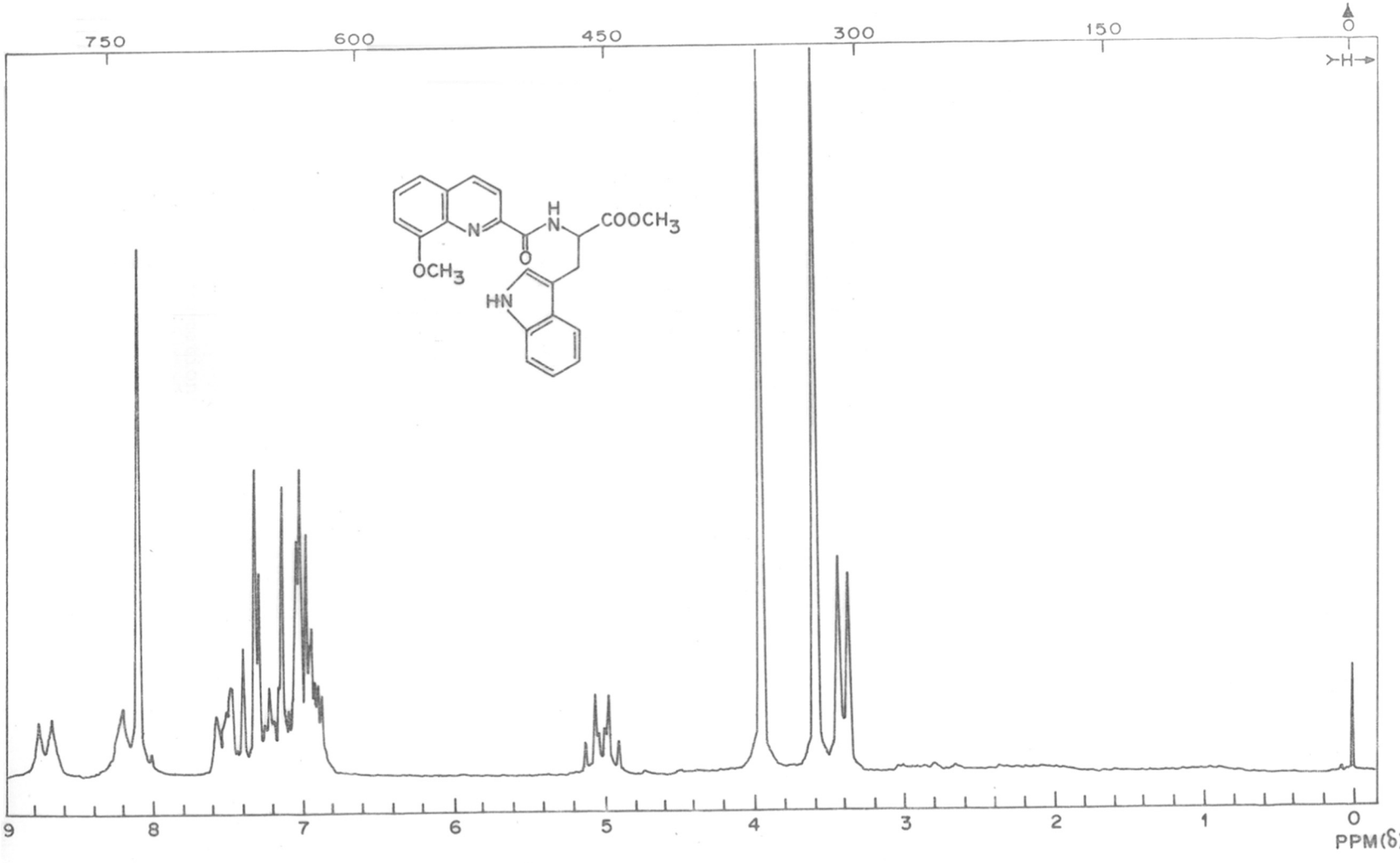


FIG. 1 : ^{1}H-NMR SPECTRUM OF COMPOUND (11) IN $CDCl_3$

The same condensation of 10 with 9 to give 11 was also effected using methylchloroformate as the condensing agent in 60% yield.

In order to effect the cyclisation of the amide 11 to get the required β -carboline system 19 several cyclo-dehydrating reagents were attempted. Cyclisation of 11 with $POCl_3$ in various solvents like benzene, toluene etc. were tried but never looked promising because in some cases the starting material was recovered while in others the conversion was very poor. In another experiment the combination of $POCl_3$-P_2O_5 was also tried but failed to yield the desired product 19.

From the above discussions it was felt that the reaction temperature might not be sufficiently high for the cyclisation to occur. With this hope in mind, the amide 11 was treated with $POCl_3$ in refluxing xylene for 6 hours, the cyclisation smoothly afforded a product which was assigned the structure 20 on the basis of spectral data. The ^{1}H-NMR spectrum (Fig.2) revealed three singlets due to two methoxy groups at 4.00 ppm and 4.10 ppm, the third at 12.3 ppm (D_2O exchangeable) ascribed to NH while rest of the protons appeared in the aromatic region of 7.2 - 7.8 ppm. The IR spectrum showed absence of band for amide, while the absorptions observed at 3340 cm^{-1} (NH) and 1720 cm^{-1} ($COOCH_3$) supported the formation of β -carboline 20. In addition the

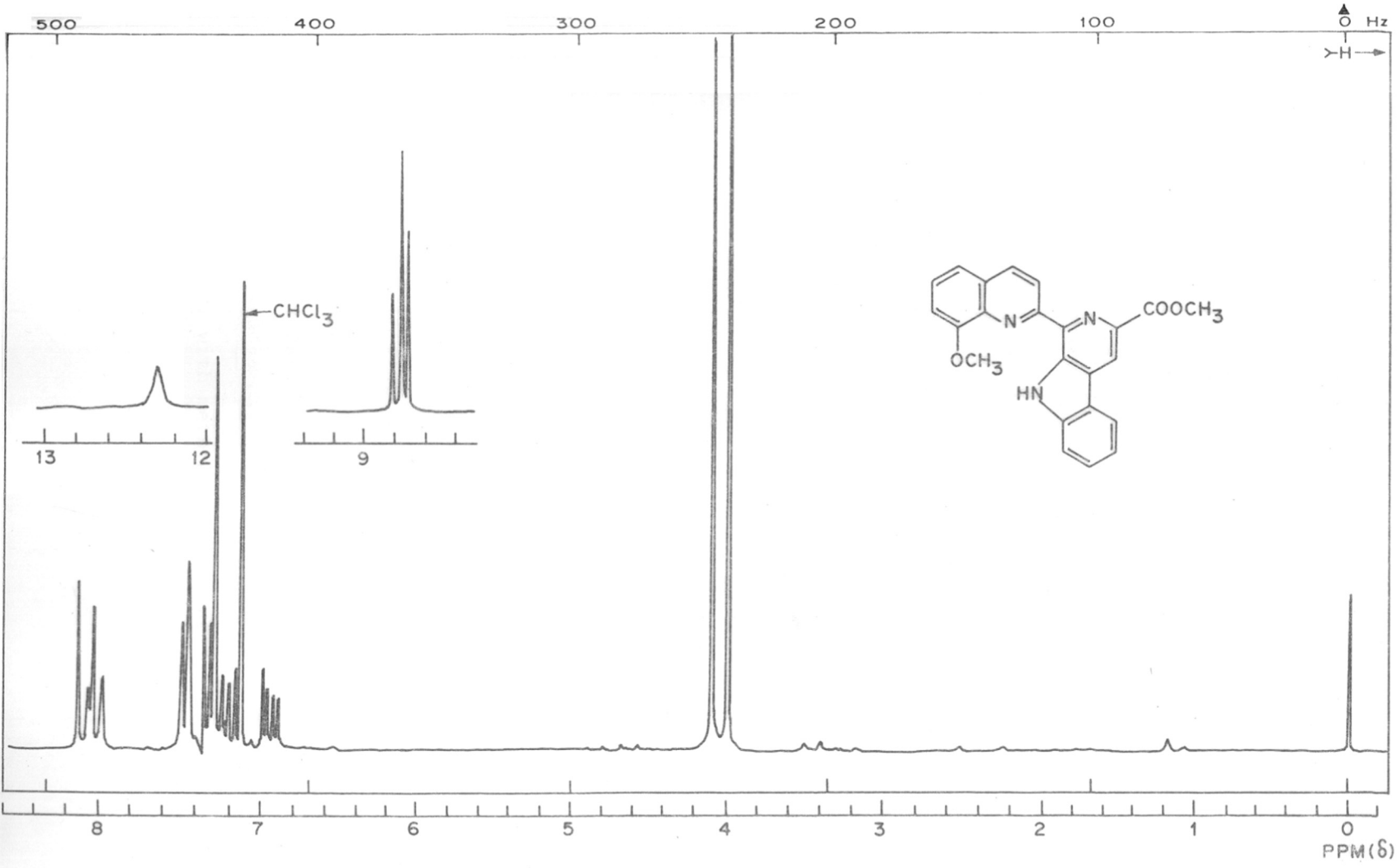


FIG. 2 : ^{1}H-NMR SPECTRUM OF COMPOUND (20) IN $CDCl_3$

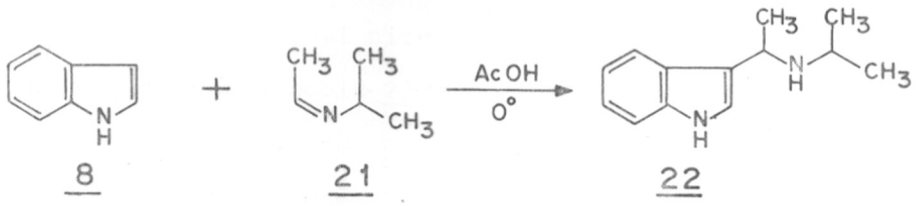
highest mass peak was recorded at m/z 383 which corresponded well with molecular weight of 20. The failure to locate peaks in the region of 3-5 ppm revealed that the expected structure of 3,4-dihydro compound 19 was not formed but probably got aromatised under the reaction conditions.

Encouraged by the success of the model studies as described above, it was thought to repeat the whole sequence but now with β -methyltryptophan 27 and 8-methoxyquinaldic acid (10).

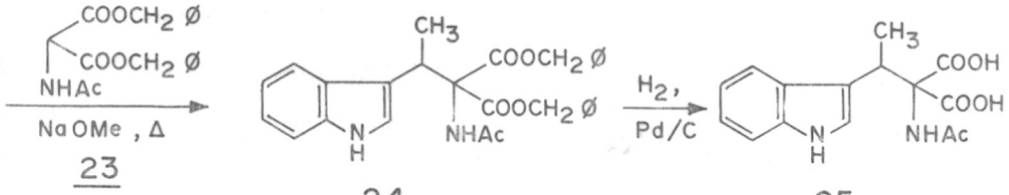
β -methyltryptophan 27 was earlier prepared by Matteson and Snyder[40] from indole (8) and the same procedure was first adopted. Indole (8) was subjected to Mannich reaction with ethylideneisopropylamine (21) (prepared from acetaldehyde and isopropylamine[41]) and the required 3-isopropylamino ethylidene indole (22) was isolated in 72% yield. 22 was then allowed to react with 23 to yield 24. Debenzylation of 24 over 5% Pd-C posed several problems because of its low solubility in various solvents and therefore this route was abandoned.

Erofeev et al.[42] have shown that 3-substituted indoles undergo condensation with nitroacetic ester to yield the corresponding β -substituted-3 indolyl-α nitropropionates in high yields. Since 22 was available (prepared earlier) the condensation of 22 with methylnitroacetate[43] (28) was studied.

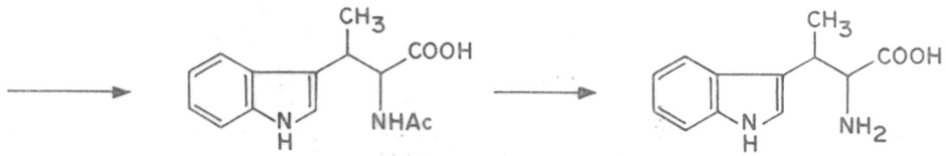
SCHEME 5



8 + 21 → 22

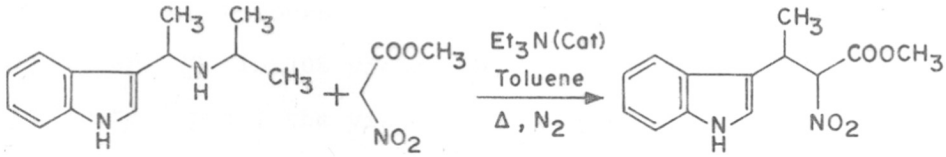


23 → 24 → 25

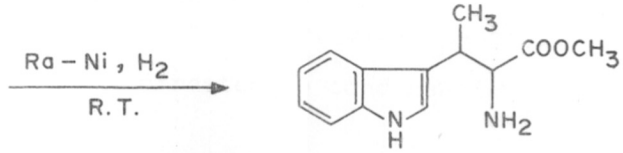


26 → 27

SCHEME 6



22 + 28 → 29



30

Condensation of methyl nitroacetate 28 with 3-(isopropyl amino ethylidene)indole 22 was effected in refluxing dry toluene in the presence of catalytic amount of triethylamine in a stream of nitrogen for 15 hours to give 29 in 85% yield. The ^{1}H-NMR spectrum (Fig.3) of 29 clearly indicated that it was a diastereomeric mixture as the methoxy group revealed two singlets at 3.50 ppm and 3.80 ppm. In addition, a multiplet at 3.90 - 4.30 ppm due to CH $\underline{CH}$ CH_3, a pair of doublets at 5.30 ppm and 5.35 ppm (J= 9.45 Hz) due to NO_2 $\underline{CH}$ $COOCH_3$ (diastereomers) were observed. A broad singlet at 8.00 ppm (D_2O-exchangeable) was assigned to $\underline{NH}$. The IR spectrum showed absorption for NH and $COOCH_3$ at 3370 cm^{-1} and 1750 cm^{-1} respectively. Further confirmation of the assigned structure of 29 was obtained when the molecular ion peak in its mass spectrum was revealed at m/z 262.

Reduction of the nitrogroup in 29 was effected in the presence of Raney-nickel at normal pressure and temperature in ethanol for 24 hours to furnish the β -methyltryptophan methylester (30) in 70% yield again as a diastereomeric mixture (Fig.4 In order to improve the yield of 30 the reduction was tried at 45 psi in the presence of Raney-nickel, however, in this case an intractable mixture of products was formed as judged by TLC.

Having obtained the required β-methyltryptophan (30) the attention was directed to condensation with 8-methoxy-

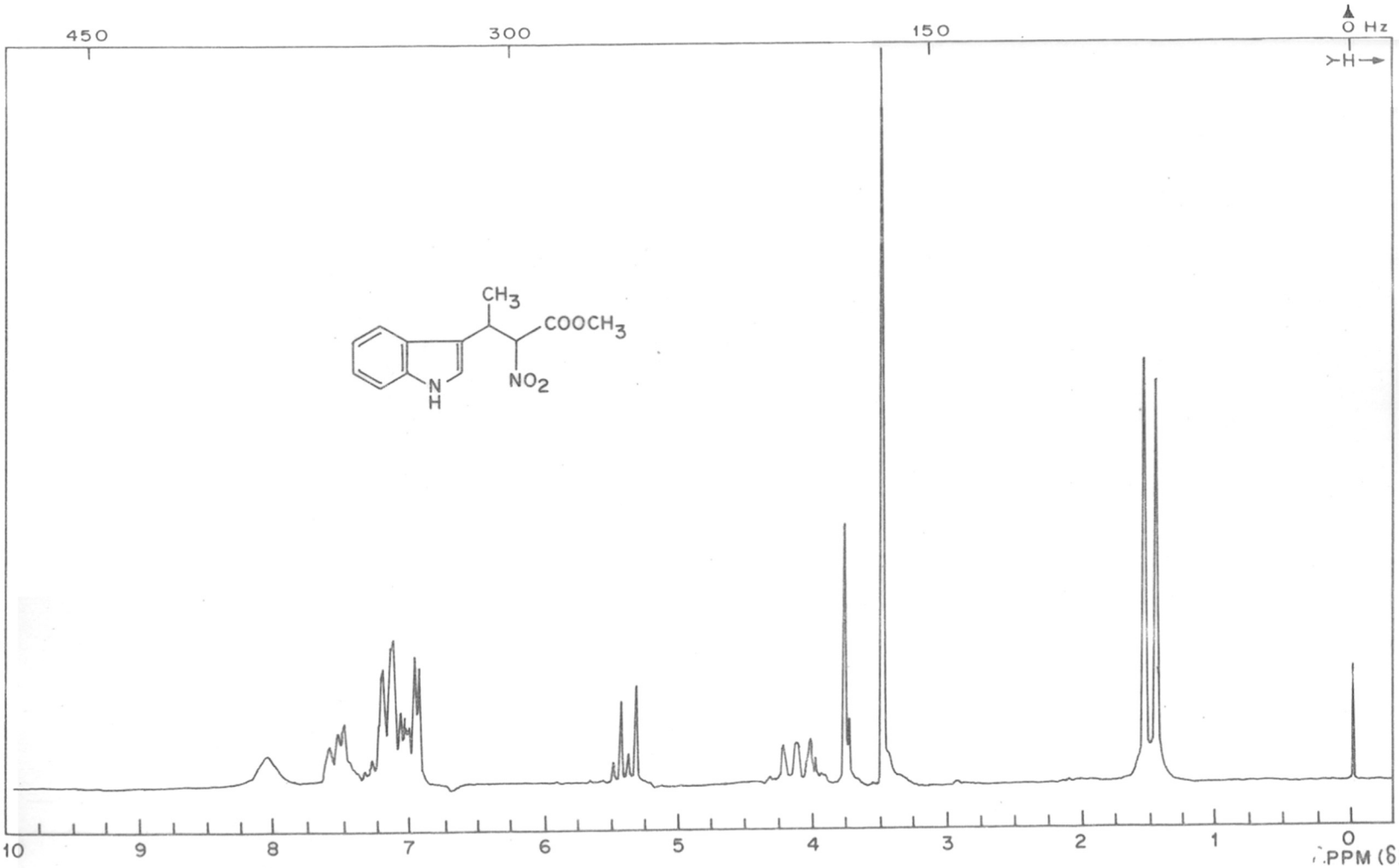


FIG. 3 : ^{1}H-NMR SPECTRUM OF COMPOUND (29) IN $CDCl_3$

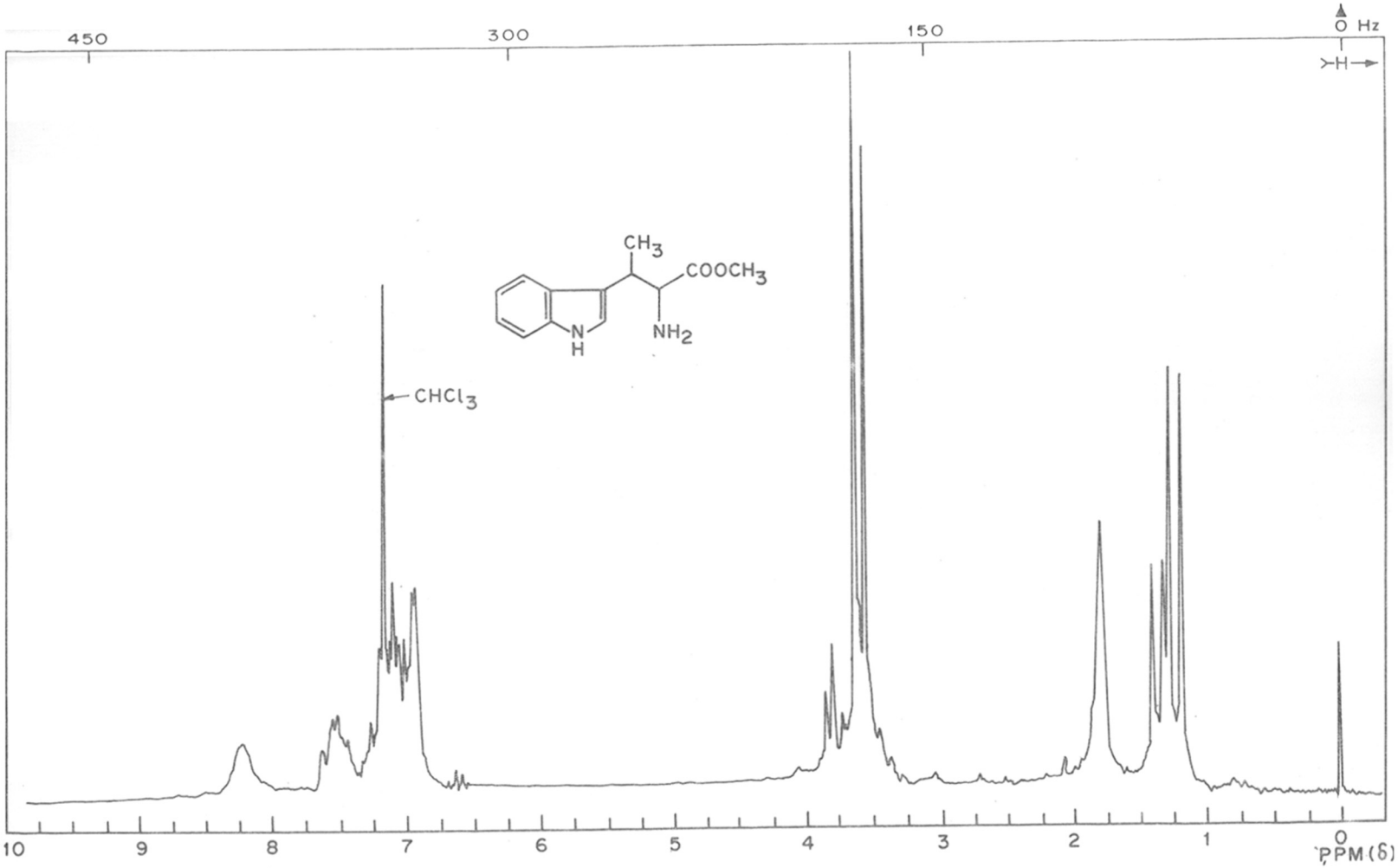


FIG. 4 : ^{1}H-NMR SPECTRUM OF COMPOUND (30) IN $CDCl_3$

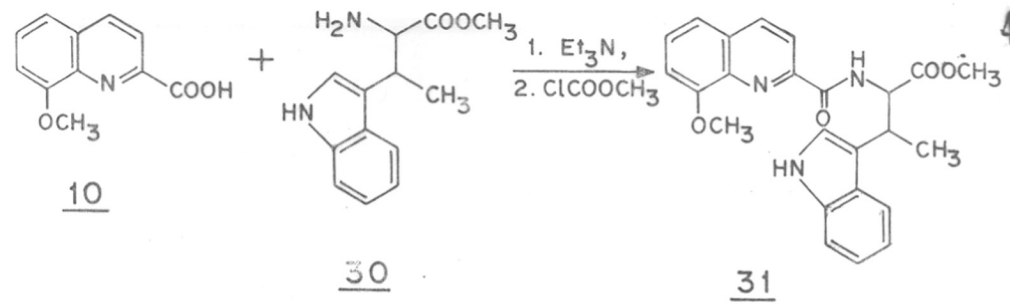
quinaldic acid and the cyclisation of the resulting product to afford β-carboline skeleton.

Accordingly (Scheme 7), 8-methoxyquinaldic acid (10) was treated with triethylamine followed by methylchloroformate at 0° in THF and to the resultant mixed anhydride was subsequently added β-methyltryptophan methyl ester (30) at 0°. After 4 hours, the desired amide 31 was obtained in 84% yield. The ^{1}H-NMR spectrum (Fig.5) of the compound 31 showed it to be a mixture of diastereomers. For instance, presence of a doublet at 1.55 (J= 6.3 Hz) was assigned to CH $C\underline{H}_3$, two singlets lay centered at 3.50 ppm and 4.05 ppm assigned to two methoxyls. Two multiplets appearing in the regions 3.6 - 4.0 ppm and 4.9 - 5.2 ppm were due to CH $C\underline{H}$ CH_3 and NH $C\underline{H}$ $COOCH_3$ respectively. Rest of the protons resonated at the expected chemical shifts. IR spectrum showed the absorption at 3690 - 3710 cm^{-1} for NH while bands at 1750 cm^{-1} and 1680 cm^{-1} were attributed to ester and amide carbonyls respectively.

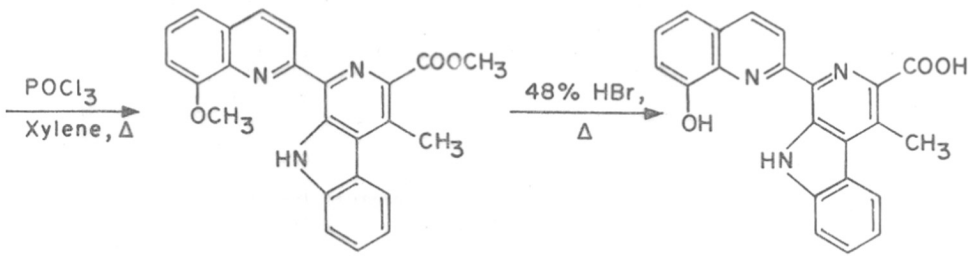
It is pertinent to mention here that since the final aim was to cyclise the amide 31 to β-carboline 32 and subsequently aromatise it thereby destroying the chiral centres in the molecule, no attempt was made whatsoever to separate the diastereomers here as well as in other cases.

The amide 31 on treatment with $POCl_3$ in refluxing xylene for 4 hours underwent smooth cyclisation with concomitant

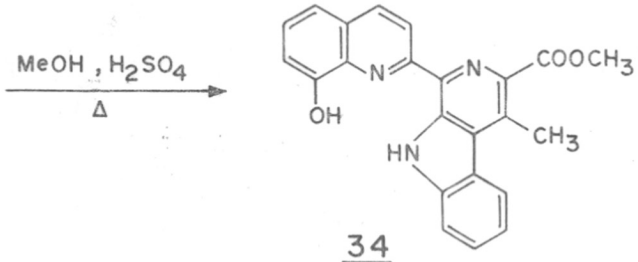
SCHEME 7



10 + 30 → 31



32 → 33



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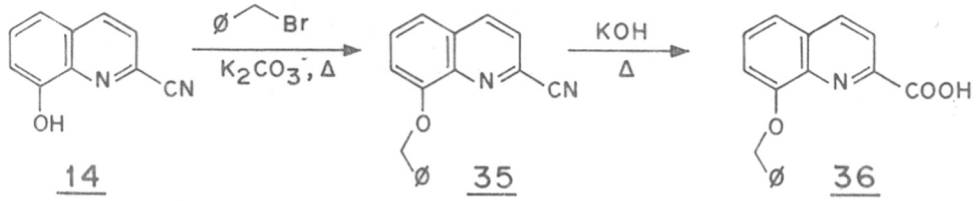
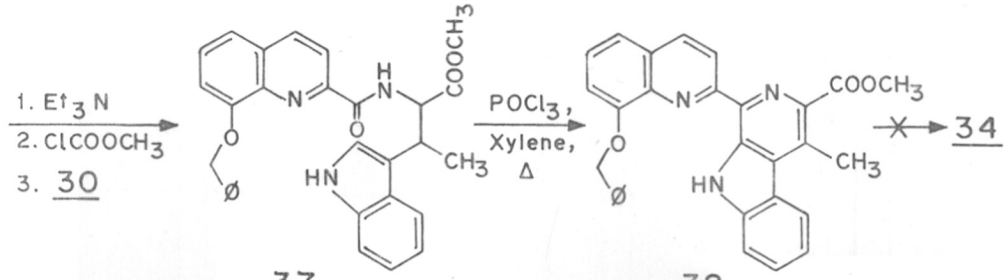
SCHEME 8



14 → 35 → 36



37 → 38

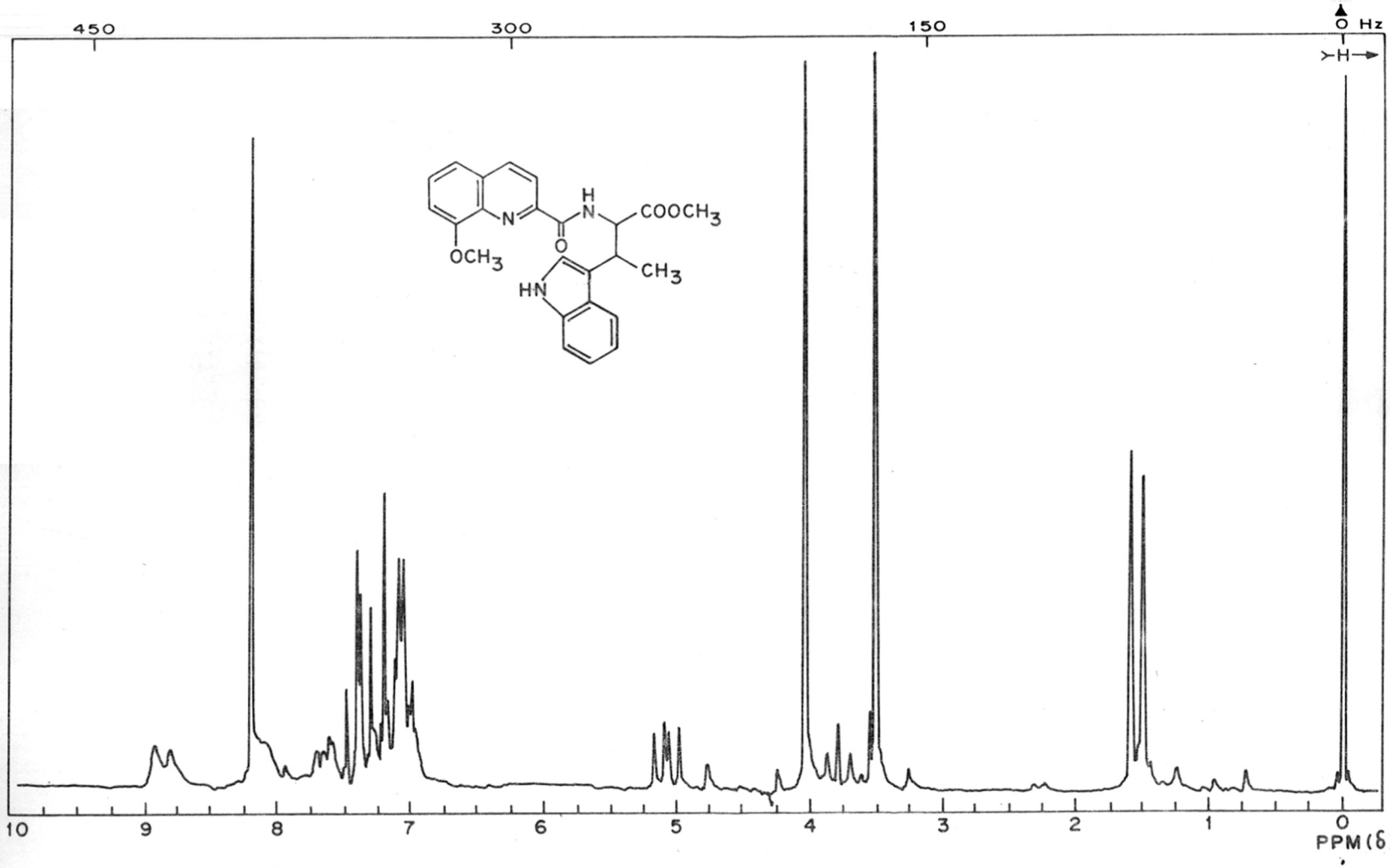


FIG. 5 : ^{1}H-NMR SPECTRUM OF COMPOUND (31) IN $CDCl_3$

aromatisation because the methyl was clearly observed(Fig.6) as a singlet at 3.20 ppm. The downfield shift of the methyl group clearly indicated that it was linked to an aromatic ring. The chemical shift of methyl at 3.20 ppm was also characteristic of β-carboline derivatives. In addition, aromatic protons appeared as multiplets in the region 7.0 - 8.9 ppm while a broad singlet at 12.60 ppm (D_2O exchangeable) was attributed to NH.

From the above experiments it was evident that the synthetic planning which had been devised for lavendamycin worked very well with moderately functionalised precursors, such as 8-methoxyquinaldic acid, tryptophan or β-methyl-tryptophan. Although the β-carboline 32 formed by the condensation and cyclisation of 8-methoxyquinaldic acid and β-methyltryptophan was initially considered as model studies, in theory, it would be possible to elaborate it to the target molecule i.e. lavendamycin by proper functionalisation of ring A. In this regard, the advantage of having OH group at C-8' of quinoline moiety could be exploited. Therefore, attempts were directed to functionalise ring A of the β-carboline 32. In order to achieve this, hydroxyester 34 was required which was obtained as follows.

The demethylation of the β-carboline 32 using EtSH-$AlCl_3$[44], BF_3 Et_2O-EtSH[45] etc. met with failure. In

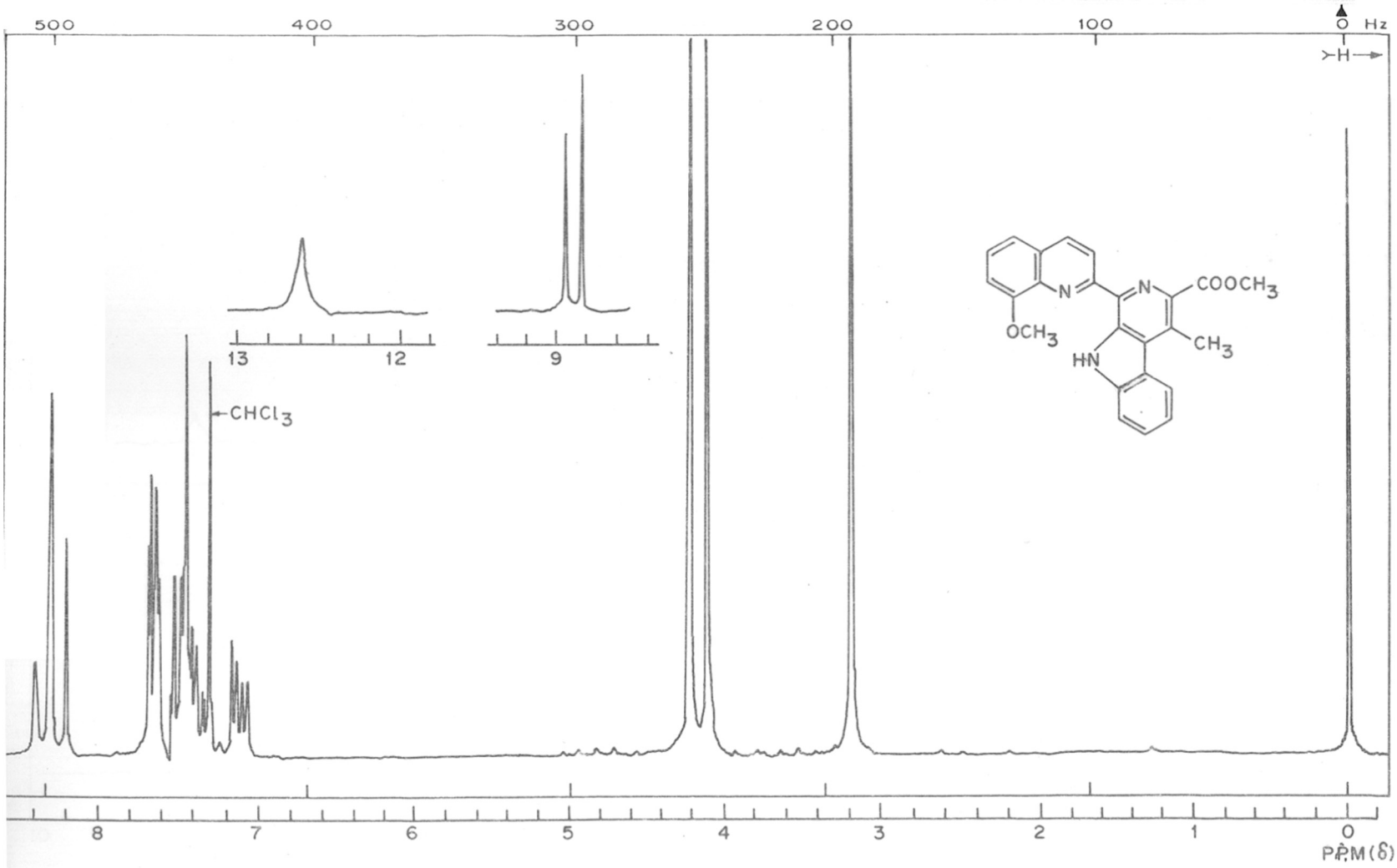


FIG. 6 : ^{1}H-NMR SPECTRUM OF COMPOUND (32) IN $CDCl_3$

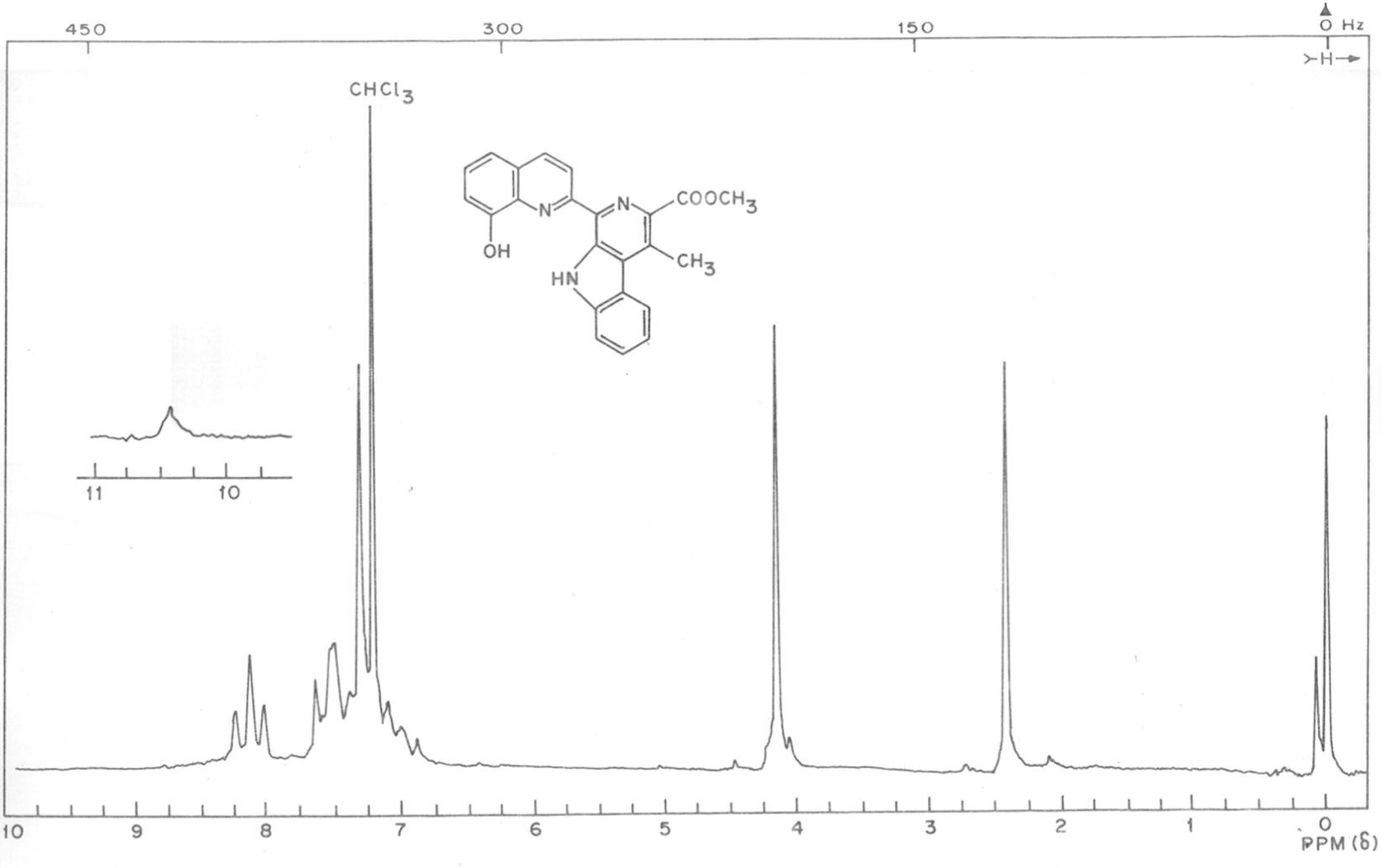


FIG. 7 : ^{1}H-NMR SPECTRUM OF COMPOUND (34) IN $CDCl_3$

order to find a suitable demethylating agent, studies on 8-methoxyquinoline as a model compound were first explored. It was interesting to note that 8-methoxyquinoline with 48% aq.HBr solution under reflux for 2 hours yielded 8-hydroxyquinoline in almost quantitative yield. Encouraged by the above findings the β-carboline 32 was subjected to the same treatment with 48% aq. HBr in an atmosphere of nitrogen. After 3 hours, the required hydroxyacid 33 was isolated. The insolubility of the hydroxyacid 33 in common solvents like chloroform, acetone etc. rendered its characterisation and purification difficult. Hence, the crude hydroxy acid 33 was esterified with methanolic sulphuric acid to furnish 85% of the hydroxy ester 34 (based on the recovery of acid). The ^{1}H-NMR spectrum (Fig.7) of 34 indicated the presence of one singlet at 2.45 ppm due to $\underline{CH}_3$ and a singlet at 4.10 ppm for methoxyl of ester function. The presence of a broad singlet at 10.1 ppm (D_2O exchangeable) was assigned to N$\underline{H}$. Further evidence to confirm this structure came from mass spectrum which revealed the molecular ion peak at m/z 383.

It was later felt that the hydroxyester 34 could be obtained with more ease if a benzyloxy substituent was present at C-8 of quinoline moiety because simple hydrogenolysis would furnish the desired product 34. Therefore 8-benzyloxyquinaldonitrile (35) was prepared as follows (Scheme 8). 8-Hydroxyquinaldonitrile 14 was heated with benzylbromide with anhydrous

potassium carbonate in refluxing acetone for 2 hours to afford 35 in 89% yield. In the ^{1}H-NMR spectrum of compound 35 benzyl protons resonated as singlet at 5.40 ppm while the aromatic protons resonated in the region 7 - 8.2 ppm. In the IR spectrum, the presence of C≡N was observed at 2220 cm^{-1} while absence of a band for OH was clearly seen which supported the structure. The mass spectrum revealed a peak at m/z 260 for its molecular ion confirming the proposed structure.

The nitrile 35 was hydrolysed by refluxing with 10% aqueous potassium hydroxide for 6 hours to the corresponding acid 36 in 88% yield. In the ^{1}H-NMR spectrum (Fig.8) of 36 the OCH_2Ph resonated at 5.20 ppm as a singlet, whereas a broad singlet at 6.65 ppm (D_2O exchangeable) was assigned to COOH. The two doublets (J= 9.5 Hz) due to H-3 and H-4 protons appeared at 8.20 ppm and 8.35 ppm while rest of the aromatic protons appeared as multiplets in the region 6.9 - 7.6 ppm. The presence of acid group was indicated by IR spectral bands at 3140 cm^{-1} and 1720 cm^{-1}. The further confirmation came from mass spectrum which revealed a peak at 279.

The acid 36 was treated with one equivalent of triethylamine followed by methylchloroformate at 0°, the resultant mixed anhydride on treatment with β-methyltryptophan 30 followed by usual work up and purification furnished the amide 37 in 80% yield. In the ^{1}H-NMR spectrum (Fig.9) of 37

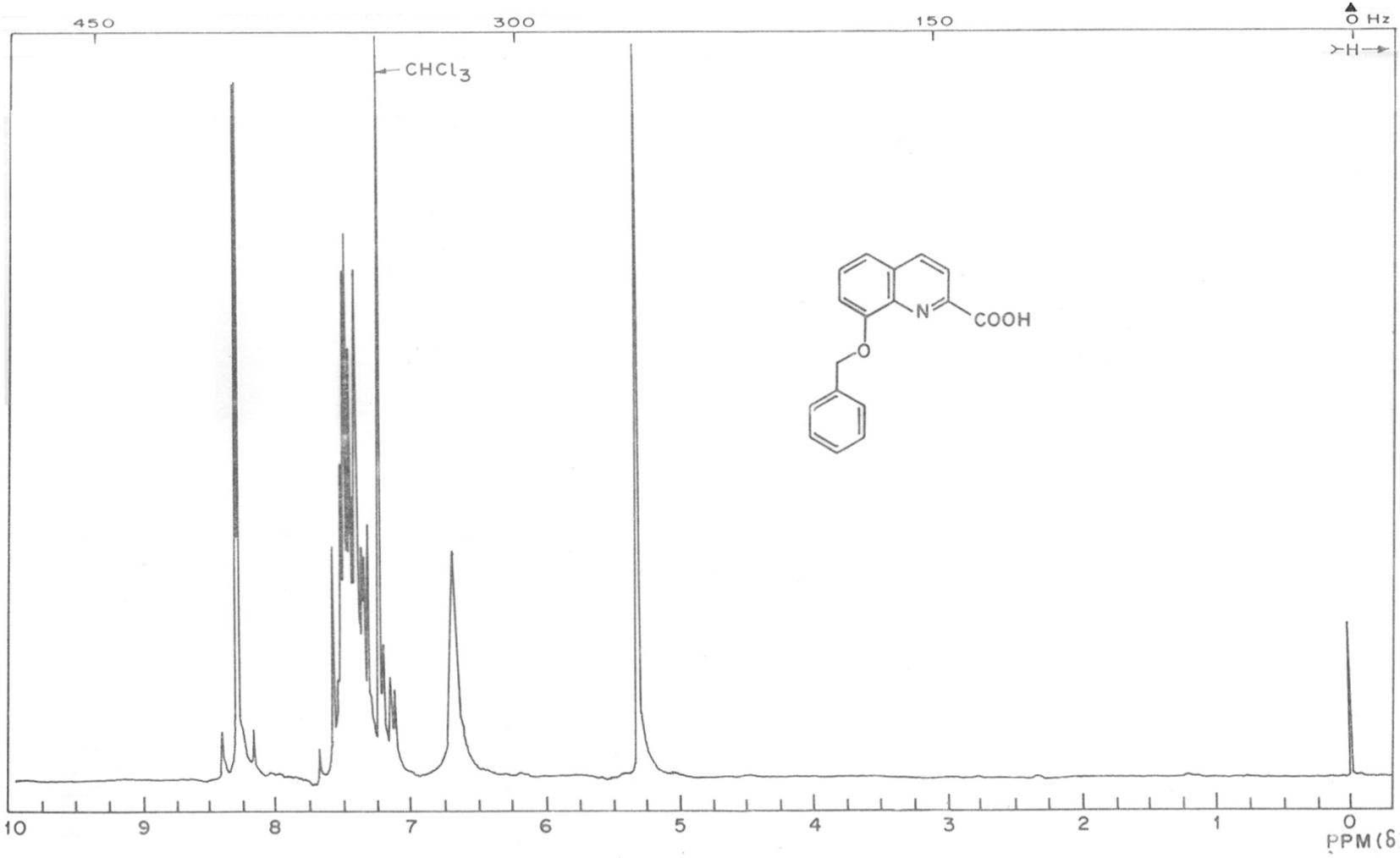


FIG. 8 : ^{1}H-NMR SPECTRUM OF COMPOUND (36) IN $CDCl_3$

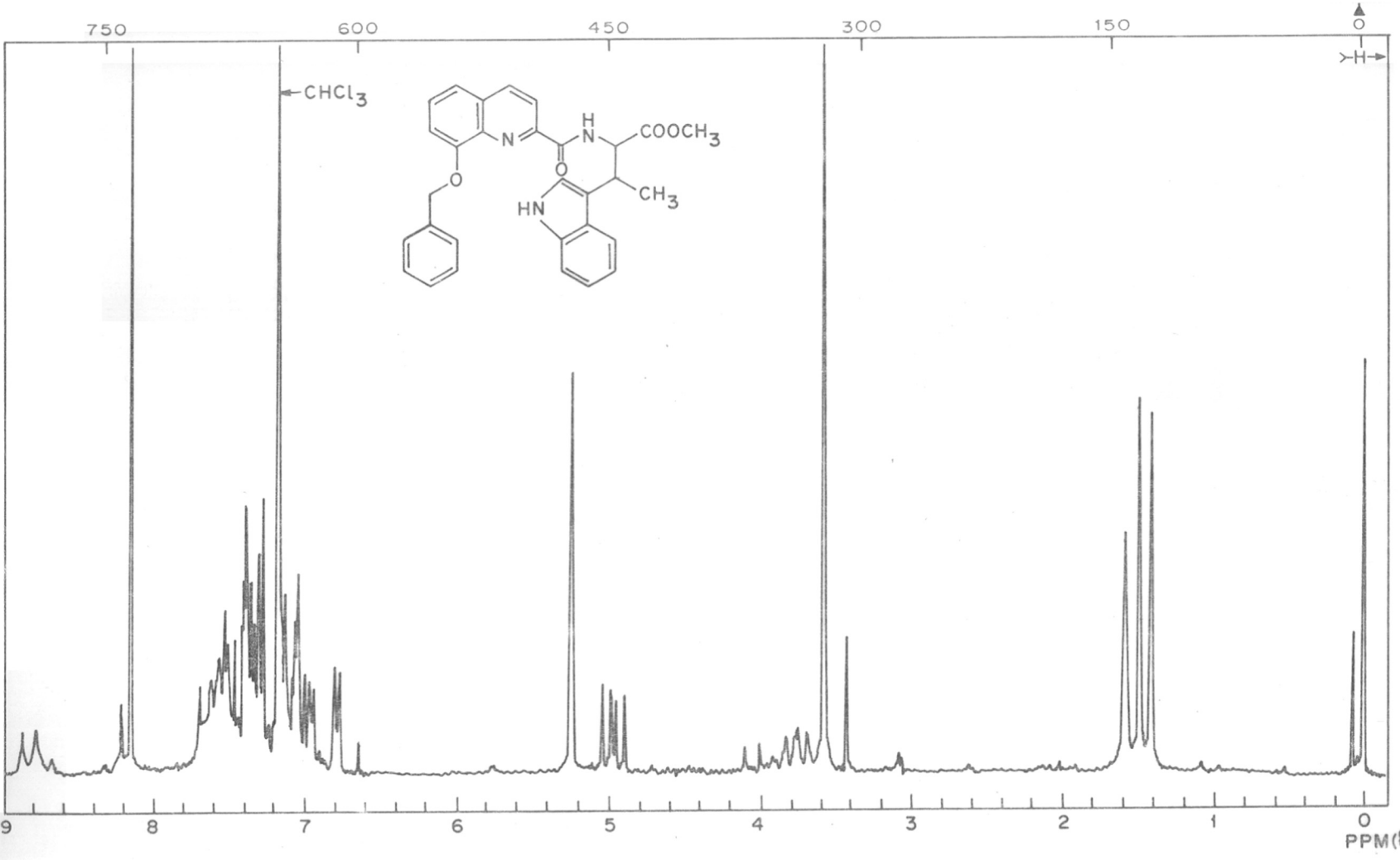


FIG. 9 : ^{1}H-NMR SPECTRUM OF COMPOUND (37) IN $CDCl_3$

the CH $C\underline{H}_3$ group resonated as triplet at 1.49 ppm (J = 8 Hz) (diastereomeric mixture), the methoxyl groups as two singlets at 3.44 ppm and 3.60 ppm (diastereomers), while the protons $C\underline{H}$ CH_3 and NH $C\underline{H}$ $COOCH_3$ appeared as multiplets at 3.7 ppm and 5.0 ppm respectively. The presence of a singlet at 5.27 ppm was attributed to $OC\underline{H}_2Ph$. The rest of the protons resonated at the expected chemical shifts. IR spectrum showed absorptions at 3360 cm^{-1}, 1740 cm^{-1} and 1680 cm^{-1} which could be attributed to $N\underline{H}$, ester carbonyl and amide carbonyl functionalities respectively. Further evidence in support of the amide 37 came from mass spectrum which revealed a peak at m/z 493.

The amide 37 obtained above was subjected to cyclodehydration reaction with $POCl_3$ in refluxing xylene for 4 hours to furnish the β-carboline 38. In the ^{1}H-NMR spectrum (Fig.10) of compound 38, the presence of two singlets at 3.20 ppm and 4.10 ppm were attributed to aromatic $C\underline{H}_3$ and $OC\underline{H}_3$ whereas, benzylic protons resonated as a singlet at 5.32 ppm. One of the aromatic protons was located at 6.27 ppm as multiplets, while rest of the aromatic protons appeared as multiplets beyond 7 ppm. The upfield shift of one of the aromatic protons was abnormal. The presence of a broad singlet at 12.43 ppm was assigned to $N\underline{H}$. The bands at 3260 cm^{-1} and 1700 cm^{-1} in IR spectrum were assigned for $N\underline{H}$ and ester carbonyl respectively.

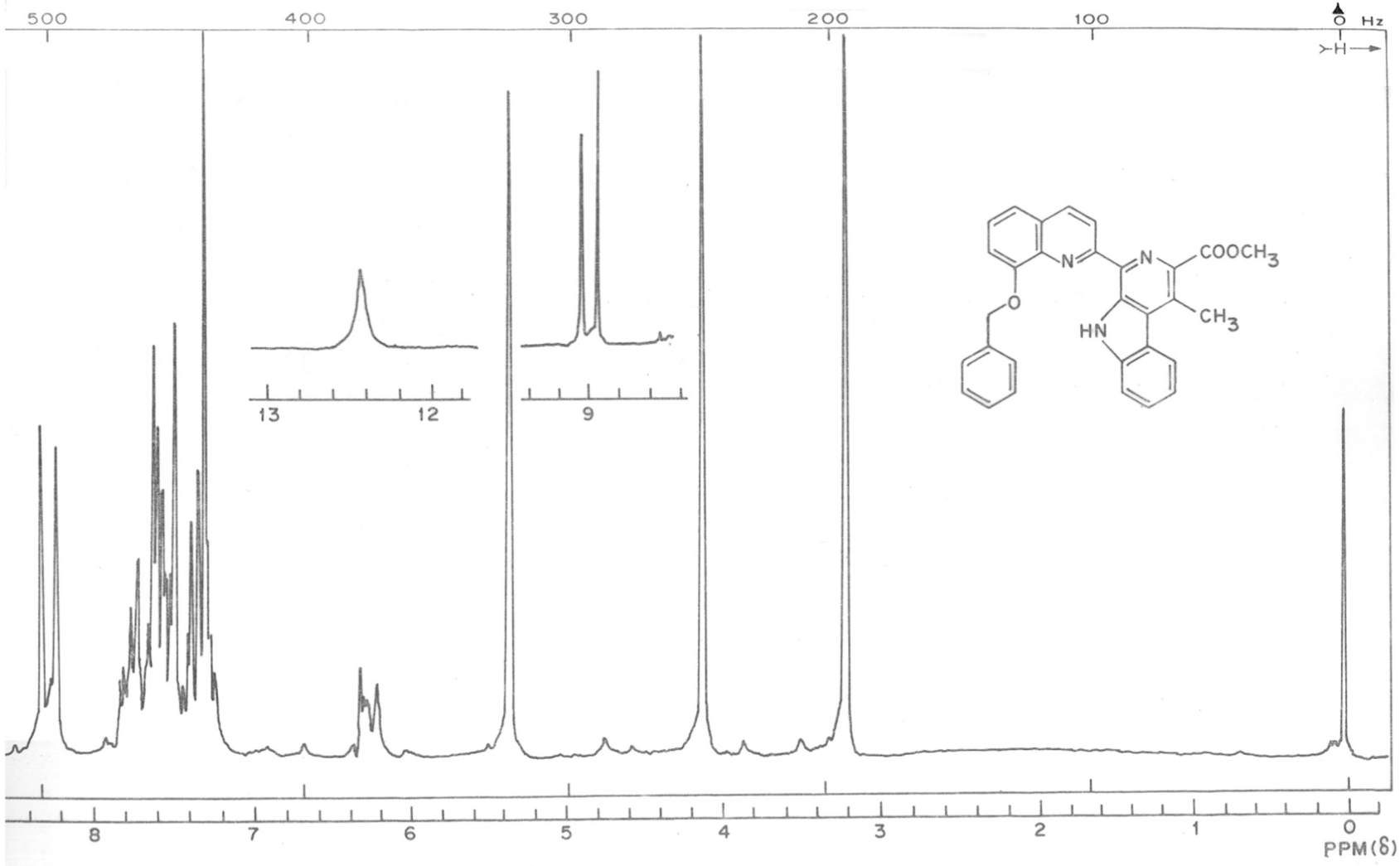


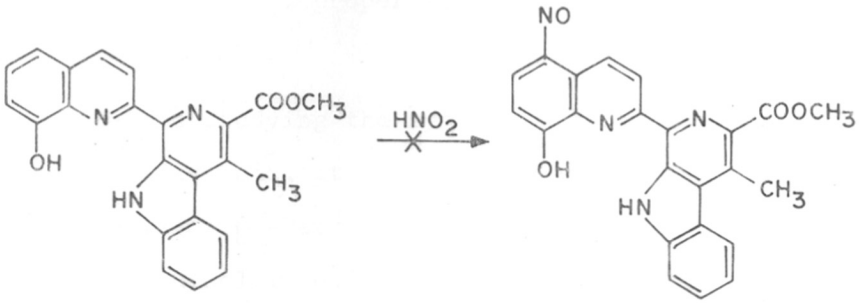
FIG. 10 : ^{1}H-NMR SPECTRUM OF COMPOUND (38) IN $CDCl_3$

The above cyclisation step provided the β-carboline, whose yields, ranging from 20-50% were not consistent. In addition to this, hydrogenolysis of the o-benzyl group in 38 over 5% Pd/C invoked some problems and the reaction was found to be critical of the activity of the catalyst used. Therefore, hydroxyester 34 in the previous route from methoxyester carboline 32 was resorted to.

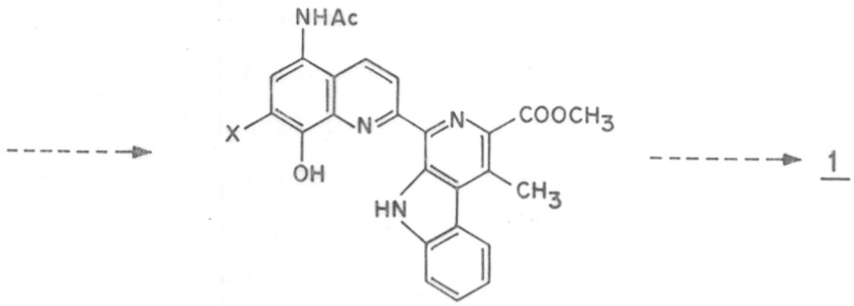
In order to functionalise ring A of lavendamycin, it was envisaged that following sequence of reactions had to be performed. Initially the 5'-position of the quinoline moiety of β-carboline 34 could be substituted with acetamido group via nitrosation followed by introduction of the bromide group at C-7' (ortho to OH group). Replacement of 7'-bromo with amino via oxidation to paraquinone would then furnish lavendamycin[46].

It has been reported that nitrosation reaction of phenols with nitrous acid generally leads to the formation of para-nitrosophenols[47]. Similar reaction was attempted with 8'-hydroxy β-carboline 34 but unfortunately failed to yield the desired product as TLC indicated that most of the starting material remained unreacted. Since the scheme planned above was critical of this reaction which met with failure after several attempts, the route was, therefore, abandoned. From these studies, it became clear that before venturing in the

SCHEME 9



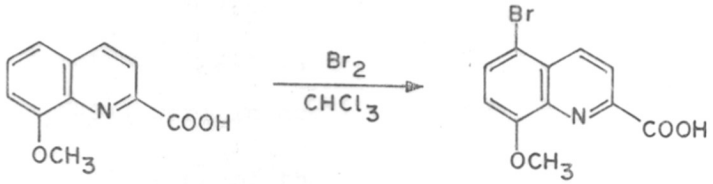
34 39



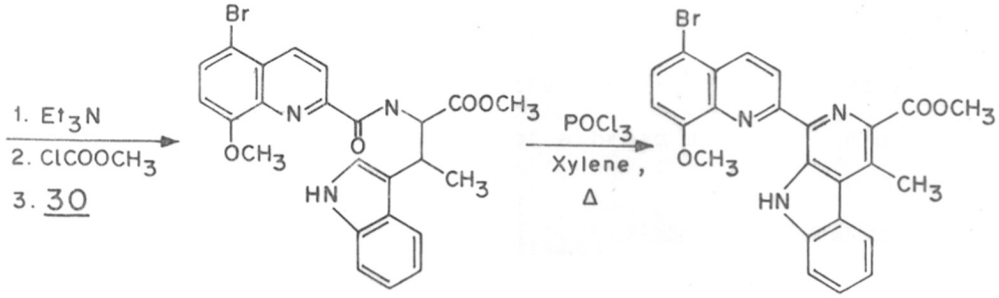
40 : X = H

41 : X = Br

SCHEME 10



10 42



43 44

total synthesis of 1, proper functionalisation of quinoline part was essential.

Before studying the synthesis with properly substituted quinaldic acid, it was thought worthwhile to attempt the synthesis of β -carboline derivative with moderately substituted quinaldic acid. The choice of 5-bromo-8-methoxyquinaldic acid 42 was made because of the ease with which it could be synthesized starting from already available 8-methoxyquinaldic acid. Thus, 42 was prepared from 8-methoxyquinaldic acid 10 by treatment with bromine in chloroform in 63% yield. The structure of the product was demonstrated by the ^{1}H-NMR spectrum where two AB quartets in the aromatic region for H-3/H-4 and H-6/H-7 protons were observed.

Condensation of the bromo acid 42 with β-methyl tryptophan 30 employing methylchloroformate at 0° then furnished the corresponding amide 43, in 95% yield. The ^{1}H-NMR spectrum (Fig.11), IR spectrum and the mass spectrum (m/z 495, 497) were in accordance with the proposed structure.

Cyclisation of the amide 43 was smoothly effected with $POCl_3$ in refluxing xylene to the desired β -carboline 44 in 31% yield whose ^{1}H-NMR spectrum (Fig.12) revealed three singlets at 3.12 ppm, 4.07 ppm and 4.15 ppm for C-CH_3 and two methoxy groups, while rest of the aromatic protons resonated beyond 6.8 ppm. The presence of a broad singlet characteristic

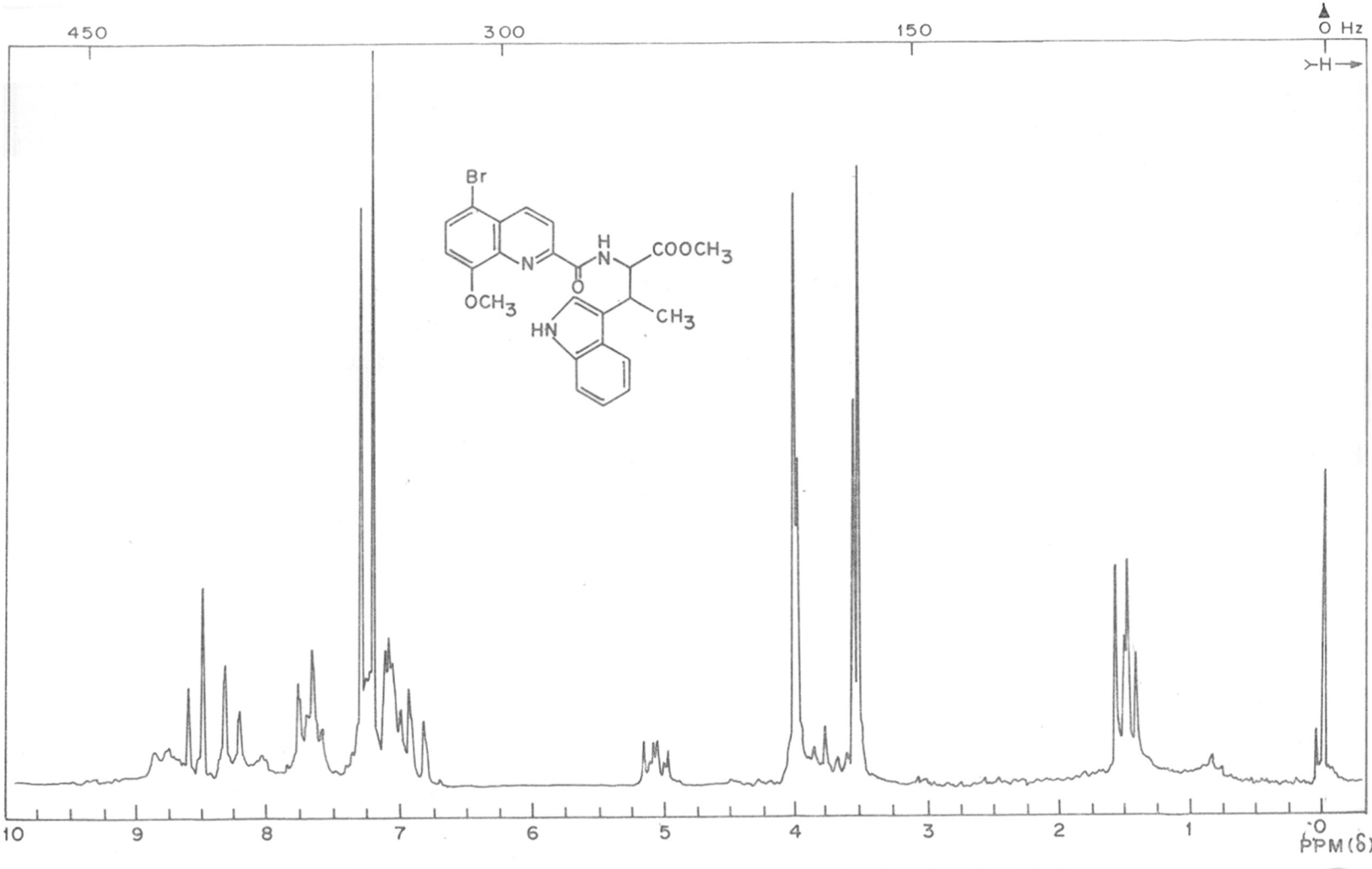


FIG. 11 : ^{1}H-NMR SPECTRUM OF COMPOUND (43) IN $CDCl_3$

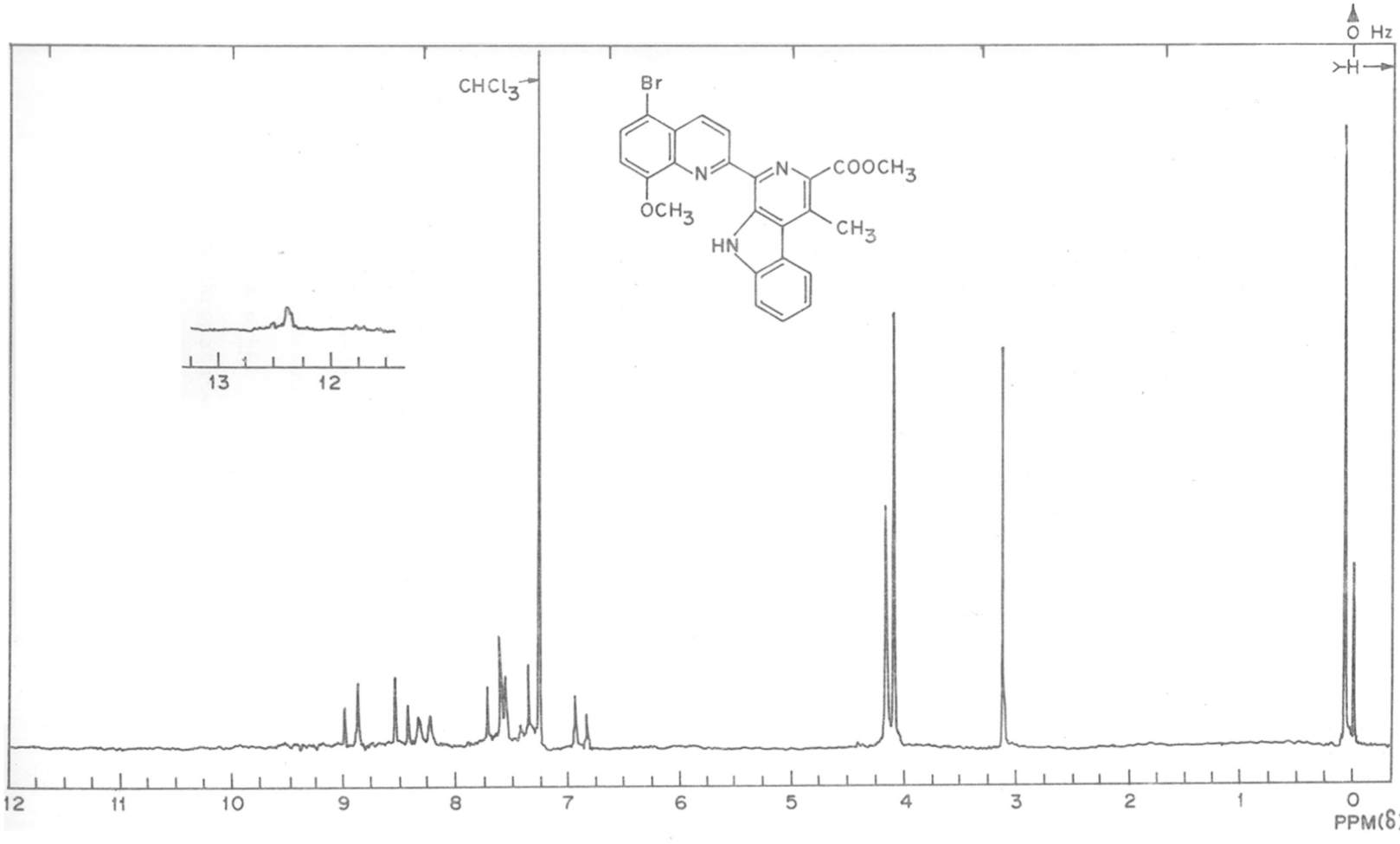


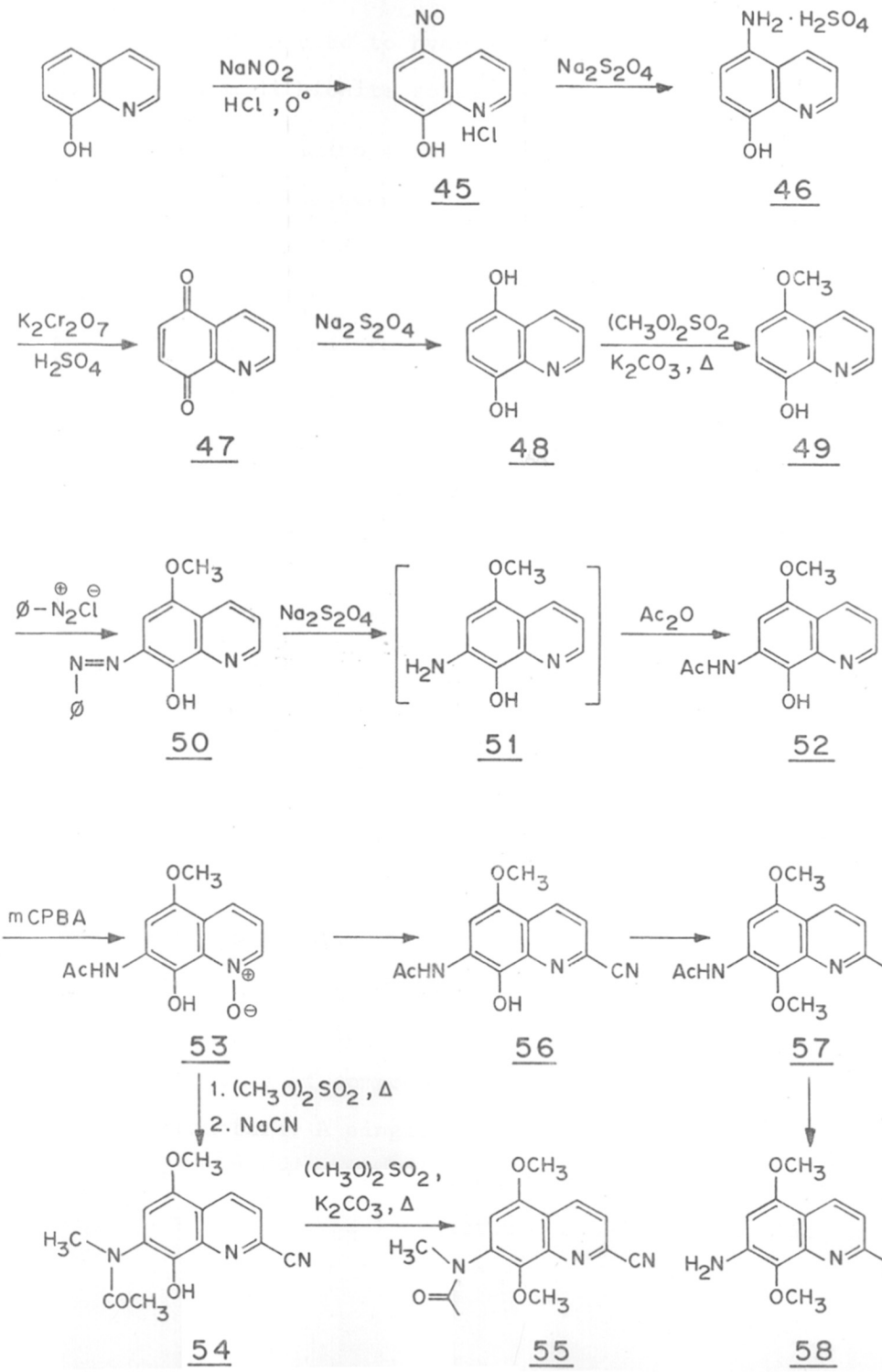
FIG. 12 : ^{1}H-NMR SPECTRUM OF COMPOUND (44) IN $CDCl_3$

of β -carboline at 12.3 ppm was attributed to NH. The IR spectrum of 44 revealed the bands for NH and ester carbonyl. The further confirmation for the structure came from mass spectrum which revealed peaks at 475 and 477.

Encouraged by the above results with 5-bromo derivative, efforts were directed to the synthesis of properly substituted quinaldic acid. The most logical method of doing this job was to introduce actual or potential substituents so that at the later stages the potential substituents could be elaborated to the required substituents.

The route as depicted in the scheme 11 was envisaged which made use of 8-hydroxyquinoline as the precursor for suitably substituted quinaldic acid. The most obvious choice was to make 5,8-dimethoxy-7-aminoquinaldic acid (58) because dimethoxy groups at the end of the sequence could be oxidised to p -quinone with ease under mild conditions. Accordingly, 8-hydroxyquinoline (7) was converted to 5,8-dihydroxy-quinoline (48) by reported procedure[48]. For e.g. 8-hydroxy-quinoline was subjected to nitrosation with sodium nitrite in hydrochloric acid to yield 87%, the required 5-nitroso-8-hydroxyquinoline hydrochloride (45). This in turn was reduced with aqueous sodium dithionite and isolated as the 5-aminosulphate 46. Two-phase oxidation of 46 with 2N potassium dichromate and 12N aq. H_2SO_4 in chloroform afforded the 5,8-quinoline quinone 47 in 54% yield. The

SCHEME 11


NaNO2
HCl , 0°
Na2S2O4
NO
NH2 · H2SO4
OH
HCl
45
46
K2Cr2O7
H2SO4
Na2S2O4
(CH3O)2SO2
K2CO3, Δ
OCH3
47
48
49
Ø–N2Cl
N=N
Ø
50
51
Ac2O
AcHN
H2N
52
mCPBA
53
56
57
1. (CH3O)2SO2, Δ
2. NaCN
CN
COCH3
54
55
58
COOH

quinone 47 was reduced to hydroquinone 48 by shaking with aqueous sodium dithionite solution in 90% yield.

Since the amino group had to be introduced at C-7 at this stage of the sequence through either diazonium salts, or nitrous acid, or nitration, 5-OH was required to be selectively protected. This was because of the fact that phenols undergo these reactions at the ortho position where the para position is blocked[49]. Had the 5,8-dihydroxyquinoline been used, a mixture of substituted (mono and di) product would have been anticipated.

It was anticipated that selective methylation of 5,8-dihydroxyquinoline (48) with one equivalent of dimethylsulphate would lead to the formation of 5-methoxy derivative 49 because of the diminished reactivity of 8-OH group which is involved in chelation with ring nitrogen. Indeed, when 5,8-dihydroxyquinoline was heated under reflux with one equivalent of dimethylsulphate and potassium carbonate in refluxing acetone followed by purification by column chromatography, 5-methoxy-8-hydroxyquinoline (49) was isolated in 48% yield. This compound showed +ve $FeCl_3$ test supporting the finding that 8-OH was free. ^{1}H-NMR spectrum (Fig.13) of compound 49 also indicated the assigned structure. A singlet at 4.13 ppm was located far OCH_3. The aromatic protons appeared in the region of 7.1 ppm - 8.23 ppm. The IR spectrum showed an absorption at

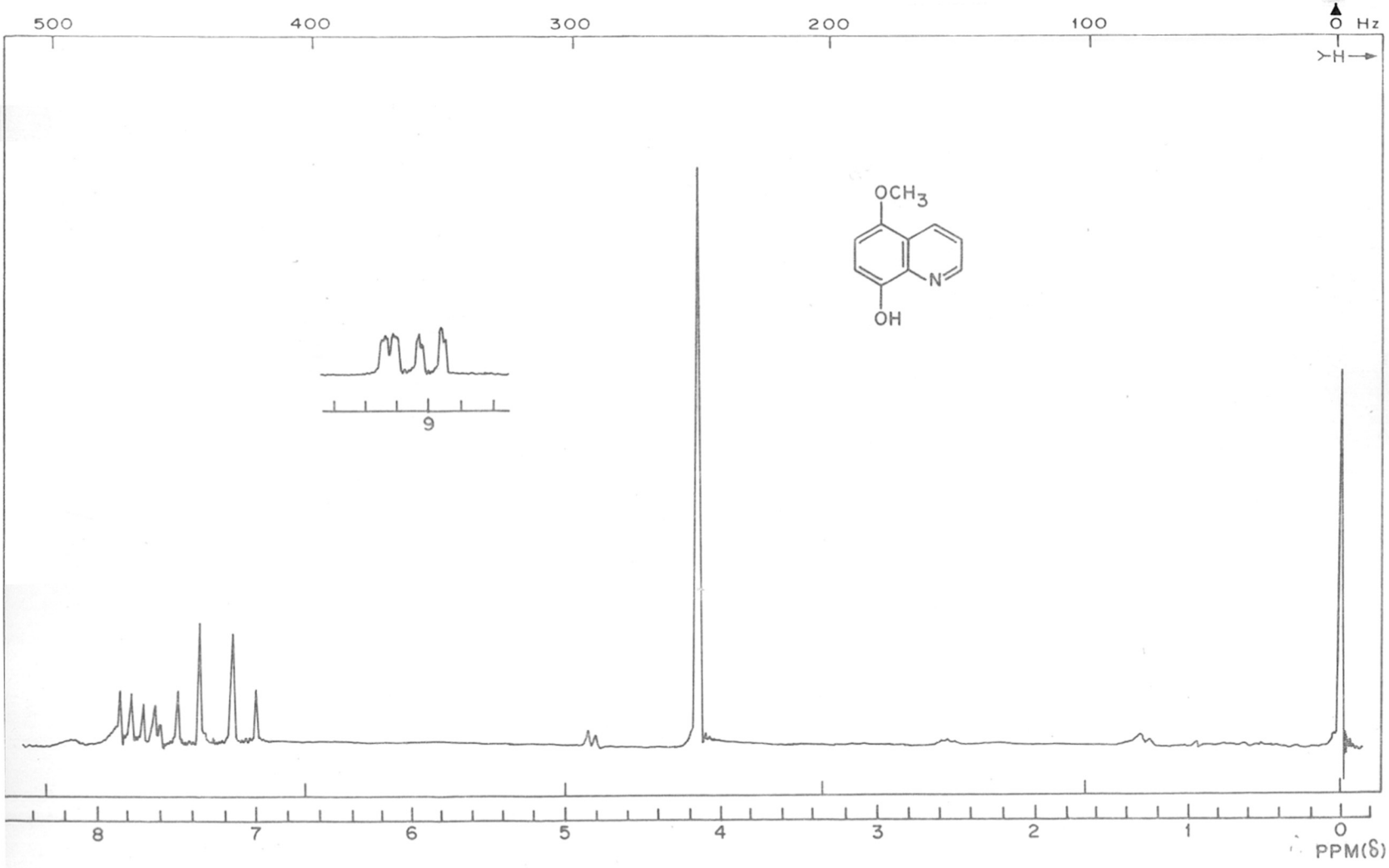


FIG. 13 : ^{1}H-NMR SPECTRUM OF COMPOUND (49) IN $CDCl_3$

3340 cm^{-1} (broad) assigned to hydrogen bonded OH. The mass spectrum revealed a peak at m/z 175 as a supporting evidence.

The azo group as a source for amino group is well known in literature[49,50]. The former, in turn could be introduced via coupling reaction with diazonium salts. This reaction was first explored when the phenol 49 was subjected to the condensation with benzenediazonium chloride at 0°, azo compound 50 was obtained in 62% as a dark violet red solid. In the ^{1}H-NMR (Fig.14) of this compound 50 a singlet at 3.93 ppm was assigned to methoxy group, a singlet at 6.57 ppm to H-6 while aromatic protons resonated in a region between 7 - 8.8 ppm as multiplets. IR spectrum showed the absorptions at 3400 cm^{-1} for OH (broad). Molecular ion peak in the mass spectrum was clearly observed at m/z 279.

Reduction of 50 using aqueous sodium dithionite reaction was carried out. All the attempts to isolate the resulting o-aminophenol 51 were unsuccessful because of its unstability. In an another experiment catalytic reduction of the azo compound 50 to yield amine 51 was also met with failure. When the reduction was effected over catalytic amount of Pd-C and attempted to isolate the free amine 52 no success was observed. These observations revealed that the amino group had to be protected immediately after its

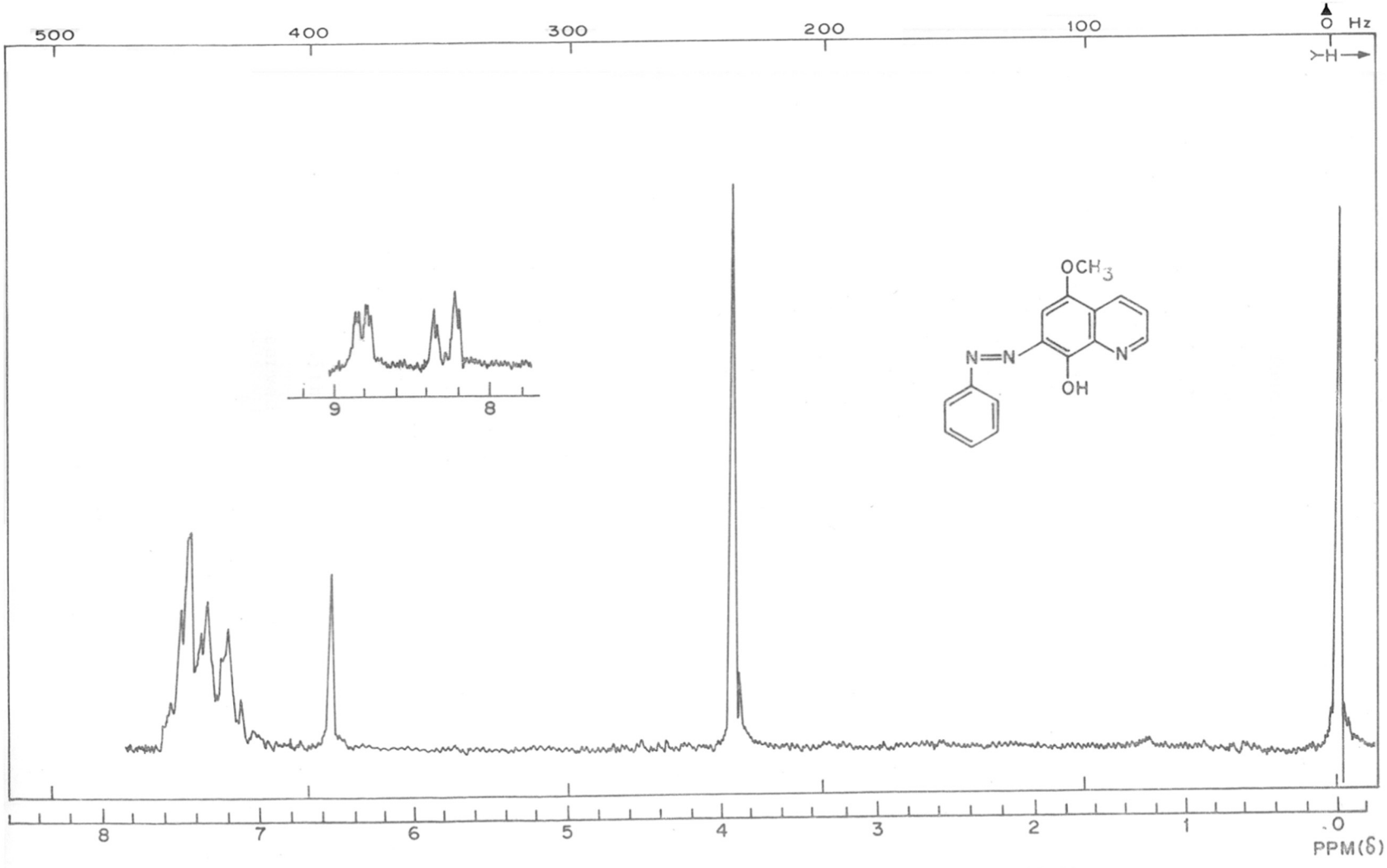


FIG. 14 : ^{1}H-NMR SPECTRUM OF COMPOUND (50) IN $CDCl_3$

formation. This was done as follows. The azo compound 50 was reduced with aqueous sodium dithionite in refluxing ethanol, after the completion of reduction, the reaction was quenched with acetic anhydride. After concentration and purification, the required amide 52 was obtained in 46% yield. The by product obtained from the reaction was acetanilide in around 65% yield (based on the aniline evolved). Comparison of the yields of the product 52 and acetanilide, it could be suggested that low yield of product 52 (by 20%) might be due to the decomposition of the free amine (earlier it had been mentioned that free amine was difficult to isolate). The ^{1}H-NMR spectrum (Fig.15) of compound 52 showed two singlets which resonated at 2.27 ppm and 3.93 ppm for NHAC and OCH_3 respectively. A singlet at 8.04 ppm for H-6 proton was clearly located. Other peaks resonated in the aromatic region. The IR spectrum showed absorptions at 3340 cm^{-1}, 3260 cm^{-1} for NH and OH and at 1670 cm^{-1} for amide carbonyl. The mass spectrum revealed m/z 232. To enhance the yield of the required amide 52, various other conditions were tried. For instance, by carrying out the reduction at room temperature followed by acetylation, lower yields were obtained. In other experiment, when the reduction was performed along with acetic anhydride no improvement in the yield was observed.

Introduction of carboxylic acid at C-2 position was envisaged as usual via the N-oxide and nitrile intermediates.

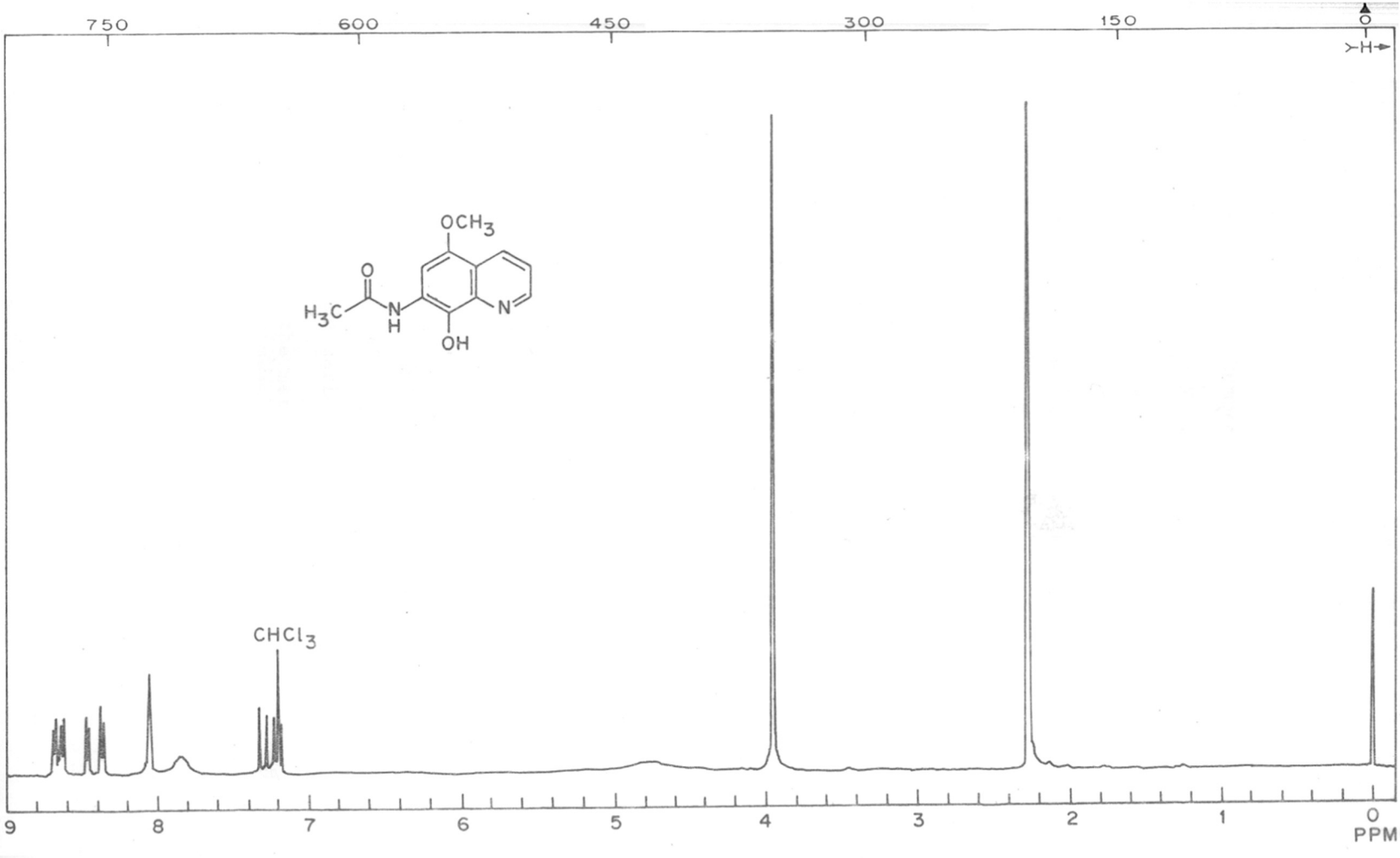


FIG. 15 : ^{1}H-NMR SPECTRUM OF COMPOUND (52) IN $CDCl_3$

Thus, reaction of the amide 52 with m-CPBA in methylene chloride at ambient temperature overnight and conventional work up furnished the N-oxide 53 in 84% yield. The ^{1}H-NMR spectrum of 53 revealed all the expected peaks. The IR spectrum showed bands for OH, NH and amide groups while the mass spectrum revealed a molecular ion peak at m/z 248.

The N-oxide 53 was heated with excess dimethyl sulphate on water bath for 4 hours to furnish the salt which was subsequently dissolved in water and treated with aqueous NaCN to give a compound. The ^{1}H-NMR suggested that the product was 7'-N-methyl acetamido 5,8-dimethoxyquinaldonitrile (54). For example, three singlets each integrating for three protons were located at 1.97 ppm, 3.33 ppm and 3.98 ppm and by comparison of this spectrum with the spectra of the previous samples it was suggested that singlets at 1.97 ppm and 3.98 ppm were due to $NCOCH_3$, and OCH_3 groups while the singlet at 3.33 ppm might be due to N-methyl group. In addition remaining protons resonated at the expected chemical shift. However, in the aromatic region AB pattern was clearly visible while the H-6 proton appeared as singlet. IR spectrum showed absorption for OH group at 3300 cm^{-1} and amide carbonyl at 1670 cm^{-1}. Further confirmation that the methyl group had been incorporated came from the mass spectrum which showed the molecular ion peak at m/z 271.

Literature survey[51] indicated that amides in general can undergo alkylation at three possible sites with dialkylsulphate viz. at oxygen and nitrogen of the amide function and at carbon α - to the amide. Examples of alkylation at all three site of amides are known. Alkylation of carbon α - to the amide usually occurs with a strong base. In the present case, no base was used and, therefore, this possibility was ruled out. Moreover, had the alkylation taken place at carbon, in the ^{1}H-NMR spectrum signals due to CH_2CH_3 group would have been observed. The second possibility that alkylation had taken place at oxygen via enolate was also ruled out because in the ^{1}H-NMR spectrum OCH_3 group would resonate around 4.0 ppm. The possibility that methylgroup has been incorporated as N-methyl was left out. There were several examples in literature, where Ar NCH_3COCH_3 group revealed N-methyl signal at 3.3 ppm. Therefore, the possibility remained in which N-methyl formation had taken place. AcNMe group in ^{1}H-NMR spectrum resonated at 3.33 ppm as observed in other known compounds. These studies clearly prove that only the N-methylation of 56 had taken place. This particular observation was rather abnormal.

To prove that 8-OH group in 54 was free, it was treated with dimethylsulphate and potassium carbonate in refluxing acetone and the anticipated 5,8-dimethoxy-7-N-methyl - acetamidoquinaldonitrile (55) was isolated in 60% yield

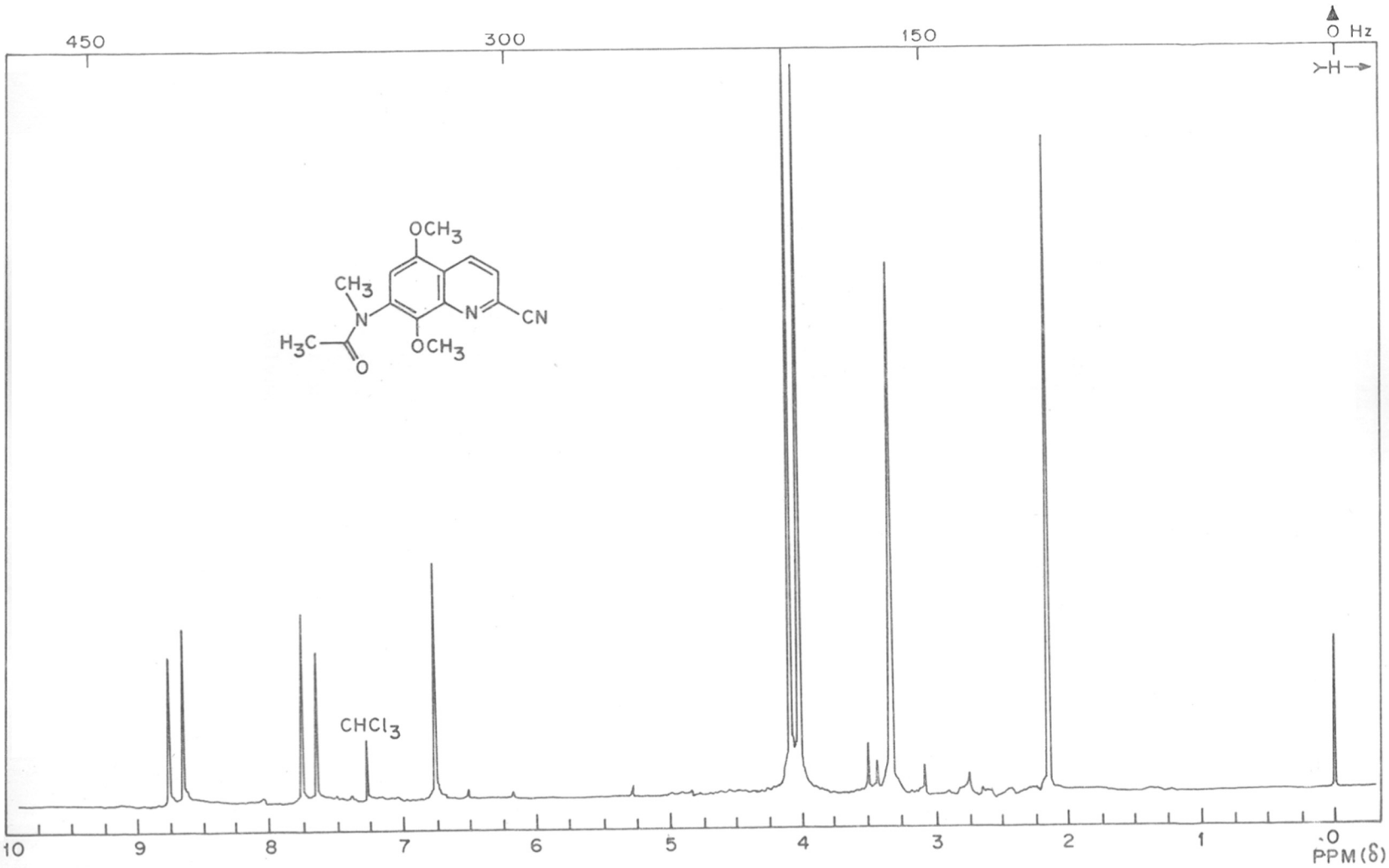
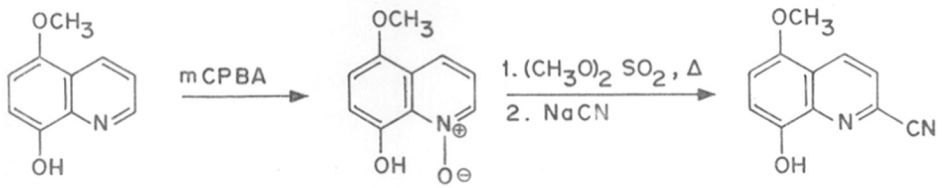


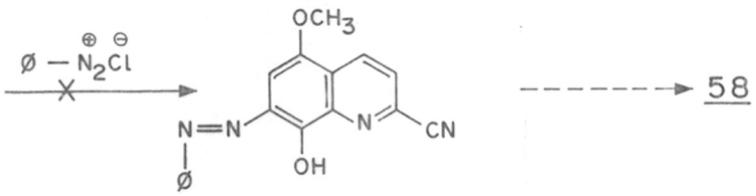
FIG. 16 : ^{1}H-NMR SPECTRUM OF COMPOUND (55) IN $CDCl_3$

whose ^{1}H-NMR (Fig.16) indicated four singlets due to N-Acetyl, N-methyl and two methoxy groups at 2.10 ppm, 3.35 ppm, 4.00 ppm and 4.10 ppm. The molecular ion peak at m/z 285 was in agreement with the proposed structure.

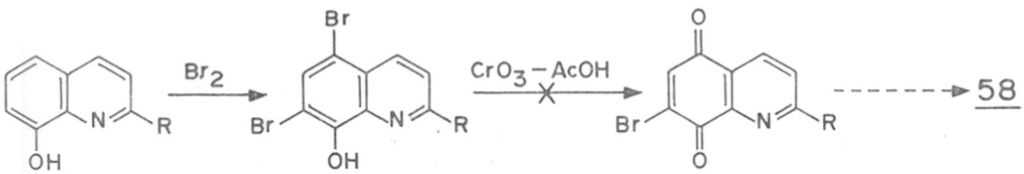
In view of the unforeseen problem of N-methylation during cyanide introduction of 53 coupled with low yield of the azo - amide step, it was decided to introduce cyanide group directly on 5-methoxy-8-hydroxyquinoline and subsequently to introduce acetamido group. Therefore, the 5-methoxy-8-hydroxyquinoline 49 was converted into its N-oxide 59 (80%) with m-CPBA and crude N-oxide 59 was then heated with excess dimethylsulphate and subsequently treated with aqueous sodium cyanide solution to give after purification 2-cyano-5-methoxy-8-hydroxyquinoline (60) in 76% yield. The ^{1}H-NMR spectrum (Fig.17) of the compound 60 revealed characteristic AB pattern for H-3, H-4 and H-6, H-7 protons. The incorporation of the nitrile in the molecule was evidenced by IR spectrum which showed an absorption at 2260 cm^{-1} for the C≡N and 3380 cm^{-1} for OH. The mass spectrum revealed a peak at m/z 200 further supporting the assigned structure. The nitrile 60 was subjected to coupling reaction with benzenediazonium chloride at 0°, TLC of the reaction mixture showed number of spots which could not be analysed. Change of reaction medium as well as pH of the reaction always gave an unanalysable mixture of products on TLC and therefore the route was abandoned.

SCHEME 12





SCHEME 13

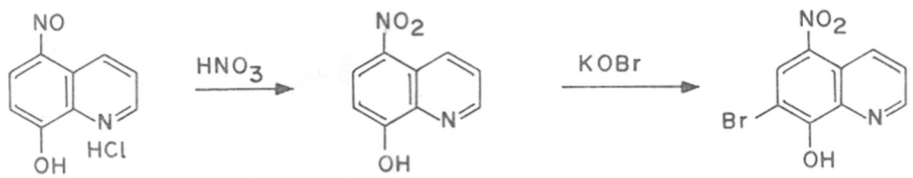


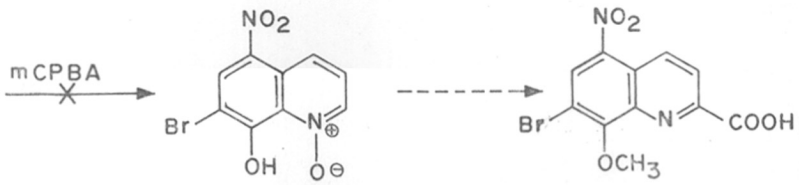
7 : R=H
14 : R=CN

62 : R=H
63 : R=CN

64 : R=H
65 : R=CN

SCHEME 14





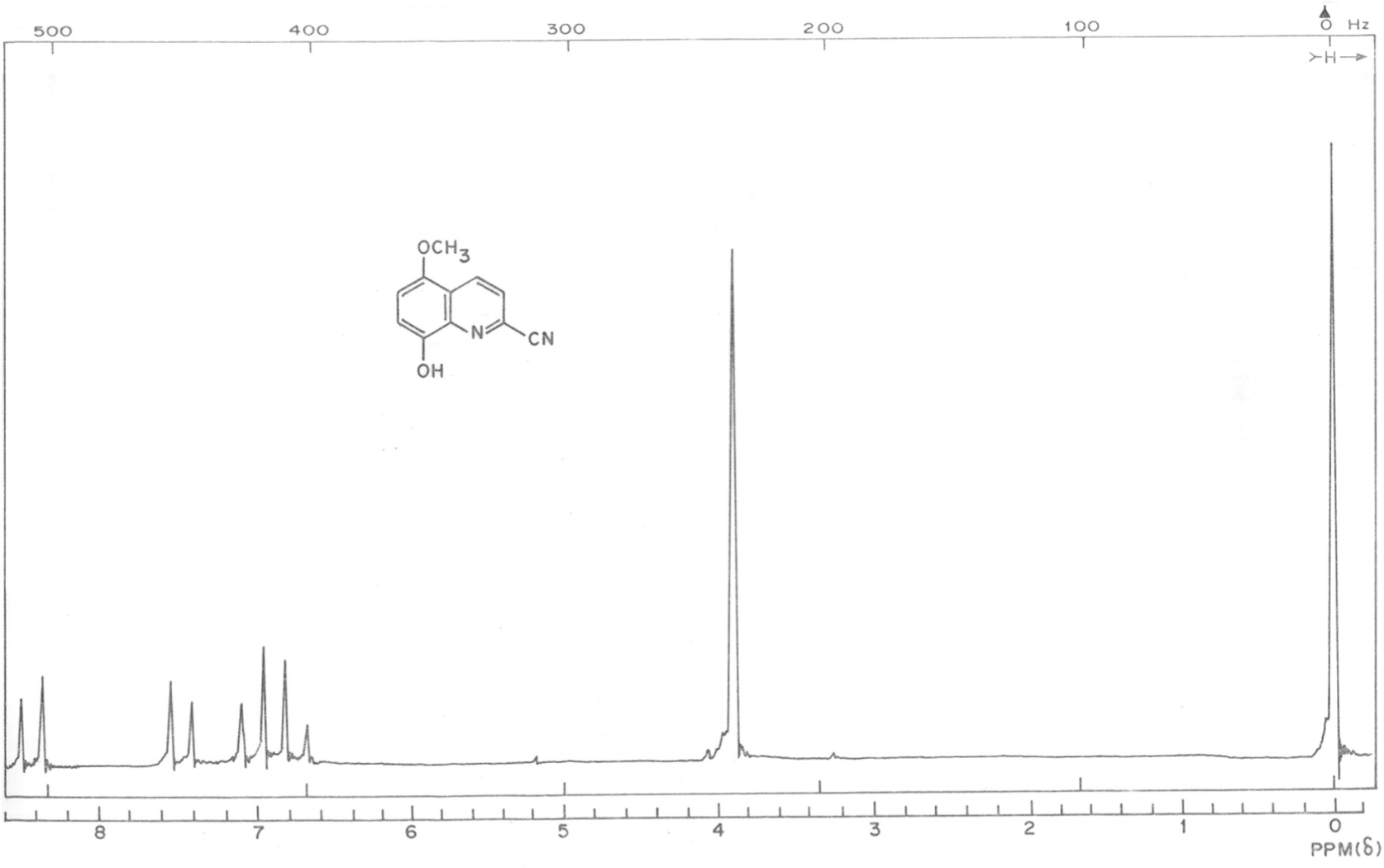


FIG. 17 : ^{1}H-NMR SPECTRUM OF COMPOUND (60) IN $CDCl_3$

The introduction of the amino group at C-7 position posed some unforeseen problems. It was decided to introduce a functionality which could be converted to the amine at the later stages.

Therefore, the strategy comprised of introduction of bromine at C-7 position as a latent amine which could be derived at later stages.

The ease with which p-bromophenols undergo oxidation to paraquinone with CrO_3-Acetic acid is well known[52]. On this basis, it was predicted that 5,7-dibromo-8-hydroxyquinoline (62) (Scheme 13) could serve as suitable starting material for the preparation of substituted quinaldic acid because 5-bromo and 8-OH groups on oxidation would give the required quinone while C-7 bromide on suitable substitution would give rise to amino group[46]. Accordingly 8-hydroxyquinoline (7) was brominated with bromine in accordance with the literature procedure[53] to yield 5,7-dibromo-8-hydroxyquinoline (62). Oxidation of 62 with CrO_3-Acetic acid at room temperature was then carried out but failed to isolate the required product 64 because of exhaustive decomposition of the starting material. When the above reaction was conducted at 0°, no success was however observed.

Since 2-cyano-8-hydroxyquinoline (14) was already

at hand, it was felt to prepare its 5,7-dibromide derivative 63 and study the oxidation. The compound 63 was easily obtained from 14 by treatment of bromine in acetic acid in 91% yield. The ^{1}H-NMR spectrum of the compound showed a singlet for H-6 proton while a AB pattern for the H-3 and H-4 protons in the aromatic region. The mass spectrum also supported the structure by revealing peaks at m/z 326, 328 and 330. The 5,7-dibromocompound 63 was then subjected to the oxidation with CrO_3-AcOH mixture. Here also formation of mixture of products was observed by TLC . With cerric ammonium nitrate as an oxidising agent, 62 and 63 failed to give required products.

The failure of the 5,7-dibromo-8-hydroxyquinoline derivatives to undergo oxidation may be attributed to the susceptibility of the molecule towards the oxidising agent (CrO_3-acetic acid). Therefore, the obvious choice which remained was to use 5-nitro-8-hydroxyquinoline 66 in which two purposes could be served. Firstly, the introduction of bromine could regioselectively be carried out at C-7 (meta to the nitro) and secondly, the nitro group at C-5 and OH at C-8 be oxidised to the para-quinone via the amine at later stages.

5-Nitro-8-hydroxyquinoline (66) has been prepared earlier[54] by direct nitration of 8-hydroxyquinoline. However,

when the above procedure was followed, a mixture of mono and di-nitro compounds were obtained. Therefore, an indirect method also reported[55] was resorted to in which 5-nitroso-8-hydroxyquinoline (45) (discussed earlier) was oxidised to 5-nitroderivative 66 with nitric acid. Bromination of the nitrophenol derivative using KOBr[55] in alkaline aqueous solution for two hours resulted in the formation of 5-nitro-7-bromoquinoline (67) in 66% yield.

When this compound 67 was subjected to N-oxidation using H_2O_2-acetic acid either at room temperature or at elevated temperature led to the destruction of the molecule because after usual work up, nothing could be isolated from the non-aqueous layer. Attempts to N-oxidise this nitrogen by stirring it with m-CPBA for 48 hours at room temperature also met with failure as the starting material 67 was recovered (TLC). The inertness of the compound 67 to undergo N-oxidation with m-CPBA was not unreasonable and may be explained in terms of the studies by other workers, related to this observation.

For instance Ramaiah and Srinivasan[56] have reported that 5,7-disubstituted derivatives such as 5,7-dihalo or 5,7-dinitro compounds were resistant to N-oxidation either with H_2O_2 (30% w/v)-acetic acid or with perbenzoic acid. This reluctance to undergo N-oxidation appeared to be due to considerably reduced basicity of the tertiary nitrogen caused by the presence of two deactivating substituents.

Landquist[57] and Israel-Day[58] have respectively reported such deactivating influence of halo and nitro substituents during N-oxidation of 5,8 or 2,3 substituted quinoxolines and substituted imido pyridines.

All the attempts reported above with regard to the synthesis of properly substituted quinaldic acid failed. However, from these studies a few important points could be deduced which would direct further efforts towards the ultimate goal.

1. With N-acetylamino group present in the 8-hydroxyquinoline, introduction of cyano-group at C-2 position could not be accomplished because of N-methylation as a side reaction.

2. Towards the oxidising agents (CrO_3-acetic acid), for the conversion of 5,7 dibromo-8-hydroxyquinoline to the bromo-quinone, the molecule was susceptible to decomposition.

3. With nitrosubstituent at C-5 and bromo at C-7 position, the reactivity of the tertiary nitrogen towards N-oxide formation was greatly diminished.

4. With cyano group at C-2, introduction of azo group at C-7 position could not be achieved.

With these points in mind, it could be suggested that for any synthesis of 1 to be successful, cyano group at C-2 had to be introduced first on 8-hydroxyquinoline. Secondly, for the introduction of amino functionality at C-7

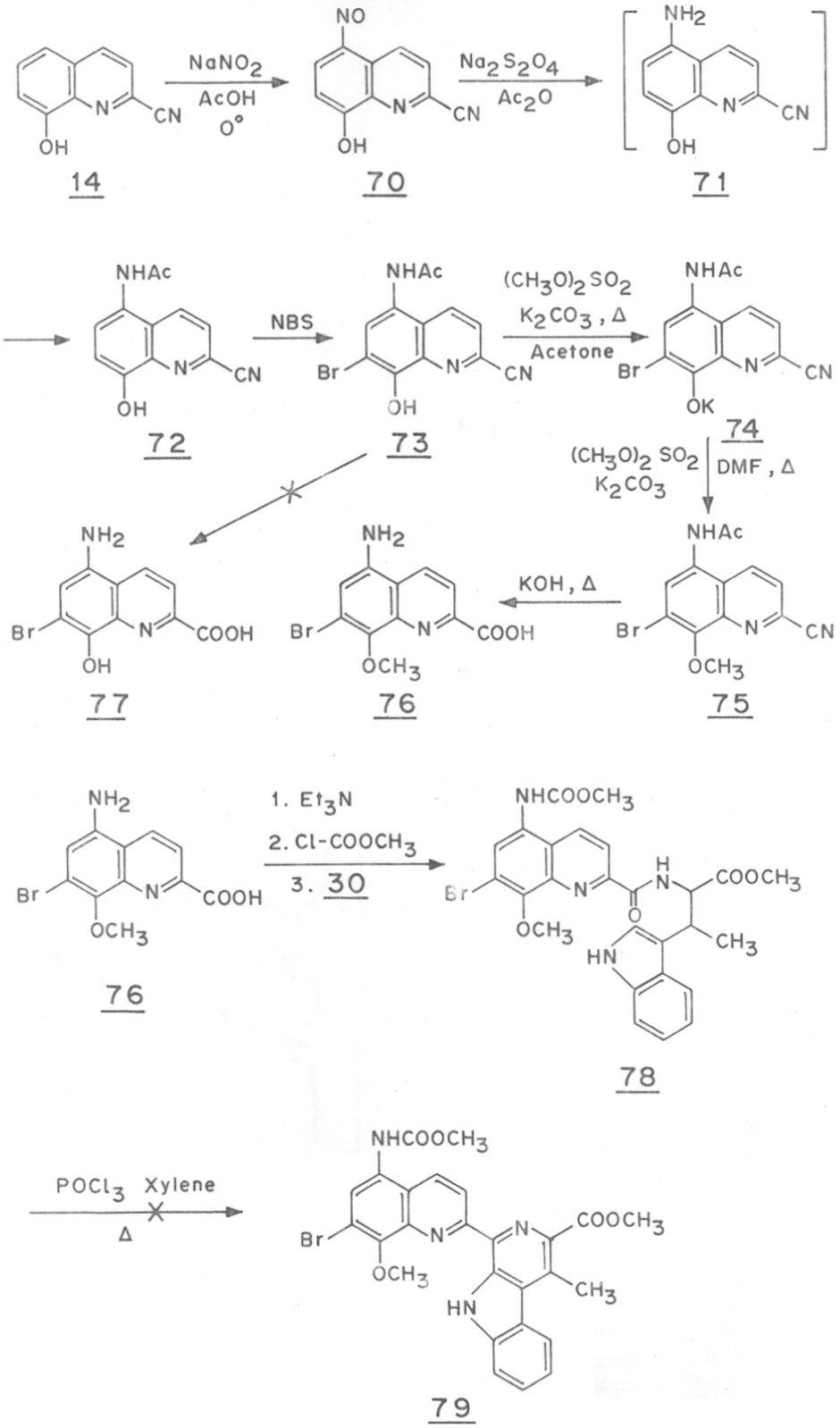
a potential group such as bromide must be present.

Hence, the strategy for the synthesis of 1 would be (a) to introduce cyano group at C-2 and (b) to functionalise the molecule with required substituents.

Accordingly, the 8-hydroxyquinaldonitrile 14 (earlier obtained from quinoline) was chosen as the starting material. It was then subjected to nitrosation reaction with sodium nitrite in acetic acid at ice-bath temperature furnishing the desired para-nitroso compound 70 in 74% yield. The ^{1}H-NMR spectrum (Fig.18) of the compound 70 showed two AB quartet in the aromatic region attributed for H-3, H-4 and H-6, H-7 which indicated that the nitroso group has gone on either C-5 or C-7 positions. However, there are several instances in literature where nitrosation of phenols has occurred on the para-position. In addition to this, earlier work described above has also demonstrated that nitrosation reactions of 8-hydroxyquinoline occurs at C-5 position (para to OH).

The next step was the introduction of a bromine at C-7 which could act as a potential amino group at later stages. There were two alternatives open for doing this. Firstly, the nitroso group could be oxidised to nitro group and then the bromine could be regiospecifically introduced at C-7 (meta to nitro). However, conversion of NO $\rightarrow$ NO_2 using nitric

SCHEME 15



14 70 71 72 73 74 75 76 77 78 79

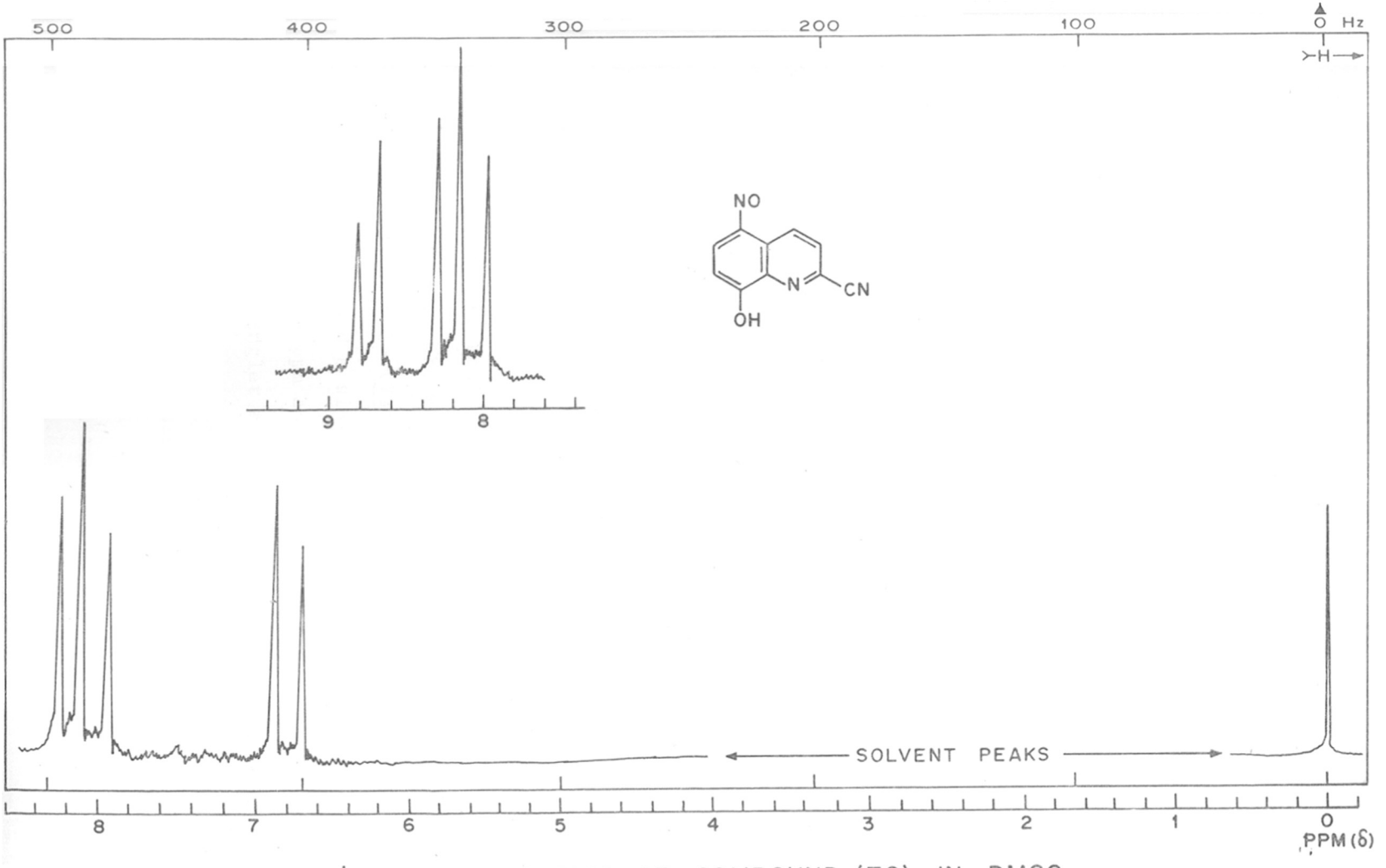


FIG. 18 : ^{1}H-NMR SPECTRUM OF COMPOUND (70) IN DMSO

acid as the oxidising agent invariably leads to the formation of mono and dinitro derivatives. Therefore, it was decided to introduce bromine after the transformation of the nitro group into N-acetyl amino function.

The nitroso compound 70 was reduced with aqueous sodium dithionite to furnish the amine 71 which was isolated as the N-acetyl derivative 72 in 88% yield. The ^{1}H-NMR spectrum (Fig.19) of 72 showed a singlet for methyl of N-acetyl at 2.24 ppm, two quartets for aromatic protons beyond 7.0 ppm. In the IR spectrum, absorptions at 3340 cm^{-1}, 3280 cm^{-1} were attributed to NH and OH groups while at 2260 cm^{-1} and 1660 cm^{-1} were ascribed to C=N and amide carbonyl respectively.

The reactivity of the amine in 72 was curtailed due to the amide formation and therefore, advantage of free hydroxyl group as a better ortho directing group towards electrophilic bromination was envisaged.

It was indeed gratifying to note that 72 underwent bromination with one equivalent of N-bromosuccinimide in DMF at 0° to furnish the required 5-acetamido-7-bromo-8-hydroxyquinaldonitrile (73) in 83% yield. The ^{1}H-NMR spectrum (Fig.20) of 73 showed a singlet for N-acetyl group at 2.19 ppm, two doublets at 8.14 ppm and 8.66 ppm (J=7.6 Hz) for H-3 and H-4 protons and a singlet at 8.00 ppm for H-6 proton and another singlet at 10.04 ppm (D_2O exchangeable) for NH. Although the structure 73 on the basis of ^{1}H-NMR spectrum was proved

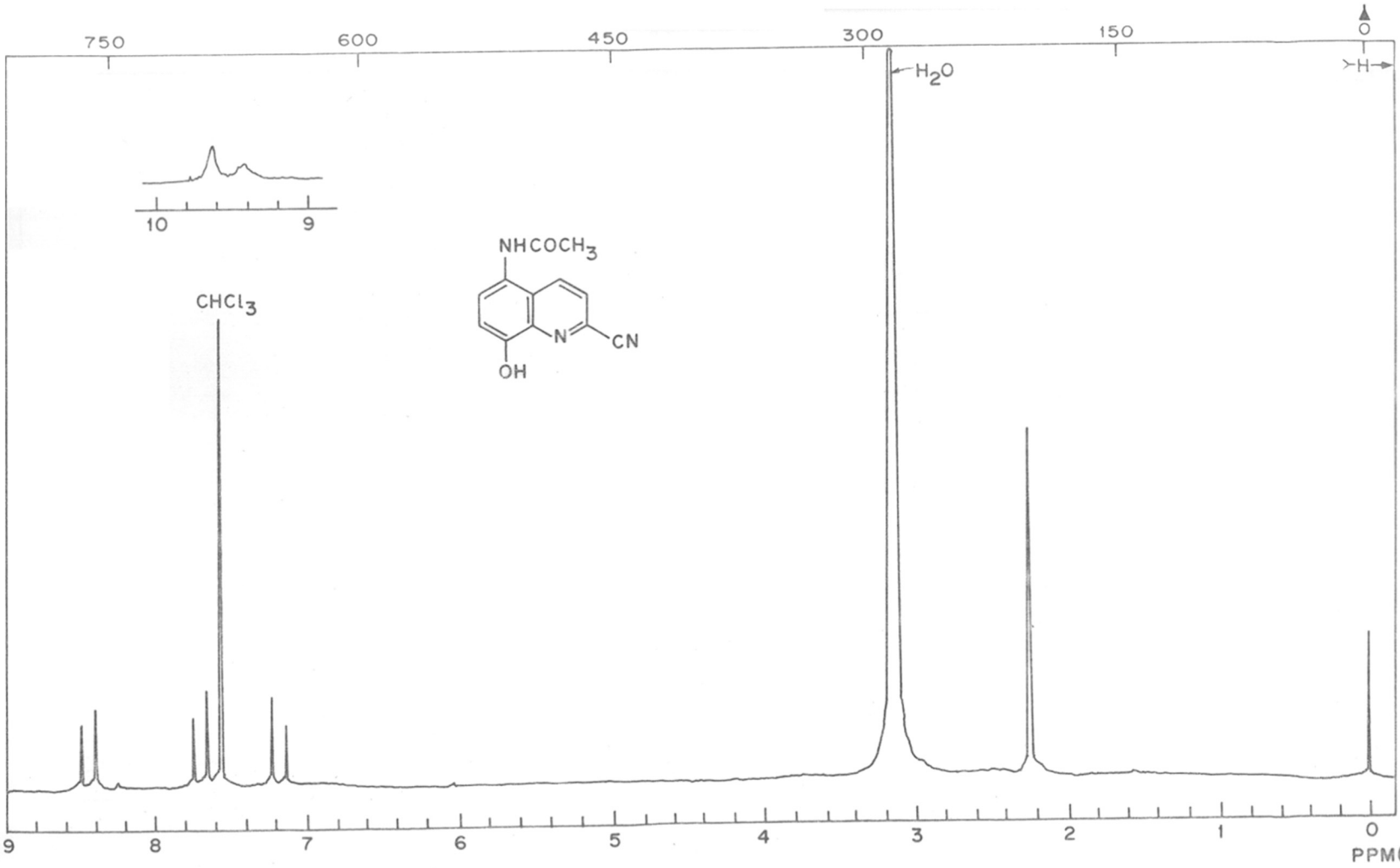


FIG. 19 : ^{1}H-NMR SPECTRUM OF COMPOUND (72) IN $CDCl_3$ + DMSO – d_6

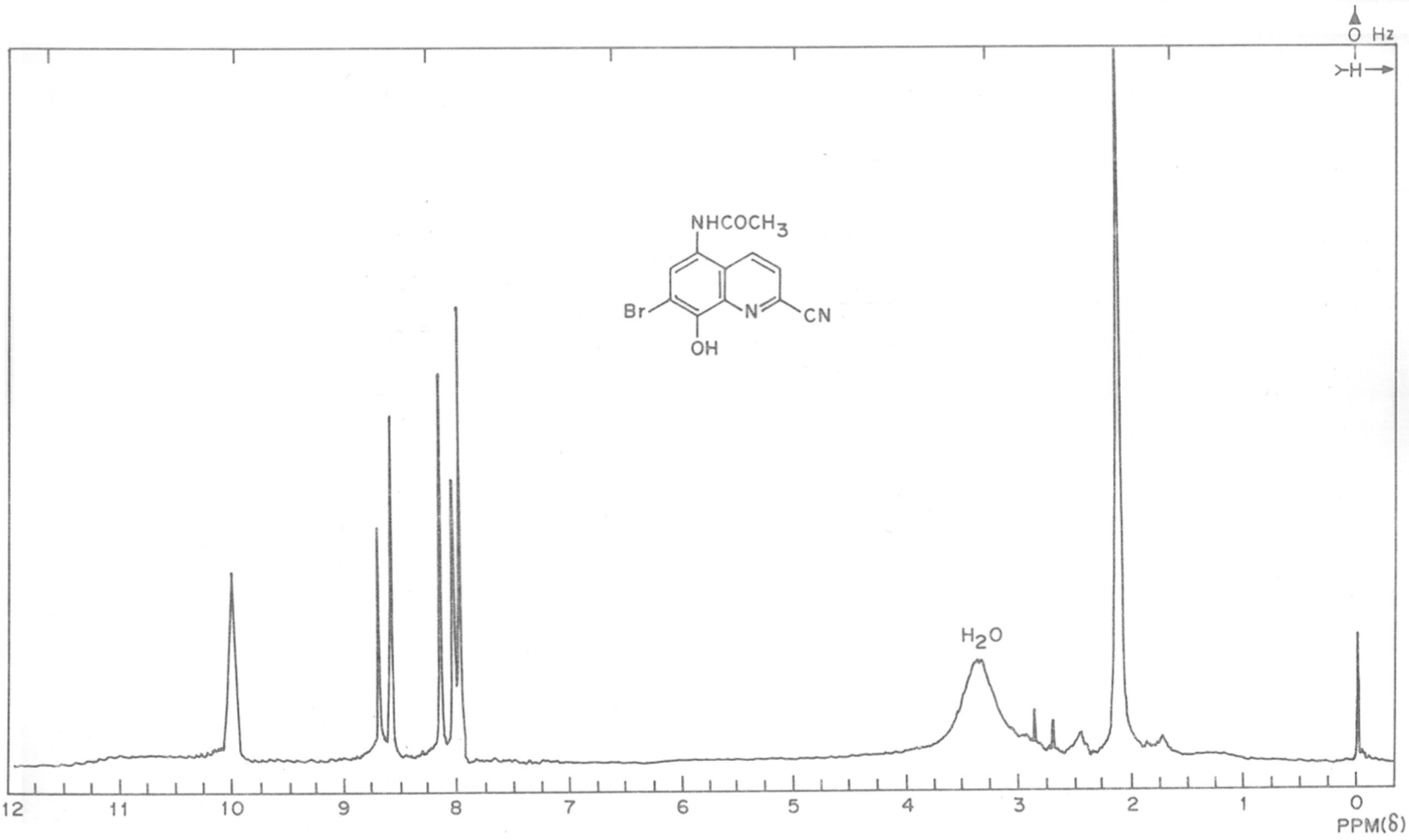


FIG. 20 : ^{1}H-NMR SPECTRUM OF COMPOUND (73) IN DMSO-d_6

without any doubt, the unambiguous proof was later established by chemical correlation (page 102).

However, bromination of phenol 72 employing variety of brominating conditions, e.g. Br_2-AcOH, Br_2-Et_3N/$CHCl_3$, Br_2-$CHCl_3$ etc. could not be achieved as it led to irretrievable devastation of the molecule.

Attempts to hydrolyse the cyano compound 73 to 77 with acid or alkali met with failure probably because of decomposition. However, when the hydroxy group was protected as O-methyl, the hydrolysis occurred with ease in alkaline conditions.

When the compound 73 was refluxed with dimethylsulphate and anhydrous potassium carbonate in boiling acetone for 8 hours during which red solid precipitate separated out, and also TLC showed the absence of starting material. After concentration of the reaction mixture followed by water treatment to dissolve potassium carbonate and filtration of the solid gave a product which was confirmed as the potassium salt 74. The potassium salt was found to be stubborn and quite unreactive towards dimethylsulphate in acetone. However, use of dipolar aprotic solvent, dimethylformamide, for methylation with dimethylsulphate in presence of anhydrous potassium carbonate, the potassium salt 74 underwent smooth methylation at 90-100°. Later, it was observed that the phenol 73 could be directly methylated

with dimethylsulphate in the presence of potassium carbonate in dimethylformamide at 90-100° to afford the methyl ether 75 in 59% yield. The ^{1}H-NMR spectrum (Fig.21) of compound 75 revealed the anticipated O-methyl signal at 4.05 ppm while rest of the protons resonated at the expected chemical shifts. The presence of a singlet at 10.2 ppm for NH indicated that NH had not undergone N-methylation and this was proved by IR spectrum in which a band at 3260 cm^{-1} (NH) was observed. Further support to this structure came from mass spectrum which revealed two peaks at m/z 319 and 321 corresponding to two isotopes of bromine.

Hydrolysis of the cyano group in 75 was effected with ethanolic potassium hydroxide for 24 hours to furnish the amino-quinaldic acid 76 in 80% yield. The ^{1}H-NMR spectrum (Fig.22) of 76 indicated the assigned structure which was conclusively proved by IR spectrum in which the absence of bands at 2200 cm^{-1} and 1670 cm^{-1} due to C=N and amide were clearly observed. In addition, three absorption bands at 3400 cm^{-1}, 3350 cm^{-1} and 3260 cm^{-1} due to NH_2 and COOH groups were also visible. Having obtained the aminoquinaldic acid 76, its condensation with β-methyltryptophan involving mixed anhydride was studied.

Condensation of compound 76 with 30 was accomplished by first converting it into the mixed anhydride by employing two equivalents of triethylamine and two equivalents of methyl-

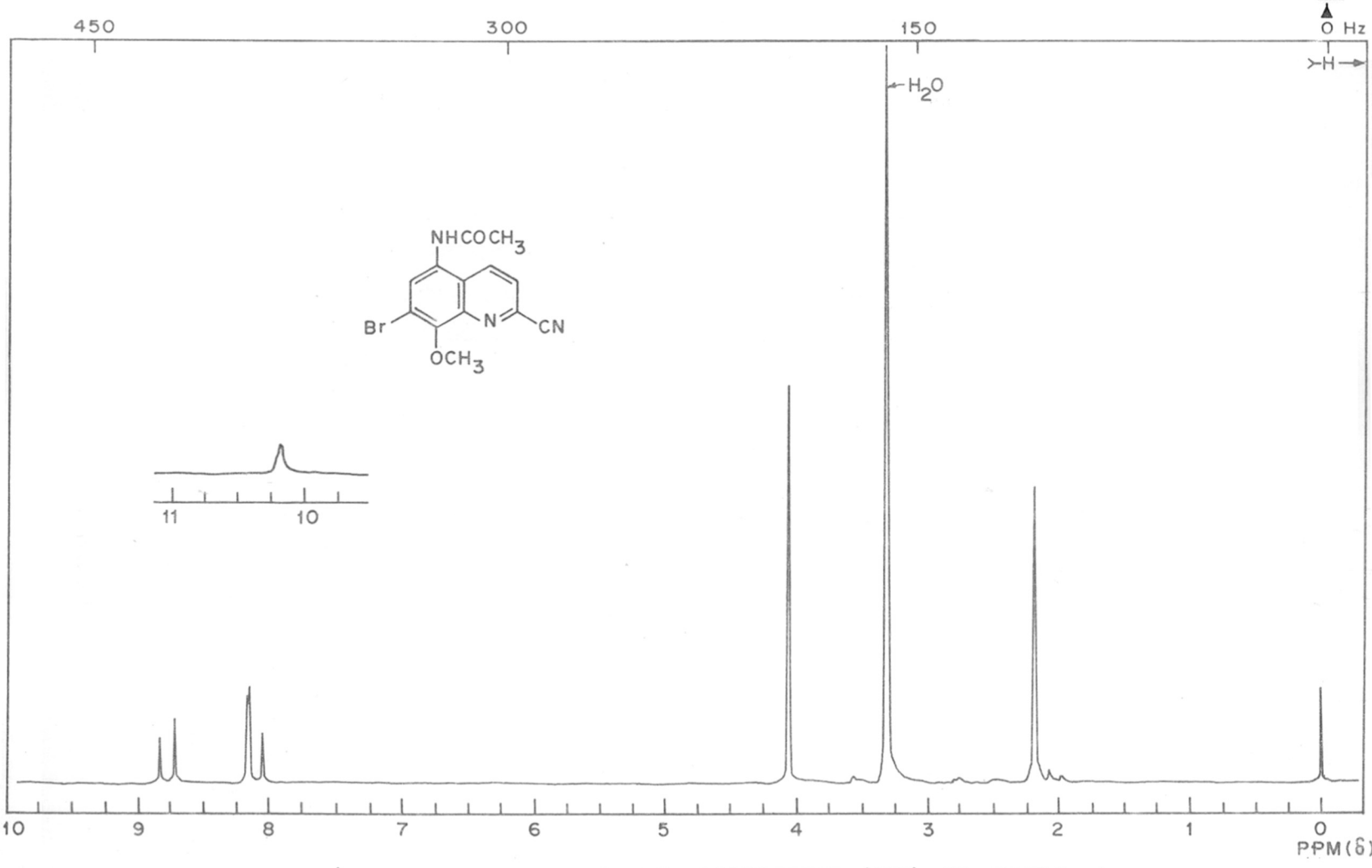


FIG. 21 : ^{1}H-NMR SPECTRUM OF COMPOUND (75) IN DMSO-d_6

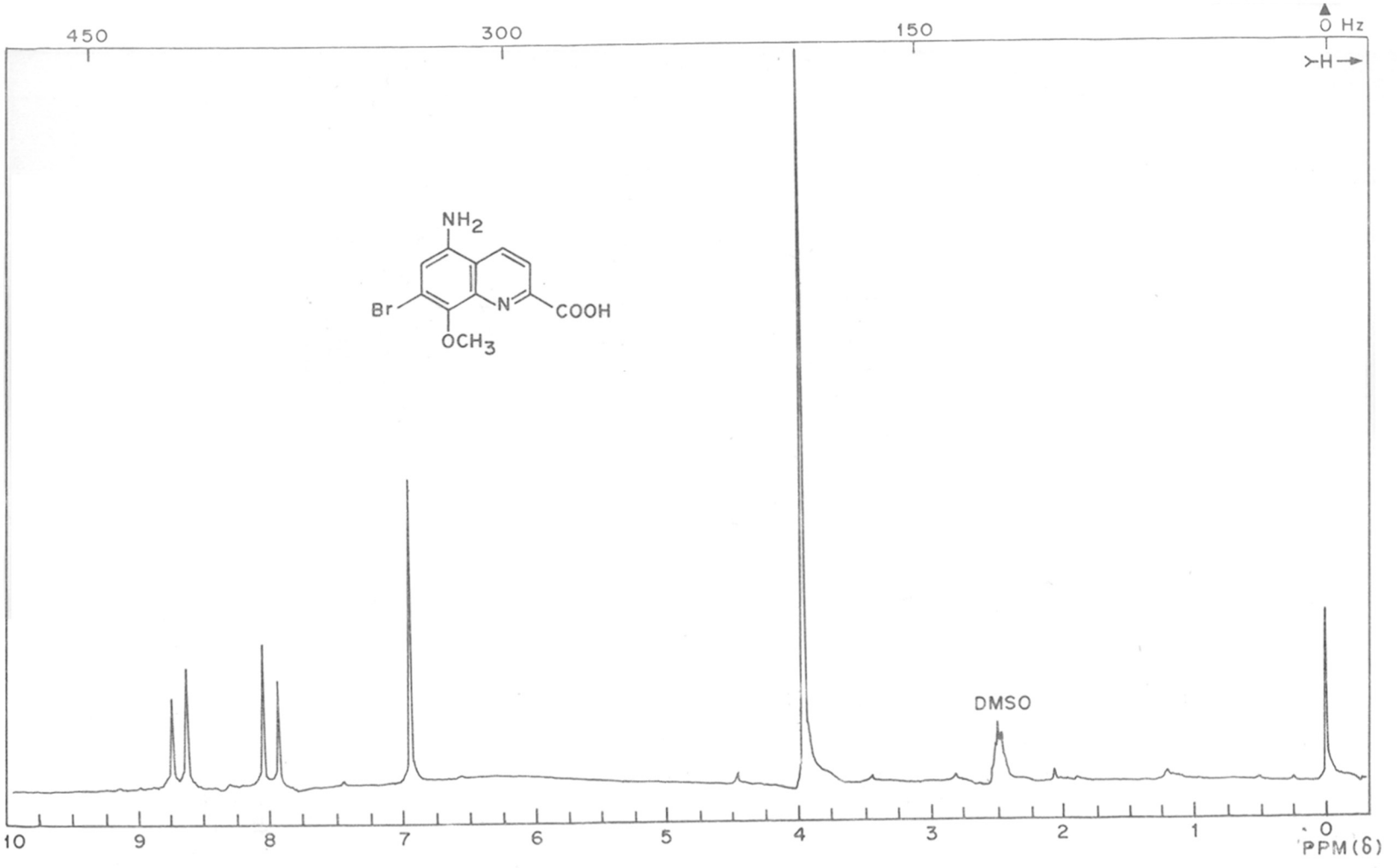


FIG. 22 : ^{1}H-NMR SPECTRUM OF COMPOUND (76) IN DMSO-d_6

chloroformate in THF at 0°. The two equivalents of methyl-chloroformate were required because of the presence of the amino group which was converted into the urethane. Subsequent addition of β-methyltryptophan 30 after purification by chromatography furnished the required amide 78 in 90% yield (based on the recovery of tryptophan). The ^{1}H-NMR spectrum (Fig.23) of 78 revealed the assigned structure as it showed a triplet at 1.51 ppm for HC-CH_3 (diastereomers), four singlets at 3.60 ppm, 3.66 ppm, 3.80 ppm and 3.93 ppm for OCH_3 groups (diastereomers) and two set of multiplets at 4.1 ppm and 5.1 ppm representing two methine protons. Aromatic protons resonated beyond 6.8 ppm.

Cyclisation of the amide 78 using previously described conditions of employing $POCl_3$ in refluxing xylene furnished a product which was found to be difficult to analyse but was definitely not the required compound. Other cyclodehydrating agents such as PPA, PPE, P_2O_5-$POCl_3$ and BF_3-etherate were tried but in all these cases, the required compound 79 could not be traced out.

Change of solvent from xylene to DMSO proved no discernible improvements. Moreover, attempted cyclo-dehydration of the amide 78 using PPA in refluxing xylene under nitrogen atmosphere led to the formation of intractable mixture. Refluxing the amide 78 in chloroform for 18 hours led to the recovery of the starting material. Heating the

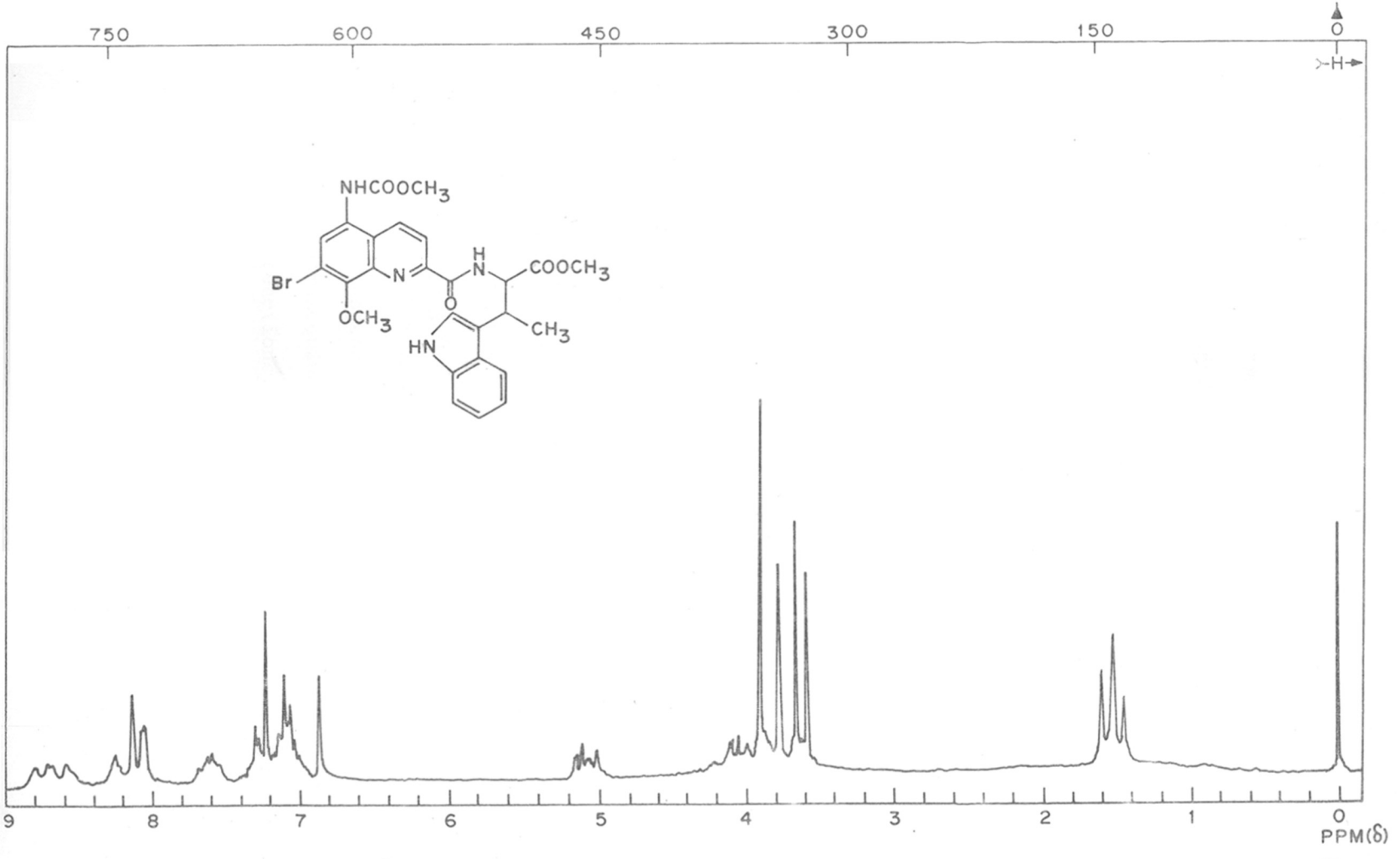


FIG. 23 : ^{1}H-NMR SPECTRUM OF COMPOUND (78) IN $CDCl_3$

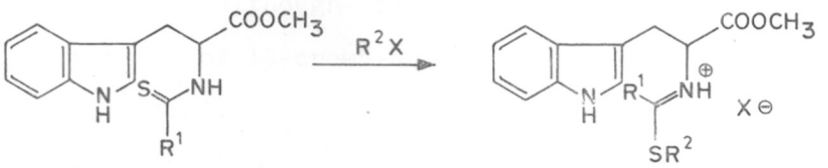
the amide 78 with neat PPA at 120° for 1 hour resulted in the formation of multispots as was evident by TLC.

There is a report in literature[59] that cyclisation of thioamides 80 to the corresponding dihydro β-carbolines 82 occurs in the presence of alkyl halides. The preparation of thioamides from amides are usually effected by Lawesson's reagent 83[60]. However, in this case the amide 78 failed to provide the thioamide 84 with Lawesson's reagent under various conditions.

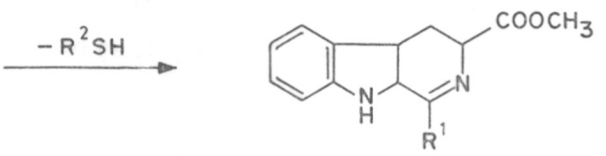
The successful cyclisation of the model compounds 11, 31, 37 and 43 to form the corresponding β-carboline derivatives 20, 32, 38 and 44 as against the failure on the part of amide 78 to undergo cyclisation under the same reaction conditions,was rather surprising and absurd. The only difference between functionalities present in all the molecules 11, 31, 37, 43 and 78 was an additional urethane group in the latter case. But if at all, the urethane group was playing such a critical role of preventing the cyclisation, then the most logical method to circumvent this problem was to replace it with some non-interfering group such as OCH_3. In essence, what was envisaged was to study 5,8-dimethoxy-7-bromoquinaldic acid (88) for condensation and cyclisation reactions. Compound 88 was therefore prepared by two different routes.

Accordingly (Scheme 17) the aminoquinaldic acid 76

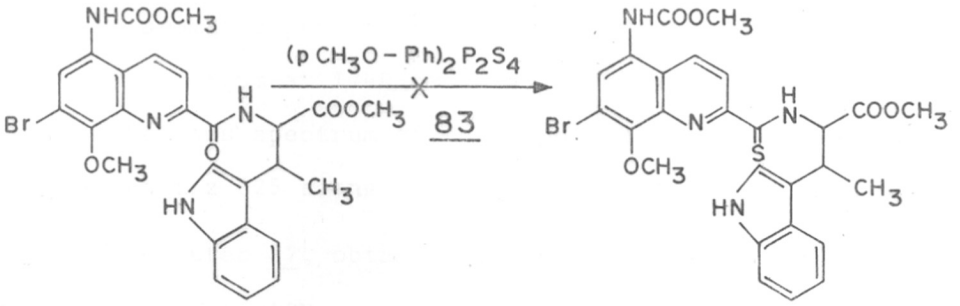
SCHEME 16



80 81



82



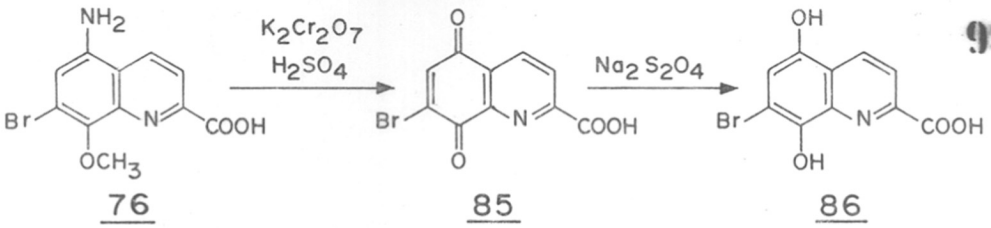
78 84

(described earlier) was oxidised using potassium dichromate sulphuric acid. Although some oxidations were performed in the presence of 18-crown-6, in this case 18-crown-6 was not essential.

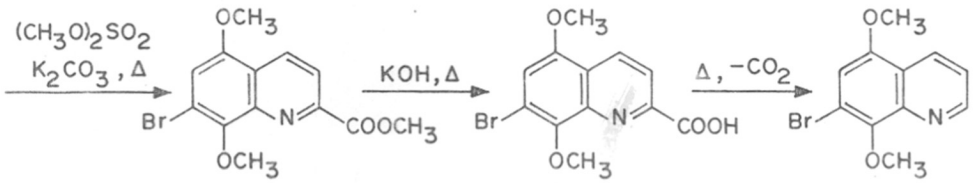
Reduction of 85 to the corresponding hydroquinone 86 was carried out using sodium dithionite. The resultant hydroquinone acid 86 was methylated with dimethylsulphate in the presence of anhydrous potassium carbonate in refluxing acetone to give 5,8-dimethoxy-7-bromo-2-carbomethoxyquinoline (87) in 30% overall yield from 76. In the ^{1}H-NMR spectrum of compound 87, all the expected resonances were revealed. For example three singlets at 3.98 ppm, 4.02 ppm and 4.17 ppm were assigned to three methoxy groups a singlet at 7.01 ppm represented H-6 proton, while two doublets appearing at 8.09 ppm (9.45 Hz) and 8.60 ppm (9.45 Hz) assigned to the remaining aromatic protons. The presence of a strong band in the IR spectrum at 1740 cm^{-1} was attributed to the ester group. The mass spectrum of the compound revealed a molecular ion peak at m/z 325 along with the isotopic peak at m/z 327.

The ester 87, obtained above, was saponified by refluxing with aqueous ethanolic potassium hydroxide for 12 hours. Usual work up furnished the desired acid 88 in 89% yield. In the ^{1}H-NMR spectrum (Fig.24) of the compound 88, the two methoxyl groups were observed at 4.00 ppm and 4.05 ppm, a broad hump at 6.90 ppm was assigned for COOH

SCHEME 17

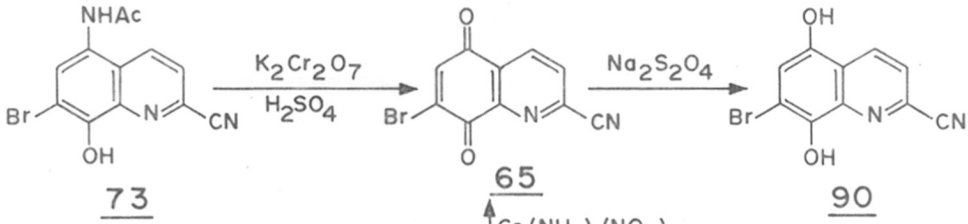


76 85 86



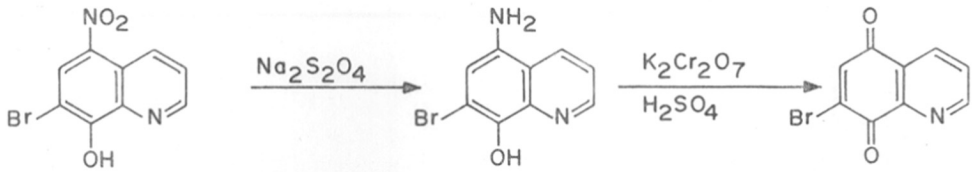
87 88 89

SCHEME 18

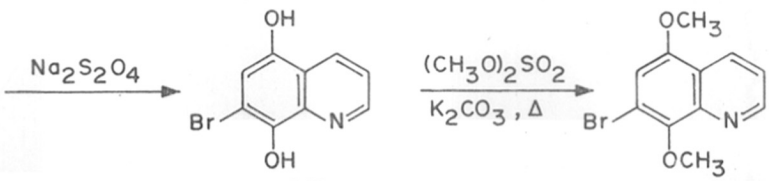


73 65 90 91 88

SCHEME 19



67 92 64



93 89

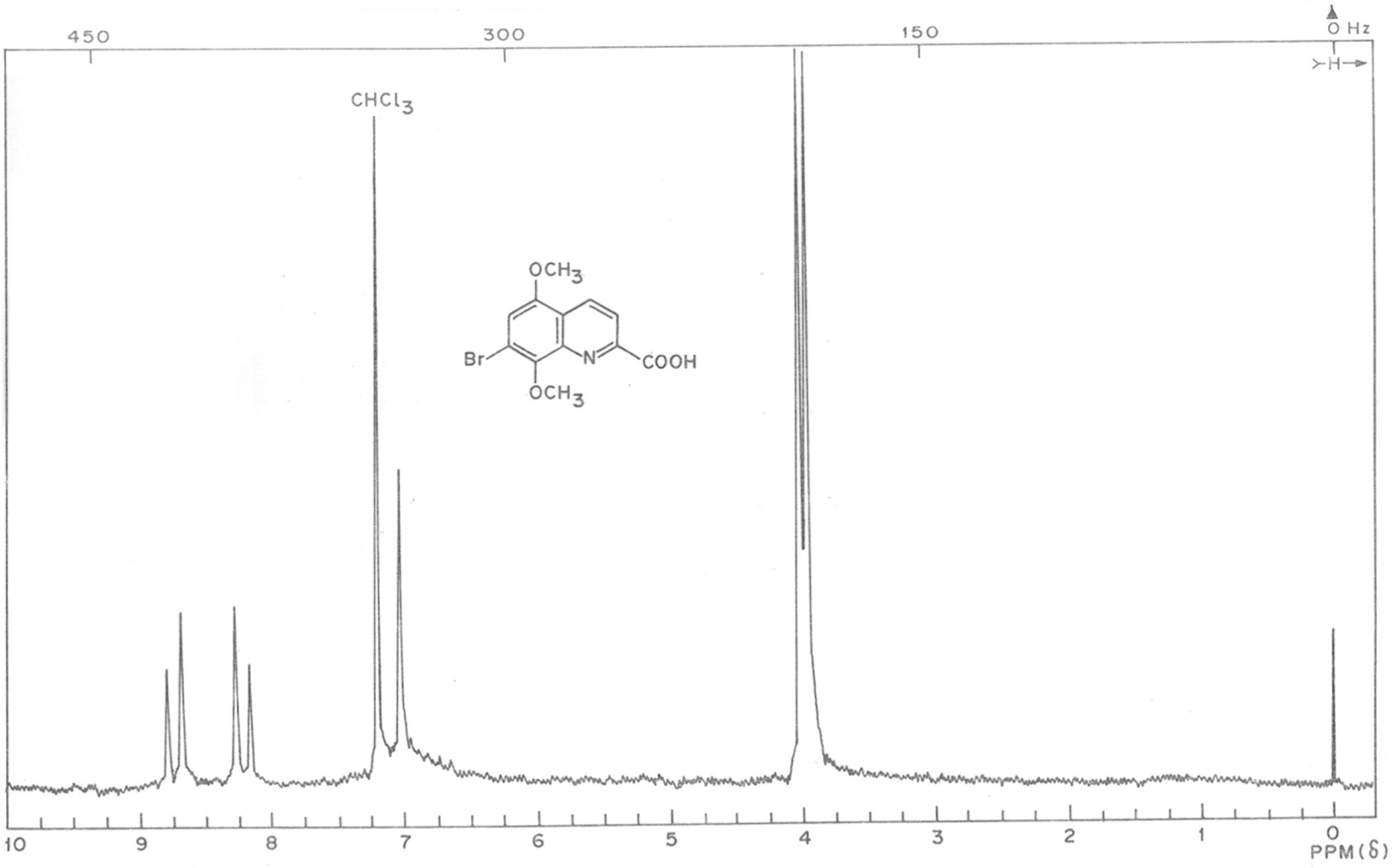


FIG. 24 : ^{1}H-NMR SPECTRUM OF COMPOUND (88) IN $CDCl_3$

proton (D_2O, exchangeable). The rest of the protons resonated at the expected chemical shifts in the aromatic region. The presence of absorption at 3340 cm^{-1} and 1720 cm^{-1} in the IR spectrum was attributed to COOH. Further evidence came from the mass spectrum which revealed peaks at m/z 311, 313.

Alternately, the same acid was obtained (Scheme 18) by the oxidation of 7-bromo-5-acetamido quinaldonitrile (73) as follows. The two phase oxidation of the compound 73 with acidic potassium dichromate at 0° led to the formation of the desired bromoquinone 65 in 73% yield. The IR spectrum showed bands for C≡N and quinone at 2230 cm^{-1} and 1700 cm^{-1}, 1670 cm^{-1}, 1600 cm^{-1}. Furthermore, the absence of NH in IR, N-$COCH_3$ in NMR and the presence of molecular ion peak at m/z 261, 263 in mass spectra gave additional proof for the structure.

Reduction of the quinone 65 using aqueous sodium dithionite resulted in the formation of the corresponding hydroquinone 90 in 94% yield which was methylated with dimethylsulphate, anhydrous potassium-carbonate in refluxing acetone to furnish after purification 7-bromo-5,8-dimethoxy-quinaldonitrile (91) in 56% yield. The ^{1}H-NMR spectrum (Fig.25) of the compound 91, showed the presence of two methoxyl protons at 4.00 ppm and 4.10 ppm. The H-6 proton appeared at 7.05 ppm as singlet while rest of the aromatic

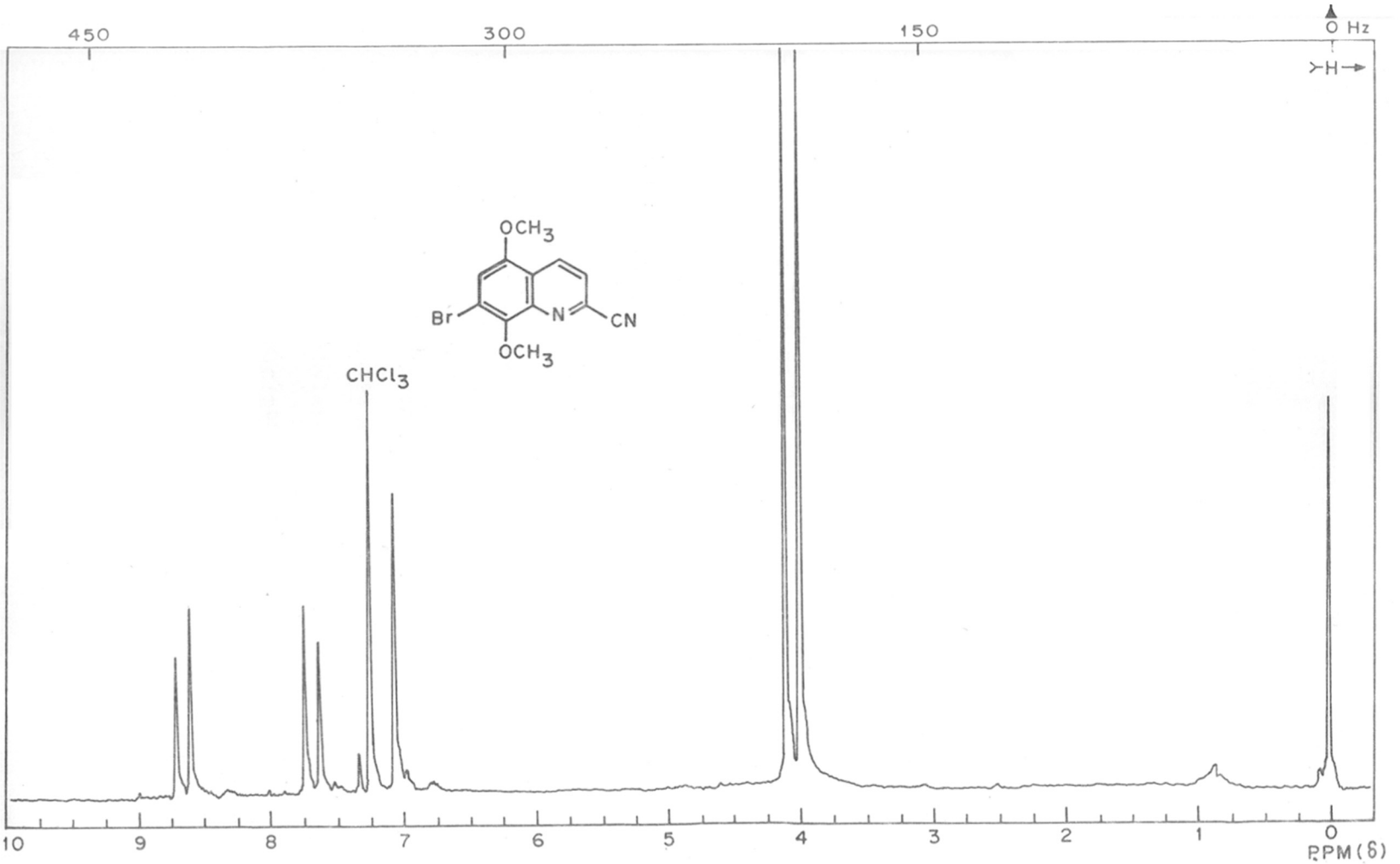


FIG. 25 : ^{1}H-NMR SPECTRUM OF COMPOUND (91) IN $CDCl_3$

protons resonated as doublets (J= 8 Hz) at 7.65 ppm and 8.65 ppm. The appearance of the peaks at m/z 291, 293 confirmed the proposed structure.

Later it was observed that the set of reactions mentioned above could be carried out without exhaustive purification of intermediates in more or less same yield.

Hydrolysis of the nitrile 91 in a refluxing solution of potassium hydroxide in ethanol gave 7-bromo-5,8-dimethoxyquinaldic acid (88) in 86% yield. This acid was found to be identical in all respects with the one obtained earlier (Scheme 17).

Regiochemistry of the bromine

In this scheme for the synthesis of 7-bromo-5,8-dimethoxyquinaldic acid (88), the structure of the intermediates were based on the spectral data. There was, therefore, a need to prove the structure of the final product 88 by some chemical means. This was done as follows.

The known 7-bromo-quinolinequinone (60)[55] (obtained from 7-bromo-5-nitro-8-hydroxyquinoline) (Scheme 9) was reduced to hydroquinone 93 with aqueous sodium dithionite and subsequently o-methylated by conventional procedure to afford 5,8-dimethoxy-7-bromoquinoline (89).

The above compound 89 was also obtained from the acid 88 as follows. The acid 88 was decarboxylated by heating

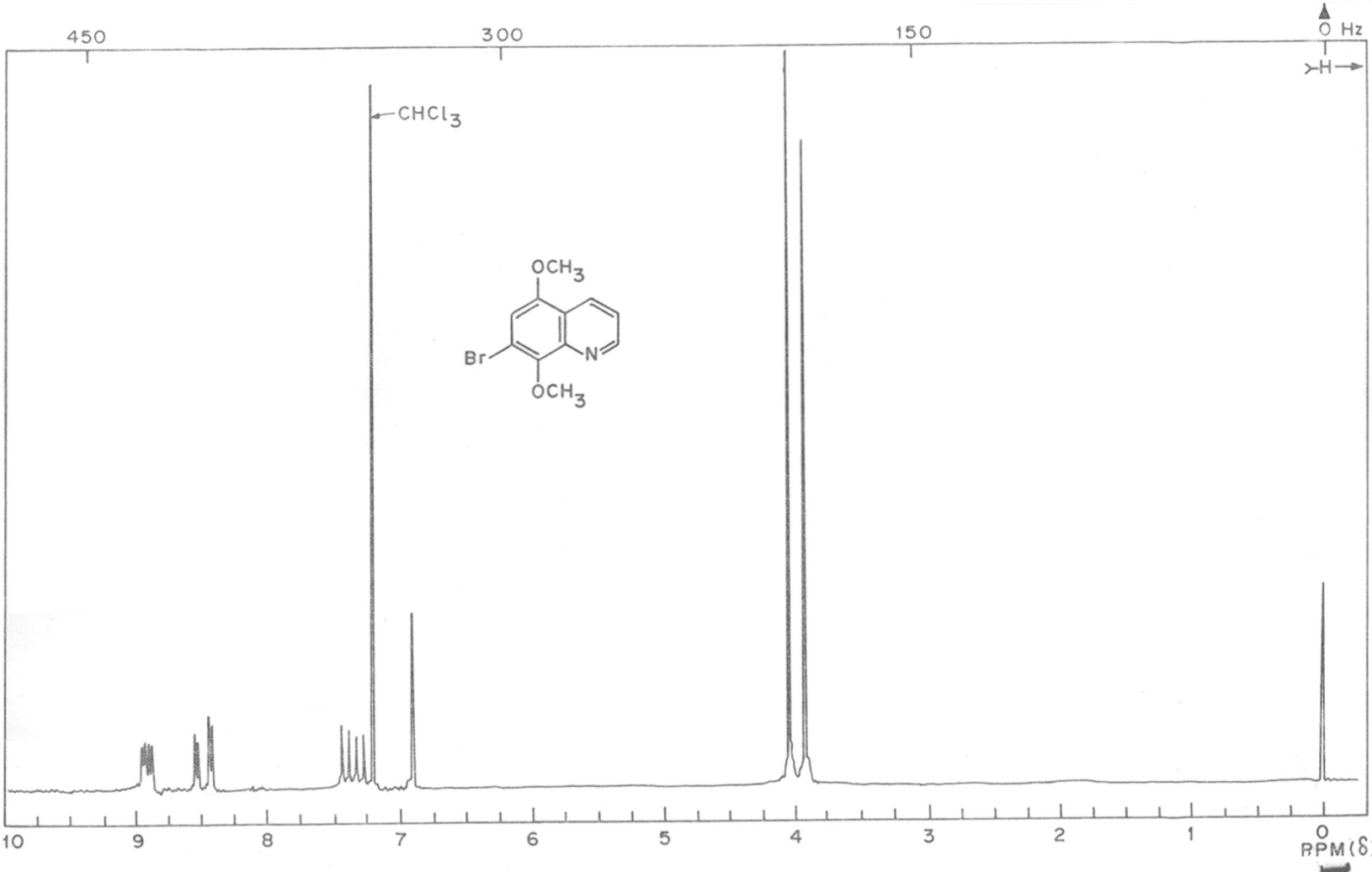


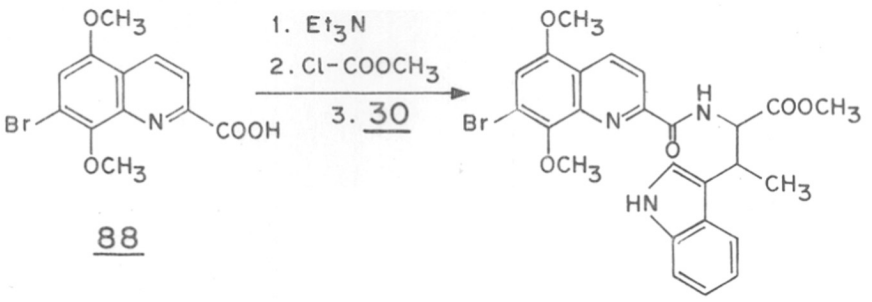
FIG. 26 : ^{1}H-NMR SPECTRUM OF COMPOUND (89) IN $CDCl_3$

at 200° in an atmosphere of nitrogen to furnish 89. The ^{1}H-NMR spectrum (Fig.26) revealed two methoxy protons at 3.95 ppm and 4.1 ppm. The H-6 proton resonated at 6.93 ppm as a singlet while H-2, H-3 and H-4 protons resonated at 7.30 ppm, 8.5 ppm and 8.94 ppm as doublet of doublets.

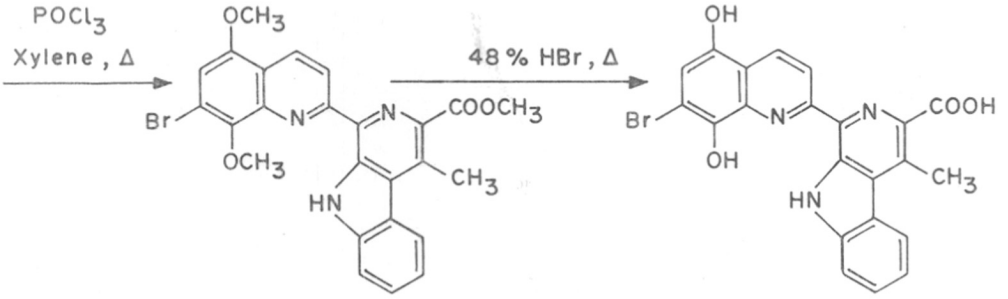
Both the products 89 (by decarboxylation and unambiguous route) were found to have identical properties. This, therefore, conclusively proved the structure assigned for 88 as well as the intermediates thereof.

Having got the required, properly substituted quinaldic acid at hand, efforts were now directed towards the condensation with tryptophan derivative. This condensation of the acid 88 with β -methyltryptophan 30 was effected using methylchloroformate as the condensing agent in presence of triethylamine at 0° to afford the anticipated amide 94 in 97% yield. The structure of 94 was demonstrated by the ^{1}H-NMR spectrum (Fig.27) in which a triplet at 1.50 ppm was assigned for CH $C\underline{H}_3$ (diastereomeric mix), while the singlets at 3.54 ppm, 3.62 ppm, 3.82 ppm and 3.94 ppm integrated for nine protons corresponding well with three methoxyl groups. One extra singlet was accounted because of the diastereomers. Multiplets at 3.94 ppm and 5.17 ppm were due to methine protons ($C\underline{H}$ CH_3 and NH $C\underline{H}$ $COOCH_3$). Presence of a singlet at 7.04 ppm may be attributed to the H-6 proton of the quinoline moiety. While rest of the aromatic

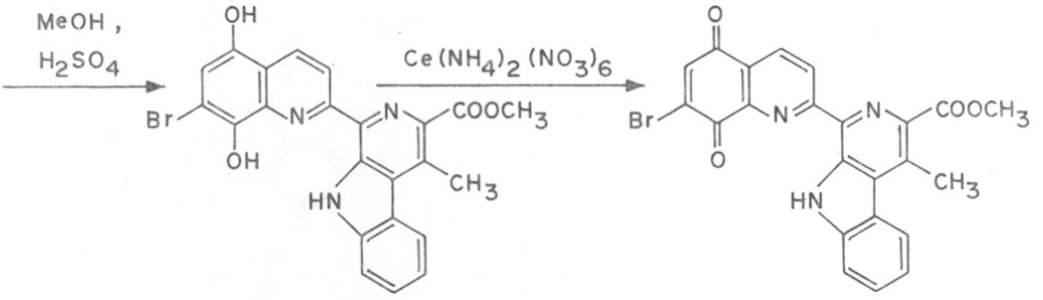
SCHEME 20



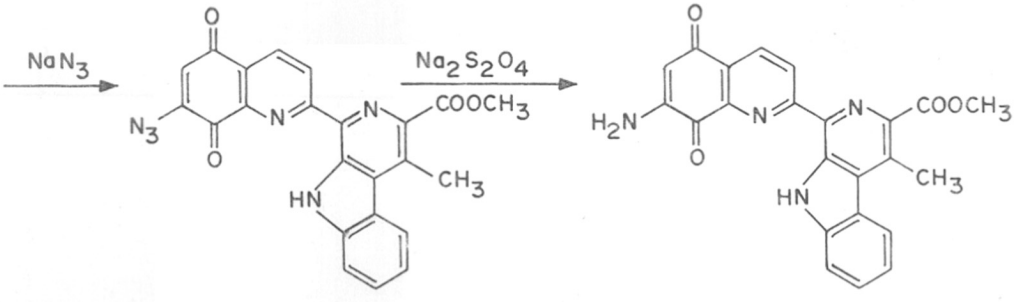
88 94



95 96



97 98



99 100

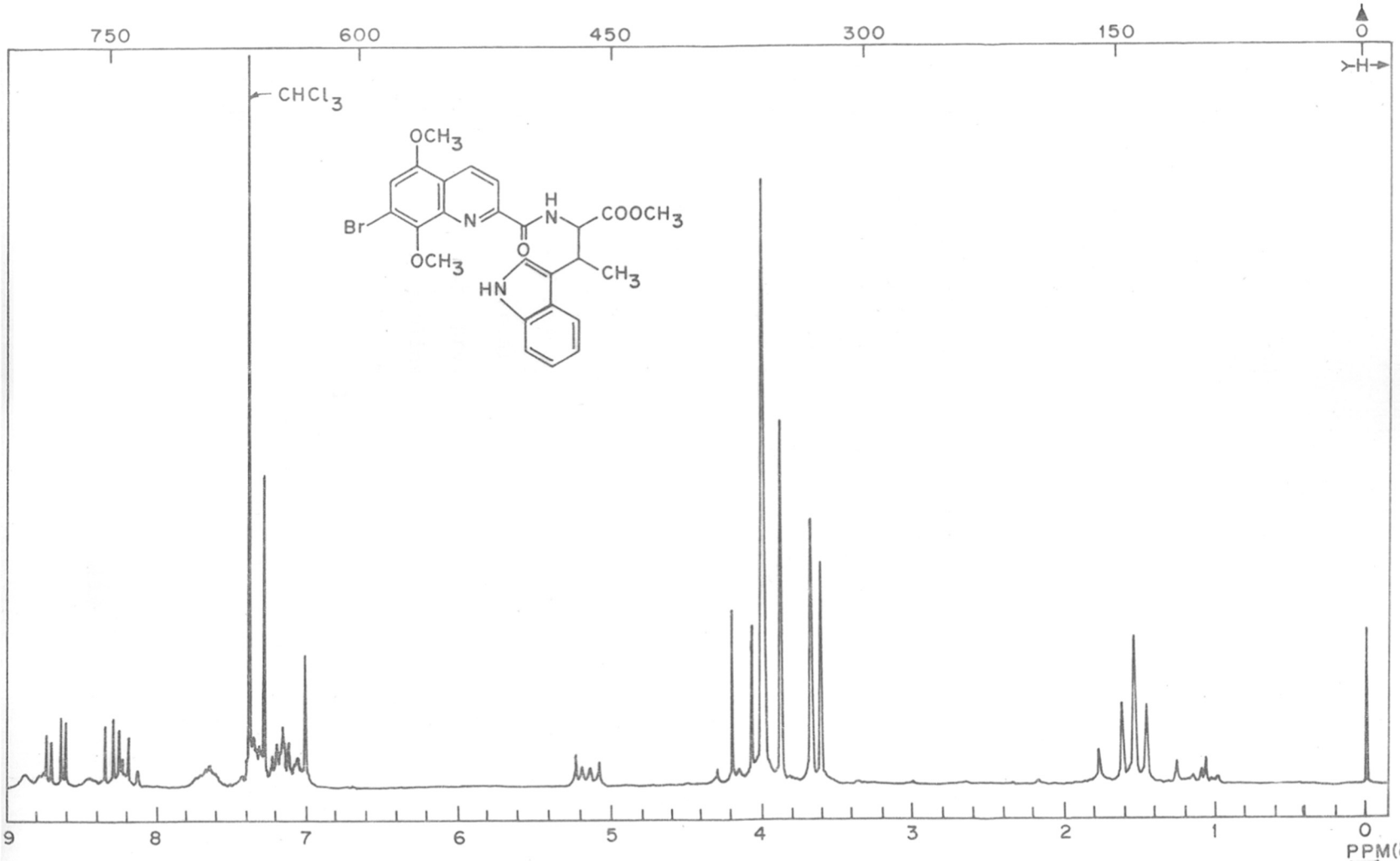


FIG. 27 : ^{1}H-NMR SPECTRUM OF COMPOUND (94) IN $CDCl_3$

protons resonated beyond 7.0 ppm. The IR spectrum showed absorptions at 3340 cm^{-1}, 1740 cm^{-1} and 1690 cm^{-1} assigned for NH, $COOCH_3$ and amide carbonyls respectively. The molecular ion peak at m/z 525, 527 further supported the structure.

Having achieved the synthesis of amide 94 (with non-interfering group OCH_3) it was subjected to cyclisation reaction. Thus, the amide 94 was heated with $POCl_3$ at xylene reflux temperature for 4 hours to furnish a product which was the desired β-carboline 95 indeed! in 88% yield. In the ^{1}H-NMR spectrum (Fig.28) of the compound 95, the presence of four singlets at 3.20 ppm, 4.00 ppm, 4.07 ppm and 4.26 ppm were attributed to C-methyl, and 3X OCH_3 groups respectively. The H-6 protons of the quinoline moiety resonated at 6.97 ppm as a singlet. The quartet typical of H-3 and H-4 protons of quinoline was also observed at 8.62 ppm (J= 9 Hz) and 8.85 ppm (J= 9 Hz) while rest of aromatic protons appeared in the region 7.23 - 8.38 ppm as multiplets. The presence of a broad singlet characteristic of β-carbolines at 12.37 ppm (D_2O-exchangeable) was attributed to NH. The IR spectrum showed stretchings at 3340 cm^{-1} and 1730 cm^{-1} for NH and ester carbonyl respectively. The mass spectrum confirmed the formation of the β-carboline 95 by the presence of m/z peaks at 505 and 507.

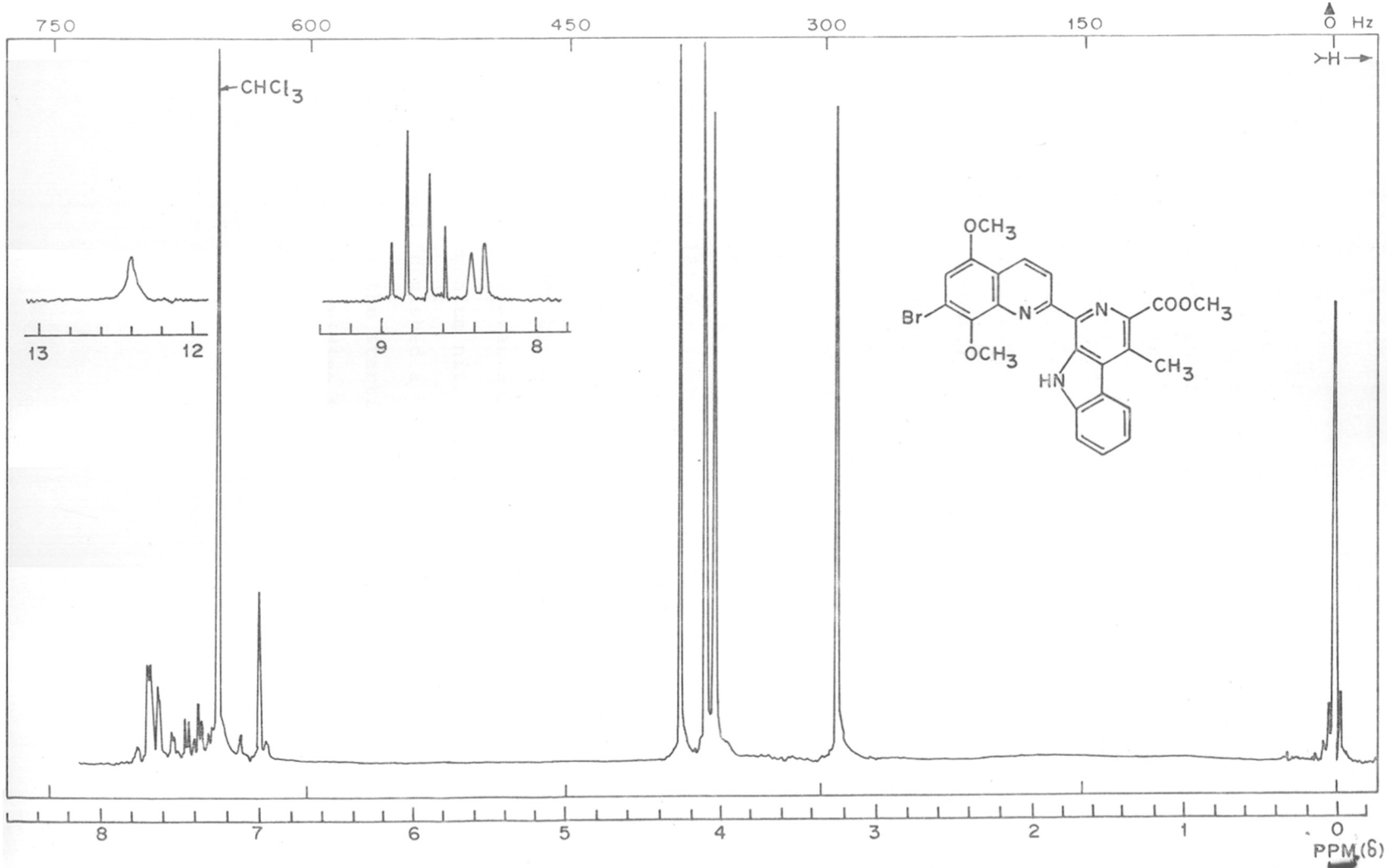


FIG. 28 : ^{1}H-NMR SPECTRUM OF COMPOUND (95) IN $CDCl_3$

It is proper to mention here the suspicion that the urethane moiety in the amide 78 might be interfering during cyclodehydrating reactions probably holds true, because of successful cyclisation of the amide 94 having a non-interfering group in its structure.

Thus, the next tasks were to get the quinone moiety and to convert bromide into amino group. The former transformation was initially carried out on the model compound viz. 7-bromo 5,8-dimethoxyquinaldonitrile(91).

The compound 91 was treated with aqueous cerric ammonium nitrate in methylene chloride at room temperature. After usual work up, 7-bromo-2-cyano-5,8-quinolinequinone (65) (Scheme 18) was isolated in 72% yield which was identical with the one obtained earlier by the oxidation of 7-bromo-5-acetamidoquinaldonitrile (73) (Scheme 18).

Encouraged by the success with the model compound, the β-carboline 95 was subjected to oxidation reaction with cerric ammonium nitrate. The reaction was very sluggish and furnished a very small amount of quinone 98, however most of the starting material was recovered. Having failed to oxidise the β-carboline 95 with CAN satisfactorily, argentic oxide was used for direct oxidation in which case also the bromoquinone 98 was isolated in poor yield with most of the starting material being unreacted.

Since the direct oxidation of 5,8-dimethoxy derivative 95 to 5,8-quinone derivative, by various methods failed, it was thought to attempt the oxidation of hydroquinone 97 (hydroquinones being more prone to oxidations to the corresponding quinones) which can be easily derived from 95. Thus 95 was treated with trimethylsilyl iodide in refluxing chloroform. After 24 hours, the product was isolated which on esterification with MeOH-H_2SO_4 furnished the starting β-carboline 95 (only the ester methyl was hydrolysed).

Therefore, exhaustive demethylation was carried out by refluxing 95 with 48% aq. HBr for 8 hours. The resulting crude hydroquinone acid 96 was converted into its methyl ester 97, which was then oxidised without purification by suspending in methylene chloride and treating with aqueous cerric ammonium nitrate at room temperature for 6 hours with vigorous stirring. Purification of the residue over silica gel column furnished the desired red bromoquinone 98 in 43% yield. The ^{1}H-NMR spectrum (Fig.29) of 98 showed two singlets at 3.20 ppm and 4.10 ppm attributed to C-CH_3 and OCH_3 respectively. In the aromatic region, careful examination revealed a singlet due to H-6 at 7.57 ppm, a characteristic quartet for H-3 and H-4 protons at 8.36 and 8.98 ppm (J= 8 Hz) while rest of the protons resonated as multiplets. The characteristic singlet for the NH indole

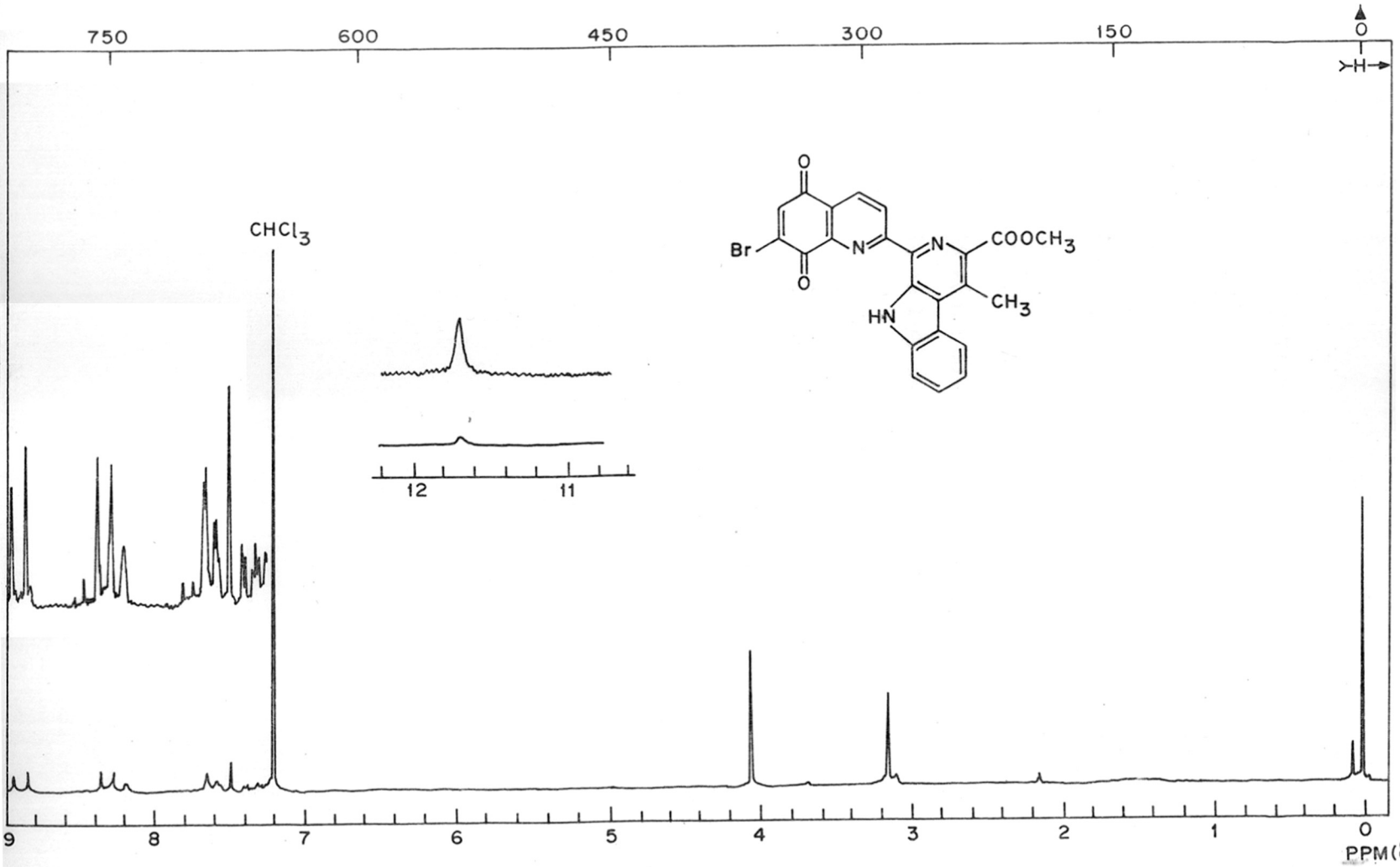


FIG. 29 : ^{1}H-NMR SPECTRUM OF COMPOUND (98) IN $CDCl_3$

was also observed at 11.77 ppm. This spectrum corresponded well with the reported spectra for 98[31]. The further confirmation came from the mass spectrum which revealed peaks at m/z 475 and 477.

Since the transformation of the bromoquinone 98 to lavendamycin methyl ester 100 via azidoquinone 99 has already been reported by Kende et al.[31] this route for the β-carboline 98 would constitute a formal total synthesis of lavendamycin methyl ester 100.

GENERAL REMARKS

Melting points were determined, either in open capillaries or on Köffler block instrument, and are uncorrected. Infrared spectra (IR) (ν_{max} in cm^{-1}) were recorded in nujol or chloroform or neat in a Perkin Elmer Model 683 Spectrophotometer with sodium chloride optics. ^{1}H-NMR spectra were recorded on a Varian T-60 or Varian FT-80A or Jeol PMX-60 or Bruker WH-90 spectrophotometer in CCl_4 or $CDCl_3$ or DMSO or DMSO-d_6 or Acetone-d_6 containing TMS as an internal standard. All chemical shifts are reported in parts per million (δ) downfield from TMS. Mass spectra were recorded on AEI MS 30 double beam mass spectrometer or CEC 21-110B spectrometer. All solvents and reagents were purified and dried by standard techniques. All evaporations were carried out under reduced pressure on Buchi rotary evaporator below 50°C. Progress of the reactions was monitored by thin layer chromatography (TLC) on 0.2 mm layers of silica gel, prepared with Acme silica gel (400 mesh) and the chromatograms were exposed to iodine vapours or ultraviolet lamp for visualisation. Column chromatography was carried out using silica gel (60-120 mesh, Acme make).

EXPERIMENTAL

8-Hydroxyquinoline-1-oxide (12)

(a) Using H_2O_2-acetic acid:

To a stirred solution of 8-hydroxyquinoline (7) (60 g, 0.41 mol) in acetic acid (120 ml) at 70-80° was added H_2O_2 (40 ml, 50%), (10 ml after every hour). After 4 hours, the mixture was cooled, diluted with water and neutralised with sodium bicarbonate. Solid was separated which was filtered, dried and recrystallised from benzene to afford yellow crystals of 12 (40 g, 60% yield), m.p. 138° (lit.[35] m.p. 139).

(b) Using m-CPBA

To 8-hydroxyquinoline (7) (0.45 g, 0.003 mol) in chloroform (20 ml) was added m-CPBA (0.75 g, 0.0043 mol) and then stirred overnight at room temperature. Excess of peracid was destroyed by successive washing of the organic layer with aqueous sodium sulphite, aqueous sodium carbonate, brine and dried (Na_2SO_4). The solvent was evaporated and the residue was dried under vacuo to furnish (12) in 83% yield.

8-Hydroxy-1-methoxyquinoline sulphate (13)

A mixture of the N-oxide 12 (18 g, 0.11 mol) and dimethylsulphate (10 ml, 0.11 mol) was heated on a water bath for three hours during which time, yellow solid dissolved to form a brown viscous mass. It was washed with ethylacetate to remove unreacted N-oxide and then used as such for further reaction.

8-Hydroxyquinaldonitrile (14)

A solution of the salt 13 in water (50 ml) was added dropwise to an aqueous solution of sodium cyanide (17 g in 50 ml water) with stirring at 0°. After 3 hours, acetic acid was added to adjust the pH of the reaction mixture at 3-4 and then the solid was filtered,thoroughly washed with water and dried to yield the 8-hydroxyquinaldonitrile (14) (13.7 g, 72% yield from 12), m.p. 134°, (lit.[36] 135°).

8-Methoxyquinaldonitrile (18)

A mixture of 8-hydroxyquinaldonitrile (14) (8 g, 0.047 mol), dimethylsulphate (7.5 ml, 0.078 mol) and anhydrous K_2CO_3 (35 g, 0.25 mol) was heated under reflux for one hour. After the removal of acetone, water was added to the reaction mixture and stirred for 0.5 hour. The solid separated which was filtered to furnish 8-methoxyquinaldonitrile (18) (8 g, 95% yield) as pale yellow crystals, m.p. 105°. IR(nujol): 2240 (C≡N); ^{1}H-NMR ($CDCl_3$): δ 4.1 (s, 3H, OCH_3), 7.0 - 7.6 (m, 3H, Ar), 7.66 (d, 1H, J=8 Hz, Ar), 8.2 (d, 1H, J=8 Hz, Ar); m/z 184.

Analysis: Calculated for $C_{11}H_8N_2O$: C, 71.74; H, 4.35; N, 15.22; Found: C, 71.57; H, 4.29; N, 15.18%.

8-Methoxyquinaldic acid (10)

The nitrile 18 (8 g, 0.043 mol) was heated under reflux with aqueous sodium hydroxide solution (200 ml, 10%) for 4 hours with stirring. The reaction mixture was cooled and then acetic

acid was added to adjust the pH at 4. The aqueous solution was repeatedly extracted with ethyl acetate. The combined organic layer was successively washed with water, brine, dried (Na_2SO_4) and concentrated to give 8-methoxyquinaldic acid (10) (5.3 g, 60% yield); m.p. 147° (lit.[38] m.p. 150°).

Methyl tryptophan hydrochloride (9): This was prepared by esterification of tryptophan by following the reported procedure[39].

Methyl N_b-(8-methoxyquinaldoyl) tryptophan (11):

(a) Using toluene-p-sulphonyl chloride

To a stirred solution of 8-methoxyquinaldic acid (10) (1 g, 0.0049 mol) in dry tetrahydrofuran (25 ml) was added triethylamine (0.7 ml, 0.005 mol) and the resulting solution cooled to 0°. Toluene p-sulphonyl chloride (0.94 g, 0.0049 mol) was added in one portion. After one hour (TLC indicated the absence of the acid 10 and formation of mixed anhydride under UV light), triethylamine (0.7 ml, 0.005 mol) and methyl tryptophan hydrochloride (1.25 g, 0.005 mol) were added. The mixture was allowed to attain room temperature during one hour. Triethylamine hydrochloride was removed by filtration and the filtrate concentrated to dryness. Purification on silica gel column with benzene yielded the amide 11 (1 g, 50% yield) as a white solid, m.p. 181-182°. IR (nujol): 3330 (NH), 1760 ($COOCH_3$), 1670 (amide). ^{1}H-NMR ($CDCl_3$): δ 3.44 (d, 2H, J= 6 Hz), 3.62 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.9-5.2 (m, 1H,

NHCHCOOCH$_3$), 6.82 - 7.67 (m, 8H, Ar), 8.0 - 8.37 (m, 3H), 8.56 - 8.66 (1H, bd). m/z 403.

Analysis: Calculated for $C_{23}H_{21}N_3O_4$: C, 68.48; H, 5.20; N, 10.40; Found: C, 68.26; H, 5.33; N, 10.27%.

(b) Using methylchloroformate

A stirred solution of 8-methoxyquinaldic acid (10) (1 g, 0.0049 mol) in dry tetrahydrofuran (25 ml) was treated with triethylamine (0.7 ml, 0.005 mol). The resulting solution was cooled to 0°, methylchloroformate (0.39 ml, 0.005 mol) added in one portion. After 1 hour (TLC), triethylamine (0.7 ml, 0.005 mol) was introduced followed by methyltryptophan-hydrochloride (1.25 g, 0.005 mol). The mixture was allowed to attain room temperature during 1 hour, triethylamine hydrochloride was filtered of and washed with tetrahydrofuran.

The filtrate was rotary evaportated to yield a residue which was purified by column chromatography over silica gel (benzene as eluent) to furnish the amide 11 (1.2 g, 60% yield) as a white solid, m.p. 181-182°.

Alternately, the crude product was also purified by crystallisation (benzene-pet. ether) m.p. 179-181°.

The compound was found identical in all respects with the sample obtained earlier.

1-[2'-(8'-Methoxyquinolyl)]-3-methoxycarbonyl-β-carboline (20): A solution of the amide 11 (0.1 g, 0.25 mol) in dry xylene (2 ml) was heated under reflux with phosphorous oxychloride

(1 ml, 6.5 m.mol) for 6 hours. It was cooled and poured over crushed ice and rendered alkaline using Na_2CO_3. The xylene layer was separated and the aqueous layer was extracted repeatedly with ethyl acetate. The combined organic layer was washed with water, brine, dried (Na_2SO_4) and rotary evaporated under reduced pressure to furnish the β-carboline 20 (0.03 g, 29% yield) as a yellow solid. After recrystallisation from benzene it melted at 233-234°; IR(nujol): 3340(NH), 1720 ($COOCH_3$), ^{1}H-NMR ($CDCl_3$): δ 4.00 (s, 3H, OCH_3), 4.1 (s, 3H, OCH_3), 7.23 - 7.87 (m, 6H, Ar), 8.07 (d, 1H, J= 9 Hz, Ar), 8.13 (d, 1H, J= 9 Hz,Ar), 8.77 (s, 1H), 8.80 (d, 1H, J= 9 Hz, Ar), 12.3 (bs, 1H, NH). M/z 383.

Analysis: Calculated for $C_{23}H_{17}N_3O_3$: C, 72.06; H, 4.43; N, 10.63; Found: C, 72.29; H, 4.72; N, 10.97%.

Ethylidene isopropylamine (21)

A 250 ml two-necked flask fitted with a reflux condenser and a dropping funnel was immersed in ice-bath. It was charged with isopropylamine (43 ml, 0.51 mol) followed by acetaldehyde (28 ml, 0.5 mol) in portions over a period of 2 hours with stirring. KOH pellets were added and the reaction mixture stirred for additional 15 minutes. It was then allowed to stand for 10 minutes and layer separated. The lower layer (dark brown) was discarded while the upper layer was kept over potassium hydroxide (crushed) in freeze overnight and distilled to give colourless aldimine 21 (31 g, 72% yield),

b.p. 55-56°/760 mm (lit.[41] b.p. 58-60°/760 mm).

3-(Isopropylamino ethylidene)-indole (22)

To a solution of indole (8) (2.93 g, 0.25 mol) in glacial acetic acid (150 ml) < 15°C was added ethylidene isopropylamine (23.4 g, 0.275 mol) in benzene (50 ml) with stirring during 10 minutes. The solution was stored in freeze at 0° for 4 days. The reaction mixture was then poured over ice-water (500 ml) and washed with ether (100 ml). The ether layer was once washed with dilute hydrochloric acid and then the combined aqueous layer was rendered basic with 10N aqueous NaOH (the temperature being maintained below 25°C). The oil was separated which solidified after keeping overnight in freezer. The solid was filtered and dried to furnish the amine 22 (30 g, 60% yield), m.p. 114°C (lit.[40] m.p. 113-117.5°C). ^{1}H-NMR ($CDCl_3$) δ 1.07 (d, 6H, J= 6 Hz), 1.47 (d, 3H, J=6 Hz), 1.7 (s, 1H, NH, exchanges with D_2O), 2.83 (m, 1H, J=6 Hz), 4.07 (q, 1H, J= 6 Hz), 6.87 - 7.77 (m, 5H), 8.27 (br.s, exchanges with D_2O).

Methyl nitroacetate (28): This was prepared from nitromethane in two steps according to the reported procedure[43].

Methyl-β-(3-indolyl)-β-methyl-α-nitropropionate (29)

In a 100 ml two-necked round bottom flask fitted with a reflux condenser, a guard tube and a gas inlet were placed 3-(isopropylamino ethylidene)-indole (22) (2.02 g, 0.01 mol), methylnitroacetate (28) (1.42 g, 0.012 mol) and triethylamine (5 drops) in dry toluene (50 ml). The resulting mixture was

heated with stirring at 110-120° in a stream of nitrogen for 15 hours. The solid was filtered, the filtrate rotary evaporated and the residue chromatographed (silica gel) using benzene-ethylacetate (9:1) as eluent to furnish the nitroester 29 (2.26 g, 85% yield), m.p. 92-95°; IR (Nujol): 3370 (NH), 1750 ($COOCH_3$); ^{1}H-NMR ($CDCl_3$): δ 1.50 (d, 3H, CH_3, J= 6 Hz), 3.50, 3.80 (s, 3H, OCH_3:diastereomers), 3.90-4.30 (m, 1H), 5.30, 5.35 (d, 1H, $NO_2C\underline{H}$ $COOCH_3$: diastereomers), 6.90 - 7.70 (m, 5H, Ar), 8.00 (br.s, 1H, N$\underline{H}$); m/z 262.

Analysis: Calculated for $C_{13}H_{16}N_2O_2$: C, 67.26; H, 6.90; N, 12.07; Found: C, 67.18; H, 7.11; N, 2.10%.

β-Methyltryptophan methyl ester (30)

A solution of the nitroester 29 (3.5 g, 0.0134 mol) in ethanol (40 ml) was stirred at room temperature in the presence of Raney nickel (3 g) under the atmosphere of hydrogen. The reaction was monitored by TLC. After 24 hours, catalyst was filtered and the filtrate concentrated to a gummy product. This compound was taken in ether, extracted with dilute hydrochloric acid (4 X 25 ml) and then, the aqueous extract was rendered neutral ($NaHCO_3$). The resulting solution was extracted with ether, dried (Na_2SO_4) and concentrated to afford a viscous liquid which solidified gradually on keeping to furnish the amine 30 (2.2 g, 71% yield), m.p. 76-80°C. IR(nujol): 3400, 3300 (NH_2), 1740 ($COOCH_3$); ^{1}H-NMR ($CDCl_3$): δ 1.2, 1.35 (d, 3H, J= 6.3 Hz, CH_3), 1.75 (br.s, 2H, exchanges with D_2O), 3.55, 3.65 (s, 3H, OCH_3), 3.80 (m, 2H, $C\underline{H}CH_3$, $NHC\underline{H}COOCH_3$)

6.7 - 7.7 (m, 5H, Ar), 8.2 (br.s, 1H, NH, exchanges with D_2O), m/z: 232.

Methyl-β-methyl-Nb(8-methoxyquinaldoyl) tryptophan (31)

To a precooled solution of 8-methoxyquinaldic acid (10) (0.39 g, 1.92 m.mol) in dry tetrahydrofuran (10 ml) at 0° was added with stirring triethylamine (0.27 ml, 1.95 m.mol) followed by methylchloroformate (0.14 ml, 2 m.mol) in one lot. After one hour, β-methyltryptophan 30 (0.57 g, 0.0025 mol) in tetrahydrofuran (10 ml) was added in one portion and stirred overnight at room temperature. Solid was separated which was filtered, washed with acetone and then the filtrate was concentrated under reduced pressure to afford a residue. It was chromatographed on silica gel using benzene-acetone (9:1) as eluent to furnish the pure amide 31 (0.675 g, 84%), as a white solid, m.p. 193-195°C; ^{1}H-NMR($CDCl_3$): δ 1.55 (d, 3H, CH_3, J= 6.3 Hz), 3.50 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 3.60 - 4.00 (m, 1H, $C\underline{H}CH_3$), 4.90 - 5.20 (m, 1H, NH $C\underline{H}$ $COOCH_3$), 6.90 - 8.30 (m, 10H, Ar), 8.7 - 9 (br.d, 1H).

Analysis: Calculated for $C_{24}H_{23}N_3O_4$: C, 69.06; H, 5.52; N, 10.07; Found: C, 68.86; H, 5.74; N, 10.18%.

1-[2'(8'-Methoxyquinolyl)]-3-methoxycarbonyl-4-methyl-β-carboline (32).

A stirred solution of the amide 31 (0.5 g, 1.19 mol) in dry xylene (50 ml) containing $POCl_3$ (5 ml, 32.5 m.mol) was

heated under reflux for 4 hours. It was cooled and poured over crushed-ice with stirring. The pH of the resulting solution was adjusted to 8 (Na_2CO_3). The xylene layer was separated. The aqueous layer was extracted with ethylacetate. The combined organic layer was washed with water, brine and dried (Na_2SO_4). The solvent was evaporated to dryness under reduced pressure to furnish the β-carboline 32 m.p. 203-205°C as a yellow solid (0.397 g, 81%). After recrystallisation from benzene the compound melted at 210-212°C. ^{1}H-NMR ($CDCl_3$): δ 3.20 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3), 4.23 (s, 3H, OCH_3), 7.03 - 7.23 (dd, 1H, Ar), 7.33 - 7.77 (m, 5H, Ar), 8.27 (d, 1H, J = 9 Hz), 8.4 (d, 1H, J= 9 Hz), 8.86 (d, 1H, J= 8.7 Hz, Ar), 12.6 (br.s, NH). IR (nujol): 3290(NH), 1730 ($COOCH_3$).

Analysis: Calculated for $C_{24}H_{19}N_3O_3$: C, 72.5; H, 4.78; N, 10.58; Found: C, 72.57; H, 5.01; N, 10.47%.

1-[2'-(8'-Hydroxyquinolyl)]-3-carbomethoxy-4-methyl-β-carboline (34).

A suspension of 32 (0.10 g, 0.25 m.mol) was stirred and refluxed with 48% aq. HBr (2 ml) under an atmosphere of nitrogen for 3 hours. The flask was cooled to room temperature and the contents poured over crushed ice with stirring. The solid thus obtained was filtered and dried to furnish 33 (0.095 g).

A suspension of crude acid 33 (0.095 g) was refluxed in dry

methanol (40 ml) in the presence of concentrated sulphuric acid (1 ml) for 6 hours. The solvent was evaporated under diminished pressure and water was added to it. The solid obtained was filtered and chromatographed using benzene, benzene-ethylacetate (9:1) to give the hydroxyester 34 (0.046 g) and unreacted hydroxy acid 33 (0.040 g) in 85% yield (based on the recovery of 33) m.p. 210-212°C. ^{1}H-NMR ($CDCl_3$): δ 2.45 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3), 6.8-8.3 (m, 9H, Ar), 10.1 (br s, NH). m/z 383.

8-Benzyloxyquinaldonitrile (35)

A mixture of 8-hydroxyquinaldonitrile (14) (3.4 g, 0.02 mol), benzylbromide (5 ml, 0.042 mol), anhydrous potassium carbonate (7.5 g, 0.054 mol) and dry acetone (100 ml) was refluxed with stirring for 2 hours. The reaction was monitored by TLC, solid was filtered and the filtrate concentrated on rotary evaporator to yield a residue which was washed repeatedly with pet. ether to remove unreacted benzyl bromide. Recrystallisation of the residual solid from benzene-pet. ether furnished the benzyl ether 35 (4.63 g, 89% yield) as white needles, m.p. 101°C; IR(nujol): 2220(CN). ^{1}H-NMR($CDCl_3$): δ 5.40 (s, 2H, CH_2), 7.00 - 7.60 (m, 8H, Ar), 7.70 (d, 1H, J= 8 Hz, Ar), 8.20 (d, 1H, J= 8 Hz, Ar). m/z 260.

Analysis: Calculated for $C_{17}H_{12}N_2O$: C, 78.45; H, 4.61; N, 10.76; Found: C, 78.19; H, 4.31; N, 10.56%.

8-Benzyloxyquinaldic acid (36)

The nitrile 35 (2.73 g, 0.011 mol) and 10% potassium hydroxide (10 ml) were heated under reflux for 6 hours. The solution was cooled and rendered acidic with acetic acid and solid was obtained which was filtered and dried to afford the acid 36 (2.5 g, 88% yield) as a white solid, m.p. 80-81°C; ^{1}H-NMR ($CDCl_3$): δ 5.20 (s, 2H, OCH_2), 6.65 (br.s, exchanges with D_2O), 6.95 - 7.6 (m, 8H, Ar), 8.20 (d, 1H, J= 9.5 Hz, Ar), 8.35 (d, 1H, J=9.5 Hz, Ar); IR(nujol): 3140 (broad), 1720 (COOH). m/z 279.

Analysis: Calculated for $C_{17}H_{13}NO_3$: C, 73.11; H,4.66; N, 5.02. Found: C, 72.90; H, 4.52; N, 5.23%.

Methyl-β-methyl-Nb-(8-benzyloxyquinaldoyl)tryptophan (37)

To a stirred solution of the acid 36 (0.487 g, 1.70 m.mol) in dry tetrahydrofuran (10 ml) at 0° was added triethylamine (0.17 g, 1.7 m.mol) followed by methylchloroformate (0.165 g, 1.74 m.mol) in one lot. After 1 hour, β-methyltryptophan 30 (0.383 g, 1.7 m.mol) in dry tetrahydrofuran (20 ml) was added in one portion and stirred overnight. The solid was filtered and the filtrate was evaporated to dryness and the residue was purified by chromatography over silica gel using benzene-ethylacetate (9:1) as eluent to furnish the amide 37 (0.685 g, 80% yield) as a white solid. m.p. 157°C. IR(nujol): 3360 (NH), 1740 ($COOCH_3$), 1680 (CONH); ^{1}H-NMR ($CDCl_3$): δ 1.49 (t, 3H, J= 8 Hz), 3.44, 3.6 (s, 3H, OCH_3),

3.78 (m,1H, $C\underline{H}CH_3$), 5.0 (d, 1H, $NHC\underline{H}COOCH_3$, diastereomers), 5.27 (s, 2H, CH_2), 6.87 - 7.73 (m, 13H, Ar), 8.20 (s, 2H, Ar), 8.84 (bd, 1H, J= 8 Hz). m/z 493.

Analysis: Calculated for $C_{30}H_{27}N_3O_4$: C, 73.00; H, 5.48; N, 8.52; Found: C, 72.81; H, 5.32; N, 5.50%.

1-[2'-(8'-Benzyloxyquinolyl)]-3-methoxycarbonyl-4-methyl-β-carboline (38).

A solution of the amide 37 (0.862 g, 0.00175 mol) and phosphorousoxychloride (10 ml) in dry xylene (20 ml) were heated under reflux with stirring for 4 hours at which time TLC indicated completion of reaction. It was cooled and poured over crushed-ice, rendered alkaline with Na_2CO_3 and the xylene layer was separated. The aqueous layer was repeatedly extracted with methylene chloride. The combined extract was washed successively with water, brine, dried (Na_2SO_4) and concentrated under diminished pressure to yield a residue which was purified by crystallisation with acetone to yield β-carboline 38 (0.41 g, 50% yield) as a white solid, m.p. 255-256°C. IR(nujol): 3260 (NH), 1700 ($COOCH_3$). ^{1}H-NMR ($CDCl_3$): δ 3.20 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 5.32 (s, 2H, OCH_2), 6.27 (m, 1H), 7.2 - 8.0 (m, 11H, Ar), 8.30 (d, 2H, J= 9 Hz), 9.10 (d, 1H, J=9 Hz), 12.43 (bs, 1H, NH).

Analysis: Calculated for $C_{30}H_{23}N_3O_3$: C, 76.11; H, 4.86; N, 8.87; Found: C, 75.56; H, 5.1; N, 8.80%.

5-Bromo-8-methoxyquinaldic acid (42)

To a stirred solution of 8-methoxyquinaldic acid (10) (3 g, 0.0015 mol) in chloroform (50 ml) was added dropwise a solution of bromine (3 ml, 0.056 mol) in chloroform (10 ml) during fifteen minutes. An orange solid was separated which was filtered and dried (3.6 g). It was dissolved in a saturated solution of sodium bicarbonate and then reprecipitated by concentrated HCl. The solid on recrystallisation from ethanol furnished the bromoacid 42 (2.60 g, 63% yield) as shining needles m.p. 180-185°. IR (nujol): 3490 (COOH), 1740 (COOH). ^{1}H-NMR (DMSO): δ 4.90 (broad, COOH), 7.20 (d, 1H, J= 9 Hz, Ar), 7.90 (d, 1H, J= 9 Hz, Ar), 8.20 (d, 1H, J= 9 Hz, Ar), 8.60 (d, 1H, J= 9 Hz, Ar).

Methyl-β-methyl-Nb(5'-Bromo-8'-methoxyquinaldoyl)tryptophan (43)

To a stirred solution of the bromoacid 42 (0.570 g, 0.002 mol) in dry tetrahydrofuran (10 ml) at 0°C were added triethylamine (0.28 ml, 0.002 mol) followed by methylchloroformate (0.16 ml, 0.0021 mol). The mixture was stirred for one hour till all the acid had been consumed (TLC). To the above mixture was added, β-methyltryptophan 30 (0.464 mg, 0.002 mol) in tetrahydrofuran (5 ml) and stirred overnight. The reaction mixture was filtered, the solid washed with acetone and the combined filtrate was concentrated under vacuum. The crude material on purification over chromatography using benzene as eluent furnished the amide 43 (0.90 g, 95% yield) m.p. 90-95°C. IR(nujol): 3340 (NH), 1740 ($COOCH_3$), 1670 (amide).

^{1}H-NMR ($CDCl_3$): δ 1.50,1.55 (d, 3H, $CHC\underline{H}_3$ diastereomers), 3.54, 3.60 (s, 3H, OCH_3), 3.80 (m, 1H, $C\underline{H}CH_3$), 4.01, 4.06 (s, 3H, OCH_3), 5.10 (m, 1H, $HNC\underline{H}COOCH_3$), 6.8-9.00 (m, 10H, Ar). m/z 495, 497.

Analysis: Calculated for $C_{24}H_{22}N_3O_4Br$: C, 58.06; H, 4.44; N, 8.47. Found: C, 58.11; H, 4.31; N, 8.23%.

1-[2'-(5'-bromo-8'-methoxyquinolyl)]-3-methoxycarbonyl-4-methyl-β-carboline (44):

The amide 43 (0.50 g, 0.001 mol) in xylene (25 ml) was heated under reflux with $POCl_3$ (5 ml) in an oil bath. After 4 hours the contents were poured over crushed-ice the xylene layer was separated. The aqueous layer was neutralised with $NaHCO_3$ and then extracted with chloroform. The combined organic extract was washed with water and dried (Na_2SO_4). Evaporation of the solvent under diminished pressure gave a residue which on purification over chromatography (silica gel) using benzene as an eluent afforded the pure β-carboline 44 (0.150 g, 31%) m.p. 282-285°C. IR (nujol): 3380 (NH), 1720 ($COOCH_3$). ^{1}H-NMR ($CDCl_3$): δ 3.12 (s, 3H, CH_3), 4.07 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 6.89 (d, 1H, J= 7.6 Hz, Ar), 7.23 - 7.6 (m, 3H, Ar), 7.65 (d, 1H, J= 7.6 Hz, Ar), 8.17 (d, 1H, J= 7.6 Hz, Ar), 8.41 (d, 1H, J= 9.45 Hz, Ar), 8.88 (d, 1H, J= 9.45 Hz, Ar), 12.30 (br.s, 1H, NH).

5-Nitroso-8-hydroxyquinoline hydrochloride (45):

To a solution of 8-hydroxyquinoline (58 g, 0.4 mol),

concentrated HCl (75 ml) and ice (200 g) in water (200 ml) was added a solution of $NaNO_2$ (30 g, in 100 ml water) in portions with vigorous shaking over 1 hour at 0°. The reaction mixture was allowed to stand overnight at 0° and the solid was filtered, washed with cold water and dried to furnish 5-nitroso-8-hydroxyquinoline hydrochloride (45) (80 g, 87% yield).

5-Amino-8-hydroxyquinoline sulphate (46):

A two litre flask was charged with freshly prepared salt 45 (40 g, 0.174 mol) dissolved in water (160 ml) and 5N NaOH solution (260 ml). The solution was heated to 40° and then treated with sodium dithionite (95 g), the temperature rose spontaneously to 75-80°. A rapid stream of nitrogen was passed and the orange solution was allowed to cool slowly to about 50°C at which temperature 12N sulphuric acid (250 ml) was added. When the evolution of sulphur dioxide had subsided, it was stirred under diminished pressure with magnetic stirring till most of the dissolved gases had been removed. The resulting precipitate was cooled and filtered to give 5-amino-8-hydroxyquinolinesulphate (46).

The crude salt was used as such for further reaction.

5,8-quinolinequinone (47):

The above crude salt 46 (20 g, 0.078 mol) was dissolved in a mixture of water (200 ml), ice (200 g) and 12N sulphuric acid (70 ml). It was placed in a separating funnel and shaken

thoroughly while 2N potassium dichromate solution (60 ml diluted to 80 ml) was added. This was followed by the addition of ice-cold chloroform (500 ml). The contents were then vigorously shaken for 5 minutes. The layers were allowed to separate and chloroform layer was removed. The aqueous solution was extracted twice with cold chloroform (400 ml). The combined extract was washed with brine, dried (Na_2SO_4) and rotary evaporated (below 50°) to give the quinone 47 (6.7 g, 54% yield) as a yellow solid, m.p. 116-118°C (lit.[48] m.p. 120-121°).

5,8-Dihydroxyquinoline (48)

A solution of quinone 47 (5.26 g, 0.033 mol) in ethyl acetate (100 ml) was taken in a 250 ml separating funnel. To it an aqueous solution of sodium dithionite (11 g in minimum water) was added and then shaken vigorously when an exothermic reaction ensued. The aqueous solution was extracted twice with ethyl acetate and the combined ethyl acetate layer was washed successively with water, brine and dried (Na_2SO_4) and rotary evaporated to furnish hydroquinone 48 (4.79 g, 90% yield) as yellow solid.

5-Methoxy-8-hydroxyquinoline (49):

A solution of hydroquinone 48 (0.668 g, 4 m.mol) in dry acetone (25 ml) was refluxed with dimethylsulphate (0.396 ml, 4 m.mol) in presence of anhydrous potassium carbonate (2 g). The reaction was monitored by TLC. After completion of the

reaction, acetone was distilled, water added and then stirred for 0.5 hour. The aqueous layer was extracted with ethyl acetate which was washed with water, brine, dried (Na_2SO_4) and evaporated. The residue was resolved by column chromatography over silica gel using benzene as the eluent to furnish the monomethyl ether 49 (0.349 g, 48% yield), m.p. 77°C. IR(nujol): 3340 (OH); ^{1}H-NMR ($CDCl_3$): δ 4.13 (s, 3H, OCH_3), 7.10 (d, 1H, J= 8 Hz, Ar), 7.47 (d, 1H, J=8 Hz, Ar), 7.80 (dd, 1H, Ar), 9.00 (dd, 1H, Ar), 8.23 (m, 1H). m/z 175.

Analysis: Calculated for $C_{10}H_9NO_2$: C, 68.57; H, 5.14; N, 8.0. Found: C, 68.63; H, 5.21; N, 7.92%.

5-Methoxy-7-azobenzene-8-hydroxyquinoline (50):

To a stirred solution of phenol 49 (3.4 g, 0.019 mol) in dilute hydrochloric acid at 0°, was added dropwise, benzene diazonium chloride solution (prepared from 2.7 g of aniline, 2.4 g of $NaNO_2$ and 10 ml HCl). The solution was rendered alkaline with sodium carbonate when the solution turned violet-red. The aqueous layer was extracted with ethyl acetate and then washed with water, brine and dried (Na_2SO_4). Solvent removal gave a residue which was purified by column chromatography over silica gel to furnish the azo compound 50 (3.34 g, 62% yield) as dark violet-red solid m.p. 125°, IR (nujol): 3440 (broad, OH), 1610, 1600, 1580. ^{1}H-NMR ($CDCl_3$): δ 3.93 (s, 3H, OCH_3),

6.57 (s, 1H, Ar), 7.0 - 7.7 (m, 6H, Ar), 8.2 (dd, 1H, Ar), 8.80 (m, 1H, Ar). m/z 279.

Analysis: Calculated for $C_{16}H_{13}N_3O_2$: C, 68.82; H, 4.66; N, 15.05. Found: C, 68.98; H, 4.70; N, 14.78%.

5-Methoxy-7-acetamido-8-hydroxyquinoline (52):

To a refluxing solution of the azo compound 50 (2 g, 7 m.mol) in ethanol (25 ml) was added sodium dithionite (6 g in minimum water) when the red colouration immediately disappeared. The hot reaction mixture was then treated with acetic anhydride (25 ml) and stirred at room temperature. After 1 hour at room temperature the solution was rendered neutral with Na_2CO_3 and extracted with $CHCl_3$. The organic layer was washed with brine and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure afforded a residue which on purification by column chromatography over silica gel with benzene-ethylacetate (9:1) and increasing polarity of ethylacetate as the eluent furnished first the acetanilide [0.118 g, 65% yield (based on aniline liberated)] as the byproduct and then the desired amide 52 (0.77 g, 46% yield) m.p. 178-180°. IR(nujol): 3340, 3260 (NH and OH), 1670 (amide); ^{1}H-NMR ($CDCl_3$): δ 2.27 (s, 3H, $COCH_3$), 3.93 (s, 3H, OCH_3), 4.76 (br, 1H, NH), 7.24 (dd, 1H, Ar), 7.84 (br.s, 1H, Ar), 8.04 (s, 1H, Ar), 8.42 (d, 1H, Ar), 8.64 (d, 1H, Ar). m/z 232.

Analysis: Calculated for $C_{12}H_{12}N_2O_3$: C, 62.07; H, 5.17; N, 12.07. Found: C, 61.92; H, 5.20; N, 12.12%.

5-Methoxy-7-acetamido-8-hydroxyquinoline 1-oxide (53):

A solution of 52 (0.39 g, 1.7 m.mol) in methylene chloride (15 ml) was stirred with m-chloroperbenzoic acid (0.436 g, 0.0025 mol) overnight at room temperature. The methylene chloride solution was washed subsequently with aqueous solution of sodium sulphite, aqueous sodium carbonate, water, brine and dried (Na_2SO_4) and rotary evaporated to furnish the N-oxide 53 (0.352 g, 84% yield), m.p. 252°; IR(nujol): 3280 (NH), 1680 (amide), 1470. ^{1}H-NMR ($CDCl_3$): δ 2.20 (s, 3H, $COCH_3$), 4.00 (s, 3H, OCH_3), 6.85 - 8.80 (m, 4H, Ar). m/z 252.

Analysis: Calculated for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.84; N, 11.29. Found: C, 57.71; H, 5.11; N, 10.97%.

5,8-Dimethoxy-7-N-methyl acetamido quinaldonitrile (54):

A mixture of phenol 53 (0.24 g, 0.89 m.mol), dimethyl sulphate (1 ml), anhydrous potassium carbonate and dry acetone was heated under reflux for 4-5 hours. Acetone was distilled off and water was added to the reaction mixture and stirred for 0.5 hour. It was extracted with ethyl acetate and washed with water, brine and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure furnished a residue which on purification on silica gel column with benzene as eluent furnished the dimethyl ether 55 (0.150 g, 60%), m.p. 189-191°C; IR(nujol): 2240 (CN), 1670 (amide). ^{1}H-NMR($CDCl_3$): δ 2.10 (s, 3H, $COCH_3$), 3.35 (s, 3H, NCH_3), 4.00 (s, 3H, OCH_3),

4.10 (s, 3H, OCH_3), 6.75 (s, 1H, Ar), 7.75 (d, 1H, J= 9.5 Hz, Ar), 8.25 (d, 1H, J= 9.5 Hz, Ar). m/z: 285.

5-Methoxy-8-hydroxyquinoline-1-oxide (59):

A stirred solution of 49 (1 g, 5.7 m.mol) in chloroform (20 ml) was treated with m-chloroperbenzoic acid (2.0 g, 0.012 mol) at room temperature overnight. The reaction mixture was washed successively with aqueous sodium sulphite, aqueous sodium carbonate, brine and dried (Na_2SO_4) and concentrated to furnish the N-oxide 46 (0.96 g, 88% yield) as a yellow solid. This crude compound was used as such without further purification for further reaction.

5-Methoxy-8-hydroxyquinaldonitrile (60):

The N-oxide 59 (1.94 g, 0.01 mol) was heated with dimethylsulphate (5 ml, 0.05 mol) on waterbath for 2.5 hours. The viscous residue was washed with ethylacetate to remove any unreacted starting material. The salt thus obtained was dissolved in water and cooled in an ice-bath. Potassium cyanide solution (1.95 g in minimum water) was added dropwise to the above solution with stirring for 2 hours. The pH of the solution was adjusted to 4-5 with acetic acid and then the aqueous layer was extracted repeatedly with chloroform. The combined extracts were washed thoroughly with water, brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave a solid which was

chromatographed over silica gel using benzene as eluent to furnish the nitrile 60 (1.55 g, 76% yield), m.p. 143°C. IR(nujol): 3380 (OH), 2260 (CN). ^{1}H-NMR ($CDCl_3$): δ 3.90 (s, 3H, OCH_3), 6.77 (d, 1H, J= 8 Hz, Ar), 7.07 (d, 1H, J= 8 Hz, Ar), 7.53 (d, 1H, J= 8 Hz, Ar), 8.50 (d, 1H, J= 8 Hz, Ar). m/z: 200.

Analysis: Calculated for $C_{11}H_8N_2O_2$: C, 66.00; H, 4.00; N, 14.00; Found: C, 65.88; H, 4.37; N, 13.64%.

5,7-Dibromo-8-hydroxyquinoline (62):

62 was prepared by bromination of 8-hydroxy quinoline by following the reported procedure[53].

5,7-Dibromo-8-hydroxyquinaldonitrile (63):

To a stirred solution of 8-hydroxyquinaldonitrile (14) (1 g, 5.9 m.mol) in glacial acetic acid (25 ml) was added dropwise bromine (2.8 g, 0.0176 mol) in acetic acid (25 ml) and the resulting solution stirred for 2 hours at room temperature. It was diluted with water and rendered alkaline using sodium carbonate. The solid was obtained which was filtered, dried and recrystallised from acetone to afford 63 (1.75 g, 91% yield), m.p. 178-180° (dec.); IR(nujol): 3340 (br. OH), 2260 (CN); ^{1}H-NMR ($CDCl_3$): δ 7.83 (d, 1H, J= 9.45 Hz, Ar), 8.03 (s, 1H, Ar), 8.23 (bs, 1H, D_2O exchangeable OH), 8.62 (d, 1H, J= 9.45 Hz, Ar). m/z: 326, 328, 330.

Analysis: Calculated for $C_{10}H_4N_2OBr_2$: C, 36.59; H, 1.22; N, 8.54; Found: C, 36.15; H, 1.34; N, 9.73%.

5-Nitro-8-hydroxyquinoline (66):

To a suspension of 5-nitroso-8-hydroxyquinoline hydrochloride (45) (2 g, 0.0095 mol) in acetic acid was added dropwise a mixture of concentrated nitric acid (3 ml) in acetic acid (15 ml) and the resulting solution was vigorously stirred at room temperature for 2 hours. It was diluted with water and the pH of the solution was adjusted to $\approx$6. The solid (1.5 g) was filtered, boiled with ethanol and filtered. The filtrate was reduced to half its volume and cooled to yield 5-nitro-8-hydroxyquinoline (66) (0.93 g, 52% yield) as yellow crystals, m.p. 175-180° (dec.) (lit.[55] m.p. 180°).

7-Bromo-5-nitro-8-hydroxyquinoline (67):

The nitrophenol 66 (0.3 g, 0.0158 mol) was dissolved in aqueous potassium hydroxide solution (2.7 g, in 900 ml) by heating on water bath and then brought to room temperature. Potassium hypobromite solution [prepared by addition of bromine (1.3 ml) to an aqueous potassium hydroxide solution (6 g in 17 ml) at -10°] was added dropwise and the resulting solution was stirred for 2 hours. The solution was rendered neutral with acetic acid. The solid was filtered, washed and dried. Recrystallisation from hot acetone furnished the bromophenol 67 (2.8 g, 66% yield) as red needles, m.p.195-197°

(lit.[55] m.p. 200°).

Attempted preparation of 7-bromo-5-nitro-quinoline-1-oxide (68):

a) With H_2O_2-acetic acid:

To a suspension of 7-bromo-5-nitro-8-hydroxyquinoline in acetic acid (15 ml) and H_2O_2 (1.5 ml, 50%) was heated on a water bath for 2 hours. The colour of the solution changed from red to pale yellow. TLC indicated the absence of starting material. The reaction mixture was diluted with water and repeatedly extracted but failed to produce any product probably due to decomposition. Moreover, when the same reaction was carried out at room temperature similar observation was noted.

b) With m-CPBA:

To a stirred suspension of 67 (0.262 g, 0.97 m.mol) in methylene chloride (25 ml) was added m-CPBA (0.348 g, 2 m.mol). After 48 hours at room temperature, TLC indicated no progress of the reaction and the starting material was recovered.

5-Nitroso-8-hydroxyquinaldonitrile (70):

To a stirred solution of 8-hydroxyquinaldonitrile (14) (13.70 g, 0.081 mol) in acetic acid (400 ml) at 0°C was added dropwise an aqueous solution of sodium nitrite (11.1 g, 0.161 mol). The reaction mixture was allowed to attain room temperature and further stirred for 4 hours. It was diluted with cold water (500 ml) and the solid was filtered

and dried to give 5-nitroso-8-hydroxyquinaldonitrile (70) (11 g, 74%) as yellow solid, m.p. > 250°. IR(nujol): 3100 (br. OH), 2240 (CN), 1670. ^{1}H-NMR (DMSO): δ 6.78 (d, 1H, J= 10 Hz, Ar), 8.05 (d, 1H, J= 10 Hz, Ar), 8.20 (d, 1H, J= 8 Hz, Ar), 8.73 (d, 1H, J= 8 Hz, Ar).

5-Acetamido-8-hydroxyquinaldonitrile (72):

A suspension of 5-nitroso-8-hydroxyquinaldonitrile (70) (4.3 g, 0.025 mol) in ethyl acetate was shaken vigorously with aqueous sodium dithionite (10 g dissolved in minimum amount of water) in a separating funnel. The reaction was exothermic and the solution became red. After the suspended solid had dissolved, the layers were separated and then the aqueous solution was repeatedly extracted with ethyl acetate. The combined ethyl acetate extract was treated with acetic anhydride (20 ml) and the solution was boiled on water bath. On cooling solid was separated which was filtered and dried to furnish 5-acetamido-8-hydroxyquinaldonitrile (72) (4.3 g, 88% yield). The compound was recrystallised from acetone m.p. 275° (dec.); IR(nujol): 3340, 3280 (NH and OH), 2260 (CN), 1660 (amide). ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 2.24 (s, 3H, $COCH_3$), 7.20 (d, 1H, J= 8 Hz, Ar), 7.63 (d, 1H, J=10 Hz, Ar), 7.72 (d, 1H, J= 8 Hz, Ar), 8.47 (d, 1H, J= 10 Hz, Ar), 9.42 (br.s, exchanges with D_2O), 9.64 (br.s, exchanges with D_2O).

5-Acetamido-7-bromo-8-hydroxyquinaldonitrile (73):

To a stirred solution of the amidophenol 72 (5.64 g,

0.025 mol) in dimethylformamide (50 ml) at 0°C was added dropwise a solution of NBS (4.86 g, 0.027 mol) in dimethylformamide (40 ml). The reaction mixture was allowed to come to room temperature. After 4 hours the solution was cooled to 0° and diluted with cold water and stirred for 15 minutes. The solid was separated which was filtered and dried to yield 5-acetamido-7-bromo-8-hydroxyquinoline (73) (6.33 g, 83% yield).. Recrystallisation from acetone furnished yellow crystals which melted at m.p. 248-250° (dec.); IR(nujol): 3300, 3240 (NH and OH), 2260 (CN), 1660 (NHCO); ^{1}H-NMR (DMSO-d_6):δ2.19 (s, 3H, $COCH_3$), 8.00 (s,1H, Ar), 8.14 (d, 1H, J= 7.6 Hz, Ar), 8.66 (d, 1H, J= 7.6 Hz, Ar), 10.04 (s, 1H). m/z 305, 307.

Analysis: Calculated for $C_{12}H_8N_3O_2Br$: C, 47.06; H, 2.60; N, 13.73; Found: C, 47.32; H, 2.80; N, 13.85%.

8-Methoxy-5-acetamido-7-bromoquinaldonitrile (75):

a) A suspension of the amidophenol 73 (0.64 g, 0.0021 mol) was refluxed while stirred with dimethylsulphate (0.53 g, 4.2 m.: anhydrous potassium carbonate (5 g, 0.036 mol) and dry acetone (50 ml) for 8 hours. The colour of the solution changed from brown to red with increase in the amount of the red precipitate. TLC of the reaction mixture indicated the absence of the starting material. Acetone was decanted and water was added to the red precipitate. Solid was filtered to yield the potassium salt of 73 (0.49 g). (The salt on acidification with acetic acid yielded the starting amidophenol 73).

To the suspension of the above salt 74 in dry acetone (50 ml), dimethylsulphate (1 ml) and anhydrous potassium carbonate (5 g) were added and then refluxed for 16 hours. TLC indicated no progress of the reaction and therefore acetone was removed and dimethylformamide (25 ml) was added. The resulting solution was heated at 90-100° using excess of dimethylsulphate. After 10 hours, the reaction mixture was cooled and poured in cold water, filtered and dried to yield a solid 75 (0.2 g, 30%) as a cream coloured solid. Recrystallisation from acetone furnished a white solid, m.p. > 250° (dec.); IR (nujol): 3260 (NH), 2260 (CN), 1670 (amide); ^{1}H-NMR (DMSO-d_6): δ 2.16 (s, 3H, CH_3), 4.05 (s, 3H, OCH_3), 8.13 (d, 1H, J=9.45 Hz, Ar), 8.14 (s, 1H, Ar), 8.79 (d, 1H, J= 9.45 Hz, Ar), 10.2 (s, 1H). m/z 319, 321.

b) Direct methylation of 73:

To a stirred solution of phenol 73 (1 g, 0.0033 mol) in dimethylformamide (10 ml) and anhydrous potassium carbonate (4 g) was heated at 90-100° for 0.5 hour during which the solution turned red with the separation of red potassium salt. Dimethylsulphate (2 ml, 0.021 mol) was then added and the reaction mixture was heated at 90-100° for further 4 hours. Additional quantity of dimethylsulphate (2 ml) was introduced and the reaction continued for 4 hours. It was then cooled, and poured over cold water and stirred for 0.5 hour. The solid which separated was filtered and dried to yield the methyl ether

75 (0.623 g, 59% yield) as white solid. It was identical in all respects with the sample obtained above.

5-Amino-7-bromo-8-methoxyquinaldic acid (76):

To a stirred solution of the nitrile 75 (2.26 g, 0.0071 mol) in ethanol (50 ml) was added an aqueous solution of potassium hydroxide (4 g in 10 ml water). The resulting solution was heated under reflux for 24 hours. The progress of the reaction was monitored by TLC (UV lamp). Ethanol was rotary evaporated and the solid obtained was dissolved in minimum amount of water and carefully acidified with acetic acid. It was repeatedly extracted with chloroform and the combined extract was dried (Na_2SO_4) and concentrated under reduced pressure to furnish the acid 76 (1.68 g, 80% yield) as a chocolate brown solid m.p. 190-193°. ^{1}H-NMR (DMSO-d_6): δ 3.93 (s, 3H, OCH_3), 6.1 (broad hump, NH_3^+, exchanges with D_2O), 6.92 (s, 1H, Ar), 8.01 (d, 1H, J= 9.45 Hz, Ar), 8.79 (d, 1H, J= 9.45 Hz, Ar).

Analysis: Calculated for $C_{11}H_9N_2O_3Br$: C, 44.44; H, 3.03; N, 9.43; Found: C, 44.04; H, 3.25; N, 9.23%.

Methyl-β-methyl-Nb(5-N-methoxycarbonyl-7-bromo-8-quinaldoyl)-tryptophan (78):

To a stirred solution of the acid 76 (0.986 g, 3.22 m.mol) in dry tetrahydrofuran (35 ml) at 0° was added triethylamine (0.67 g, 6.63 m.mol) followed by methylchloroformate (0.63 g, 6.63 m.mol) in one portion. The stirring was continued for 1 hour

after which time TLC indicated no starting material. To this solution, β-methyltryptophan 30 (0.77 g, 3.32 mol) in dry tetrahydrofuran (35 ml) was then added in one lot. After stirring overnight, tetrahydrofuran was rotary evaporated and the residue purified by chromatography over silica-gel using benzene-ethylacetate (9:1) as eluent to furnish the amide 78 (0.744 g, 90% yield based on the recovery of tryptophan) m.p. 110-115°; IR ($CHCl_3$): 3460, 3360 (NH), 1740 ($COOCH_3$), 1670 (amide); ^{1}H-NMR ($CDCl_3$): δ 1.51 (t, 3H, J= 6 Hz, $CHCH_3$, diastereomers), 3.60, 3.66, 3.80 (s, 3H, 2X OCH_3, diastereomers), 3.93 (s, 3H, OCH_3), 4.04 (m, 1H, $CHCH_3$), 5.13 (m, 1H, NH CH $COOCH_3$), 6.82 - 8.86 (m, 11H, Ar). m/z 510, 512 (M^+ -$COOCH_3$).

Attempted cyclisation of amide 78 with $POCl_3$:

To a stirred solution of amide 78 (0.1 g, 0.178 m.mol) in dry xylene (20 ml) was added phosphorous oxychloride (1 ml). The solution became brownish-red with the formation of an insoluble precipitate. The reaction mixture was heated under reflux for 6 hours. Xylene was removed under diminished pressure and the residue neutralised with $NaHCO_3$. The solid was obtained which was found insoluble in all the solvents such as acetone, chloroform and DMSO rendering the characterisation difficult.

b) Using PPA

To a stirred solution of PPA (prepared by heating 2 g P_2O_5 and 1.2 ml orthophosphoric acid) was added the amide 78

(0.1 g, 0.178 m.mol) in dry xylene (20 ml). The resulting mixture was heated under reflux for 5 hours in an atmosphere of nitrogen. It was then cooled, poured over crushed-ice and neutralised with $NaHCO_3$. The xylene layer was separated and rotary evaporated under reduced pressure. TLC of the residual product revealed number of spots.

c) With PPE

Preparation of PPE[61]

P_2O_5 (25 g) and dry ether (50 ml) were refluxed in chloroform (50 ml) for 36 hours till the P_2O_5 dissolved. The solvent was removed in vacuo and the syrupy liquid was stored in dessicator over P_2O_5.

Attempted cyclisation with PPE

To a stirred solution of the amide 78 (0.2 g, .356 m.mol) in dry chloroform (10 ml) was added PPE (1 ml) under nitrogen. The resulting mixture was heated under reflux for 18 hours. TLC indicated no progress of the reaction. Chloroform was then removed and the temperature of the reaction mixture was raised to 120°. After 1 hour, TLC indicated the formation of number of products which could not be analysed by column chromatography.

7-Bromo-5,8-dimethoxy-2-carbomethoxy quinoline (87):

To a solution of 5-amino-7-bromo-8-methoxyquinaldic acid (76) (0.513 g, 1.7 m.mol) in ethyl acetate (75 ml) at

0° was added 7N sulphuric acid (25 ml) followed by potassium dichromate (0.5 g dissolved in minimum amount of water) and the resultant solution stirred for 2 hours. Ethyl acetate layer was separated and then aqueous layer repeatedly extracted with ethyl acetate. The combined organic layer was shaken vigorously with sodium dithionite solution (2 g in minimum amount of water) in a separating funnel. The colour of the organic layer changed from red to yellow. The aqueous layer was extracted repeatedly with ethyl acetate. The combined organic layer was dried (Na_2SO_4) and concentrated to yield a crude yellow residue. 86 which was directly put for methylation. Thus, the crude residue was refluxed with dimethylsulphate (5 ml), anhydrous potassium carbonate (10 g) in dry acetone (50 ml) for 12 hours. Acetone was distilled off, water was added to the reaction mixture and stirred for 0.5 hour. The solid was obtained which was filtered and dried. Purification of the crude residue on column chromatography of silica gel using benzene as eluent yielded a pale yellow solid 87 (0.168 g, 30% yield), m.p. 156-157°. IR(nujol): 1740 ($COOCH_3$), ^{1}H-NMR($CDCl_3$): δ 3.98 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.17 (s, 3H, OCH_3), 7.01 (s, 1H, Ar), 8.09 (d, 1H, J= 9.45 Hz, Ar), 8.60 (d, 1H, J= 9.45 Hz, Ar). m/z: 325, 327.

7-Bromo-5,8-dimethoxyquinaldic acid (88):

A solution of 87 (0.436 g, 1.3 m.mol) in ethanol (50 ml) was heated under reflux with aqueous potassium hydroxide

(2 g in 10 ml water) for 12 hours. Ethanol was removed under reduced pressure, the solid dissolved in hot water and filtered. The filtrate was cooled and acidified with concentrated HCl to yield 88 (89%) as yellow solid, m.p. 186-188° (dec.). IR(nujol): 3340 (br, OH), 1720 (COOH), ^{1}H-NMR ($CDCl_3$): δ4.00 (s, 3H, OCH_3), 4.05 (s, 3H, OCH_3), 6.90 (broad, exchanges with D_2O); 7.05 (s, 1H, Ar), 8.23 (d, 1H, J= 6.3 Hz, Ar), 8.72 (d, 1H, J= 6.3 Hz, Ar). m/z: 311, 313.

Analysis: Calculated for $C_{12}H_{10}NO_4Br$: C, 46.15; H, 3.21; N, 4.49; Found: C, 45.99; H, 3.32; N, 4.28%.

7-Bromo 5,8-dimethoxyquinoline (89):

A stirred solution of quinaldic acid 88 (0.22 g, 0.71 m.mol) was heated at 200°C for 5 minutes in an atmosphere of nitrogen. The residue obtained after cooling was chromatographed using benzene-acetone (95:5) as eluent to furnish the dimethyl ether 89 (0.161 g, 85% yield) as a pale yellow solid. m.p. 102-104°C. IR(nujol): 1620, 1600. ^{1}H-NMR ($CDCl_3$): δ 3.95 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 6.93 (s, 1H, Ar), 7.36 (dd, 1H, J_1=9.45 Hz, J_2=4.7 Hz, Ar), 8.50 (dd, 1H, J_1= 9.45 Hz, J_2= 1.5 Hz, Ar), 8.94 (dd, 1H, J_1= 4.7 Hz, J_2= 1.6 Hz, Ar). m/z: 267, 269.

Analysis: Calculated for $C_{11}H_{10}NO_2Br$: C, 49.25; H, 3.73; N, 5.22; Found: C, 50.06; H, 3.91; N, 5.00%.

2-Cyano-7-bromo-5,8-quinoline quinone (65):

To a stirred cold suspension of 5-acetamido-7-bromo-

quinaldonitrile (67) (0.430 g, 1.41 m.mol) in ethyl acetate at 0° was added 12N sulphuric acid (15 ml) followed by potassium dichromate (0.5 g in minimum water). The reaction was allowed to attain room temperature. The progress of the reaction was indicated by the disappearance of the suspension and formation of two clear layers. The organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated to afford a residue which was purified by column chromatography on silica gel using benzene as eluent to furnish the bromo quinone 65 (0.315 g, 73% yield), as yellow solid, m.p.165°, IR(nujol): 2230 (CN), 1700,1670, 1600; ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 7.65 (s, 1H,Ar), 8.17 (d, 1H, J= 7.54 Hz, Ar), 8.55 (d, 1H, J= 7.54 Hz, Ar). m/z: 261, 263.

Analysis: Calculated for $C_{10}H_3N_2BrO_2$: C, 45.63; H, 1.14; N, 10.65. Found: C, 45.52; H, 1.14; N, 10.45%.

7-Bromo-5,8-dihydroxyquinaldonitrile (90):

7-Bromo-5,8-quinoline quinone (65) (2.12 g, 0.0081 mol) dissolved in ethyl acetate (100 ml) was vigorously shaken with aqueous sodium dithionite solution (5 g dissolved in minimum water) in a separating funnel. An exothermic reaction ensued and the original yellow colour of the solution darkened slightly. The product had identical Rf value with the starting material on TLC. The organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined

non-aqueous layer was dried (Na_2SO_4) and rotary evaporated under reduced pressure to yield the hydroquinone 90 (2.1 g, 94% yield), recrystallised from benzene to give yellow crystals, m.p. 216-218°(dec.). IR(nujol): 3340 (OH), 2260 (CN); ^{1}H-NMR (Acetone-d_6): δ 7.31 (s, 1H, Ar), 6.88 (d, 1H, J= 10 Hz, Ar), 7.71 (d, 1H, J= 10 Hz, Ar), 9.27 (s, 1H, D_2O exchanges), 9.69 (s, 1H, D_2O exchanges). m/z: 265, 267.

Analysis: Calculated for $C_{10}H_5N_2BrO_2$: C, 45.28; H, 1.89; N, 10.57; Found: C, 45.28; H, 2.09; N, 10.43%.

7-Bromo-5,8-dimethoxyquinaldonitrile (91):

7-Bromo-5,8-dihydroxyquinaldonitrile (90) (2.12 g, 0.008 m.mol) was treated with dimethylsulphate (5 ml) in the presence of anhydrous potassium carbonate (10 g) in boiling acetone for 4 hours. Acetone was distilled off and water was added to the residue. After stirring for 0.5 hour at room temperature, solid was obtained which was filtered and dried. The compound was purified on a short column of silica gel using benzene to furnish 7-bromo-5,8-dimethoxyquinaldonitrile 91 (1.19 g, 56%) as yellow solid, m.p.180°; IR(nujol): 2260 (CN); ^{1}H-NMR ($CDCl_3$): δ 4.00 (s, 3H, OCH_3), 4.10 (s, 3H, OCH_3), 7.05 (s, 1H, Ar), 7.65 (d, 1H, J= 8 Hz, Ar), 8.65 (d, 1H, J= 8 Hz, Ar). m/z: 291, 293.

Analysis: Calculated for $C_{12}H_9N_2O_2Br$: C, 49.15; H, 3.07; N, 9.56. Found: C, 48.96; H, 3.28; N, 9.25%.

7-Bromo-5,8-dimethoxyquinaldic acid (88):

To a stirred solution of 7-bromo-5,8-dimethoxy-quinaldonitrile (91) (2.12 g, 0.007 mol) in ethanol (150 ml) was added an aqueous solution of potassium hydroxide (8 g in 20 ml). The resulting mixture was heated under reflux for 12 hours. Ethanol was removed and the solid was dissolved in hot water, cooled and acidified (concentrated HCl) to give 7-bromo-5,8-dimethoxy quinaldic acid 88) (1.95 g 86% yield) as a yellow solid, m.p. 186-188° (dec.). This compound was identical in all respects with the sample obtained earlier.

7-Bromo-5-amino-8-hydroxyquinoline (92):

Finely powdered 7-bromo-8-hydroxy-5-nitroquinoline (67) (1.5 g, 0.0056 mol) was added to a solution of KOH (2.5 g, 0.045 mol) in water (20 ml) with vigorous stirring so as to produce a fine suspension of potassium salt. The mixture was gently heated to boiling and sodium dithionite (5 g) added. Reduction was completed by boiling for 1 minute. It was cooled and neutralised with acetic acid to afford a solid precipitate which was filtered and dried to furnish the amine 92 (0.85 g, 68% yield) as brown solid, m.p. 180-182° (lit.[55] m.p. 184°).

7-Bromo-5,8-quinolinequinone (64):

To a solution of 7-bromo-5-amino-8-hydroxyquinoline (92) (0.480 g, 0.002 mol) in ethyl acetate (50 ml) at 0° was added

4N sulphuric acid (20 ml) followed by potassium dichromate solution (0.5 g in minimum amount of water) and stirred for 2 hours. Ethyl acetate layer was separated. The aqueous layer was repeatedly extracted with ethyl acetate and the combined organic layer was washed with brine, dried (Na_2SO_4) and concentrated under reduced pressure to furnish the 7-bromo-5,8-quinoline quinone (0.295 g, 62% yield) as red solid m.p. 179-180° (lit.[55] m.p. 182°).

7-Bromo-5,8-dihydroxy quinoline (93):

A suspension of the bromoquinone 64 (0.295 g, 0.00012 mol) in ethyl acetate (50 ml) and sodium dithionite (1 g in minimum amount of water) was vigorously shaken in a separating funnel. The organic layer was separated. The aqueous layer was extracted repeatedly by ethyl acetate. The combined organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give 93 (0.25 g, 84% yield). This was used as such without further purification for the next step.

7-Bromo-5,8-dimethoxyquinoline (89):

A mixture of hydroquinone 93 (0.25 g, 0.0011 mol), anhydrous potassium carbonate (3 g, 0.022 mol) and dimethylsulphate (1 ml, 0.011 mol) in dry acetone (60 ml) were heated under reflux for 12 hours. Acetone was distilled off, water was added to the reaction mixture and stirred for 0.5 hour. The aqueous layer was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated under reduced pressure to yield a residue which

was chromatographed on silica gel to give the dimethyl ether 89 (0.1 g, 35% yield) as a pale yellow solid. This was identical in all respects with the sample obtained by decarboxylation of 88.

Methyl-β-methyl-Nb(7'-bromo-5',8'-dimethoxyquinaldoyl) tryptophan (94):

To a stirred solution of 7-bromo-5,8-dimethoxyquinaldic acid (88) (0.1 g, 0.321 mol) in dry tetrahydrofuran (10 ml) was added triethylamine (0.032 g, 0.317 m.mol). The mixture was cooled to 0° and then methylchloroformate (0.030 g, 0.32 m.mol) was introduced. After 0.5 hour β-methyltryptophan 30 (0.089 g, 0.384 m.mol) in dry tetrahydrofuran (5 ml) was added to the above cold solution in one lot. Stirring was continued for 4 hours during which it was allowed to come to room temperature. Tetrahydrofuran was rotary evaporated and the residue was resolved by column chromatography using benzene as eluent to furnish the amide 94 (0.164 g, 97% yield) as a white solid; m.p. 85-90°; IR(nujol): 3400 (NH), 1750 ($COOCH_3$), 1690 (amide); ^{1}H-NMR ($CDCl_3$): δ 1.50 (t, 3H, $CHC\underline{H}_3$), 3.54, 3.62, 3.82, 3.94 (s, 9H, 3X OCH_3), 3.94 (m, 1H, $C\underline{H}$ CH_3), 5.17 (m, 1H, NH $C\underline{H}$ $COOCH_3$), 7.04 (s, 1H, Ar), 7.07-7.84 (m, 5H, Ar), 8.38 (d, 1H, J= 9 Hz, Ar), 8.56 (d, 1H, J= 9.00 Hz, Ar), 8.69 (d, 1H, J= 9 Hz, Ar), 8.73 (d, 1H, J= 9 Hz, Ar). m/z 525, 527.

Analysis: Calculated for $C_{25}H_{24}N_3O_5Br$: C, 57.03; H, 4.56; N, 7.98; Found: C, 57.39; H, 4.76; N, 7.66%.

1-[2'-(7'-bromo-5',8'-dimethoxyquinolyl)]-3-methoxycarbonyl-4-methyl-β-carboline (95):

A solution of the amide 94 (0.28 g, 0.53 m.mol) and $POCl_3$ (2 ml) in dry xylene (20 ml) was heated under reflux with stirring for 4 hours. The flask was cooled and the contents poured over crushed-ice with stirring. The pH of the resulting solution was adjusted at 8 with sodium carbonate. Xylene was separated and the solid was extracted with dichloromethane. The combined organic layer was washed with water and dried (Na_2SO_4) and rotary evaporated to dryness. Purification of the residue by chromatography using benzene as eluent furnished β-carboline 95 (0.184 g, 68% yield) as a white solid.

Alternately, the crude cyclised product was purified by boiling in refluxing acetone followed by cooling to yield 95 (0.238 g, 88% yield), m.p. 280°; IR ($CHCl_3$): 3340 (NH), 1730 ($COOCH_3$); ^{1}H-NMR ($CDCl_3$): δ 3.2 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 4.07 (s, 3H, OCH_3), 4.26 (s, 3H, OCH_3), 6.97 (s, 1H, Ar), 7.23-7.57 (m, 2H, Ar), 7.66 (d, 1H, J= 7.5 Hz, Ar), 8.38 (d, 1H, J= 7.5 Hz, Ar), 8.62 (d, 1H, J= 9 Hz, Ar), 8.85 (d, 1H, J= 9 Hz, Ar), 12.37 (bs, 1H, NH D_2O exchangeable). m/z: 505, 507.

Analysis: Calculated for $C_{25}H_{20}N_3O_4Br$: C, 59.28; H, 3.95; N, 8.29. Found: C, 59.01; H, 4.03; N, 8.23%.

7-Bromo-2-cyano-5,8-quinoline quinone (65):

To a stirred suspension of 7-bromo-5,8-dimethoxy

quinaldonitrile (91) (0.2 g, 0.69 m.mol) in methylene chloride-acetonitrile mixture (8:2) was added cerric ammonium nitrate (0.5 g in minimum water) and the reaction continued overnight. Methylenechloride was separated and aqueous solution was extracted with methylene chloride. The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give the bromoquinone 65 (0.13 g, 72% yield). This compound was identical with the sample prepared earlier.

Attempted oxidation of 95 → 98 using AgO:

To a solution of 95 (0.047 g, 0.093 m.mol) in dry dioxane (5 ml) and AgO (0.042 g, 0.34 m.mol) were stirred vigorously for 1 hour. 3N $HClO_4$ (5 ml) was then added, when the colour of the solution turned brown. After 15 minutes, the reaction was quenched by adding aqueous chloroform (10 ml, 2:8). Separation of the chloroform layer followed by repeated extraction of aqueous with chloroform gave combined organic layer which was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography over silica-gel using benzene as the eluent to furnish red coloured bromoquinone 98 (0.007 g) and the unreacted starting material 95 (0.022 g).

Attempted oxidation with CAN (95 → 98):

To a solution of the β-carboline 95 (0.05 g, 0.099 m.mol) in methylene chloride (25 ml) was added aqueous cerric ammonium

nitrate (0.25 g in 10 ml water). The solution was stirred vigorously for 4 hour at room temperature. The organic layer was separated and the aqueous layer repeatedly extracted with methylene chloride. The combined organic layer was successively washed with water, brine, dried (Na_2SO_4). Concentration of the solvent yielded a red solid which was purified by column chromatography over silica-gel using benzene as eluent to yield the bromoquinone 98 (0.002 g) along with the unreacted starting material (0.027 g). The bromoquinone 98 was identical with the product obtained in earlier experiment.

Demethylation using trimethylsilyl iodide

A two-necked flask fitted with 3-way stopcock was charged with the β -carboline 95 (0.05 g, 0.099 m.mol) in dry chloroform (30 ml). NaI (0.045 g, 0.3 m.mol) was added and then the flask was flushed with nitrogen and also maintained under positive pressure of nitrogen. Trimethylchlorosilane (.032 g, 0.047 ml) was introduced with the aid of a syringe and the mixture heated (60°C) for 24 hours. Methanol (5 ml) was then introduced, solvent removed in vacuo and the residue washed with acetone. The residual solid was suspended in methanol (40 ml) and refluxed in the presence of concentrated sulphuric acid (1 ml) for 12 hours. Methanol was removed under reduced pressure, diluted with water and the solid was filtered and dried to furnish β -carboline 95.

1-[2'-(7'-bromo-5',8'-quinone quinolyl)]-3-methoxycarbonyl-4-methyl-β-carboline (98):

A suspension of the β-carboline 95 (0.095 g, 0.188 m.mol) with 48% aqueous HBr (10 ml) was refluxed with stirring under nitrogen for 8 hours. The reaction mixture was cooled and poured over crushed-ice and the precipitate was filtered and dried to give 96 (0.069 g) as a brown solid. This was used for esterification without further purification.

The suspension of 96 (0.069 g) in dry methanol (30 ml) containing concentrated sulphuric acid (1 ml) was refluxed for 12 hours. Methanol was removed under reduced pressure and cold water added to the reaction mixture. The solid was formed which was filtered and dried to yield 97 (0.07 g) as brown solid. This was subjected to oxidation without further purification.

A suspension of 97 (0.07 g) in methylene chloride (50 ml) was vigorously stirred at room temperature with aqueous solution of cerric ammonium nitrate (0.5 g in 10 ml water) for 6 hours. As the reaction progressed, the suspension dissolved and the colour of the solution changed from brown to red. The organic layer was separated and the aqueous layer extracted repeatedly with chloroform. The combined organic layer was washed with water, brine, dried (Na_2SO_4) and evaporated to dryness to yield a red residue which was purified by chromatography using benzene as eluent to furnish

the bromoquinone 98 (0.038 g, 43% yield). m.p. 286-289° (lit.[31] m.p. 285-287°). IR($CHCl_3$): 3360 (NH), 1730 ($COOCH_3$), 1700, 1670. ^{1}H-NMR ($CDCl_3$): δ 3.20 (s, 3H, CH_3), 4.10 (s, 3H, OCH_3), 7.36 (t, 1H, Ar), 7.57 (s, 1H, Ar), 7.63 (t, 1H, Ar), 7.67 (d, 1H, J= 8 Hz, Ar), 8.34 (d, 1H, J= 8 Hz, Ar), 8.36 (d, 1H, J= 8 Hz, Ar), 8.98 (d, 1H, J= 8 Hz, Ar), 11.77 (bs, NH). m/z: 475, 477.

Analysis: Calculated for $C_{23}H_{14}N_3O_4Br$: C, 57.98; H, 2.94; N, 8.82; Found: C, 57.83; H, 3.09; N, 8.88%.

Analysis: Calculated for $C_{10}H_4N_2OBr_2$: C, 36.59; H, 1.22; N, 8.54; Found: C, 36.15; H, 1.34; N, 9.73%.

5-Nitro-8-hydroxyquinoline (66):

To a suspension of 5-nitroso-8-hydroxyquinoline hydrochloride (45) (2 g, 0.0095 mol) in acetic acid was added dropwise a mixture of concentrated nitric acid (3 ml) in acetic acid (15 ml) and the resulting solution was vigorously stirred at room temperature for 2 hours. It was diluted with water and the pH of the solution was adjusted to $\approx$6. The solid (1.5 g) was filtered, boiled with ethanol and filtered. The filtrate was reduced to half its volume and cooled to yield 5-nitro-8-hydroxyquinoline (66) (0.93 g, 52% yield) as yellow crystals, m.p. 175-180° (dec.) (lit.[55] m.p. 180°).

7-Bromo-5-nitro-8-hydroxyquinoline (67):

The nitrophenol 66 (0.3 g, 0.0158 mol) was dissolved in aqueous potassium hydroxide solution (2.7 g, in 900 ml) by heating on water bath and then brought to room temperature. Potassium hypobromite solution [prepared by addition of bromine (1.3 ml) to an aqueous potassium hydroxide solution (6 g in 17 ml) at -10°] was added dropwise and the resulting solution was stirred for 2 hours. The solution was rendered neutral with acetic acid. The solid was filtered, washed and dried. Recrystallisation from hot acetone furnished the bromophenol 67 (2.8 g, 66% yield) as red needles, m.p.195-197°

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