Synthesis of non-mutagenic anticancer drugs

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SYNTHESIS OF NON-MUTAGENIC ANTICANCER DRUGS

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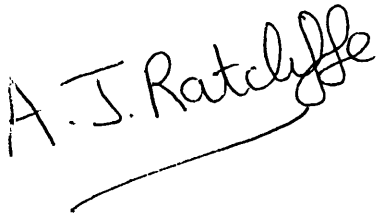

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To my parents, for all their help and encouragement throughout these last years

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SUMMARY

The work described in this thesis was carried out at the University of Bath between October 1984 and August 1987, and is concerned with the preparation of dimeric derivatives of 6H-pyrido[4,3-*b*]carbazoles (ellipticines).

3-{1-[3-(4-Cyanopyridyl)]ethyl}indole (114) was prepared by a known procedure and converted to the aminoketene thioacetal (132) by reaction with 2-lithio-1,3-dithiane. Subsequent hydrolysis led to 5-(1,3-dithian-2-yl)-11-methyl-6H-pyrido[4,3-*b*]carbazole (30). Deprotection of the 1,3-dithiane ring using silver nitrate in the presence of acid gave the 5-formyl derivative (29) (17-oxoellipticine), a known alkaloid from *Strychnos dinklagei*. Attempted deprotection using silver nitrate alone resulted in formation of the N-oxide of the thioacetal (30). The 5-formyl group was converted to the 5-methyliminomethyl analogue (141), which was reduced with sodium borohydride in methanol to give the corresponding amine (139). This amine was acylated with adipic acid to give the 6H-pyrido[4,3-*b*]carbazole dimer (140, n = 4). Attempts to the amine by reductive amination of 17-oxoellipticine using sodium cyanoborohydride in methanol at pH 6 led to the production of 5-(methoxymethyl)-11-methyl-6H-pyrido[4,3-*b*]carbazole (142) only. Repetition of the reaction at pH 3 resulted in a mixture of the methyl ether (142) and 5-(hydroxymethyl) derivative (72).

The reaction sequence for the formation of the dimer was repeated for the 9-methoxylated series, except now deprotection of the 1,3-dithiane ring to the aldehyde (159) was accomplished using N-chlorosuccinimide and silver nitrate. A small amount of the sulphone (163c)

was generated as well as the aldehyde. Attempted deprotection using silver nitrate in the presence of acid resulted in an oxidative coupling reaction at C-7 in the ellipticine ring and formation of the dehydro-dimer (160). The oxidative behaviour of both 9-methoxyellipticine (2) and 9-hydroxyellipticine (5) were studied, but dimerisation was only noted for the former compound.

O-Demethylation of the bis-9-methoxy dimer (148) was achieved using hot pyridine hydrochloride, but with concomitant cleavage of the amide linking unit. In an alternative route 5-formyl-9-methoxy-11-methyl-6H-pyrido[4,3-*b*]carbazole (159) was O-demethylated using boron tribromide and the formyl group converted to the corresponding 5-methyliminomethyl derivative (172). Reduction of this imine with sodium borohydride in methanol resulted in formation of 2-methylpyrido[3,4-*h*]pyrimido[5,6,1-*jk*]-carbazol-10-ol (173), which was fully characterised as its dimethyl-*t*-butylsilyl protected analogue (174). The desired amine was not isolated.

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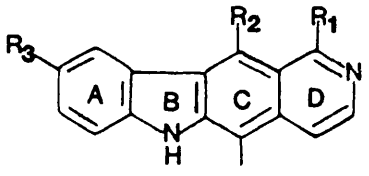
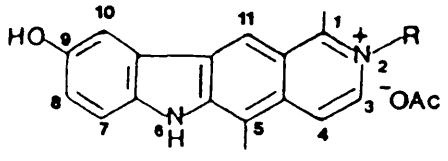
PREFACE

The plant alkaloid ellipticine (1) (5,11-dimethyl-6H-pyrido[4,3-*b*]-carbazole) was first isolated in 1959 from the leaves of *Ochrosia elliptica* Labill.[1] Subsequently further quantities of ellipticine, along with 9-methoxyellipticine (2) and olivacine (3) (1,5-dimethyl-6H-pyrido[4,3-*b*]-carbazole), were isolated from various plants in the *Aspidosperma*, *Ochrosia* and *Tabernaemontane* genera of the *Apocynaceae* family.[2] Since the discovery that these alkaloids exhibited pronounced anticancer activity against animal tumours,[3] there has been intense synthetic activity in the search for improved anticancer activity within this class of compound. As a result, numerous routes to ellipticine and its derivatives exist, and from these labours 9-hydroxy-N-2-methylellipticinium acetate (4) is produced by SANOFI in France and marketed as an effective treatment for myleoblastic leukaemia, advanced breast cancer and other solid tumours.[4]





(1) $R_1 = R_3 = H$, $R_2 = CH_3$

(2) $R_1 = H$, $R_2 = CH_3$, $R_3 = OCH_3$

(3) $R_1 = CH_3$, $R_2 = R_3 = H$

(4) $R = CH_3$

(5) $R_1 = H$, $R_2 = CH_3$, $R_3 = OH$

Biometabolic studies of ellipticine have indicated that ellipticine is oxidised *in vivo* to 9-hydroxyellipticine (5).[5] It has been known for some years that the presence of an alkoxy or hydroxy at position 9 in the ellipticine ring results in both enhanced cytotoxicity and antitumour activity.[6] Indeed, 9-hydroxyellipticine is forty times more active than ellipticine against leukaemia L1210 implanted in mice. An important

discovery is that the ellipticines are associated with a high affinity for DNA and have the ability to intercalate between the base pairs of the nucleic acid.[7] However, recent investigations on the bioxidation of 9-hydroxyellipticines (4) and (5) *in vitro* have suggested that their anticancer activity may be related both to the DNA intercalation phenomena and the fact that the hydroxylated tetracycle undergoes bioxidation to an iminoquinone which may function as an alkylating agent.[8]

As a result of the intercalation effect, a seemingly rational approach in the search for increased antitumour activity lies in the design of compounds that exhibit very large DNA binding affinities, even though no direct correlation between DNA binding affinity and pharmacological properties has been proven. In related drugs, a dramatic increase in the DNA binding affinity and antitumour activity has been demonstrated for dimeric compounds, in which two drug molecules are linked *via* a spacer unit.[9]

In common with many intercalating drugs such as daunomycin and adriamycin, the ellipticines exert a mutagenic affect,[10] probably because at sites where the drug causes damage to the DNA, repair brings about frame shifts and consequently transmission of incorrect coding information.[11] We argue that should two ellipticines linked by a spacer chain be intercalated into the same strand of DNA, then repair is improbable and mutagenicity is repressed. Therefore, the design of ellipticine dimers potentially represents a class of non-mutagenic drugs of enhanced anticancer activity. Thus a structure is required in which two ellipticine units are linked by a spacer chain of the correct length and geometry to ensure di-intercalation.

The intercalation of daunomycin into DNA is well documented. Projections based on a similar DNA intercalation by ellipticine, and related studies,[12] have suggested the optimum length of the spacer chain for di-intercalation to be 10-12 Å, and that the atoms of the chain should be be largely outside the DNA helix. Further information regarding the orientation of the ellipticine molecule in the DNA cavity has been obtained from a sophisticated computer graphic analysis using quantitative *X*-ray data of several 5-, 7- and 8-alkylated ellipticines.[13] The results have suggested that the ellipticine molecule is orientated diagonally in the cavity between the base pairs with only positions 5-, 6-, 7-, 8- and 9- 'open', and that substitution at any of these positions would not be expected strongly to influence intercalation of the chromophore between the base pairs. Given the recent evidence that anticancer activity of 9-hydroxyellipticines may in part be due to their bioxidation to reactive iminoquinones,[8] positions 5-, 7- and 8- are consequently the more suitable anchorage points for linkage of the spacer chain.

The introduction to this thesis is laid out under the following section titles:

(A) Syntheses of ellipticine and its derivatives from 1983-87: a comprehensive review of endeavours made in this direction.

(B) The biochemical properties of ellipticine and its derivatives: aspects of the antitumour activity and mechanism of action of ellipticine and its derivatives, including the bi oxidation of 9-hydroxyellipticines.

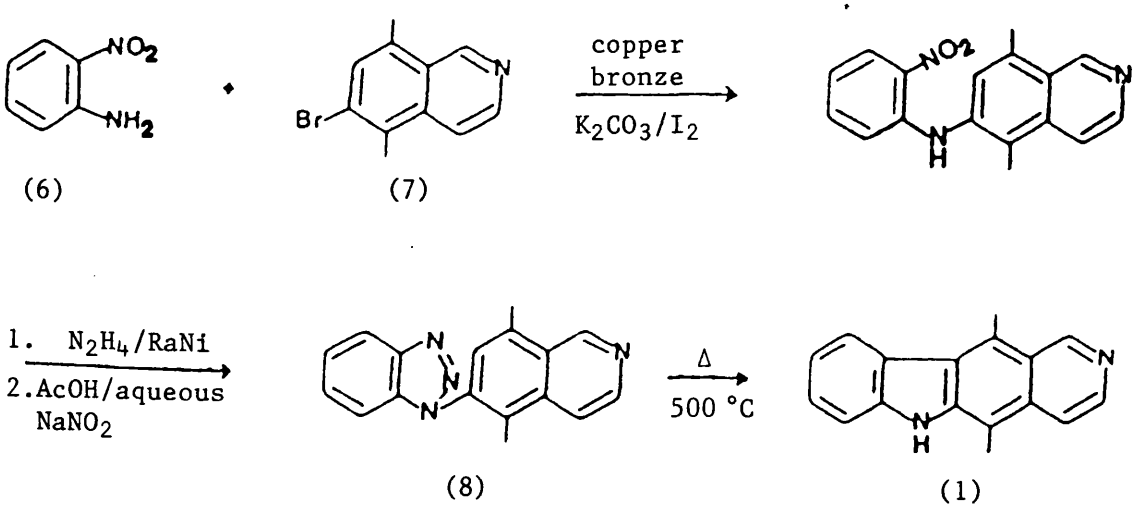
(C) Bis-intercalators: some pyridocarbazole dimers related to the ellipticines.

(A) Syntheses of Ellipticine and its Derivatives from 1983-87

Efforts to synthesise ellipticine and its derivatives, from their initial isolation to the end of 1982,[14] have been thoroughly reviewed. Recently, two further reviews have appeared extending coverage to March, 1985.[15] The approaches have been classified either based on the last ring constructed or according to the last bond to be formed. The former classification is much simpler and will be followed throughout this introduction. Since a new synthetic method involving the simultaneous construction of two rings (B + C) has recently been discovered,[16] routes to ellipticines may be classified into four classes B, C, D and B + C.

B-type syntheses

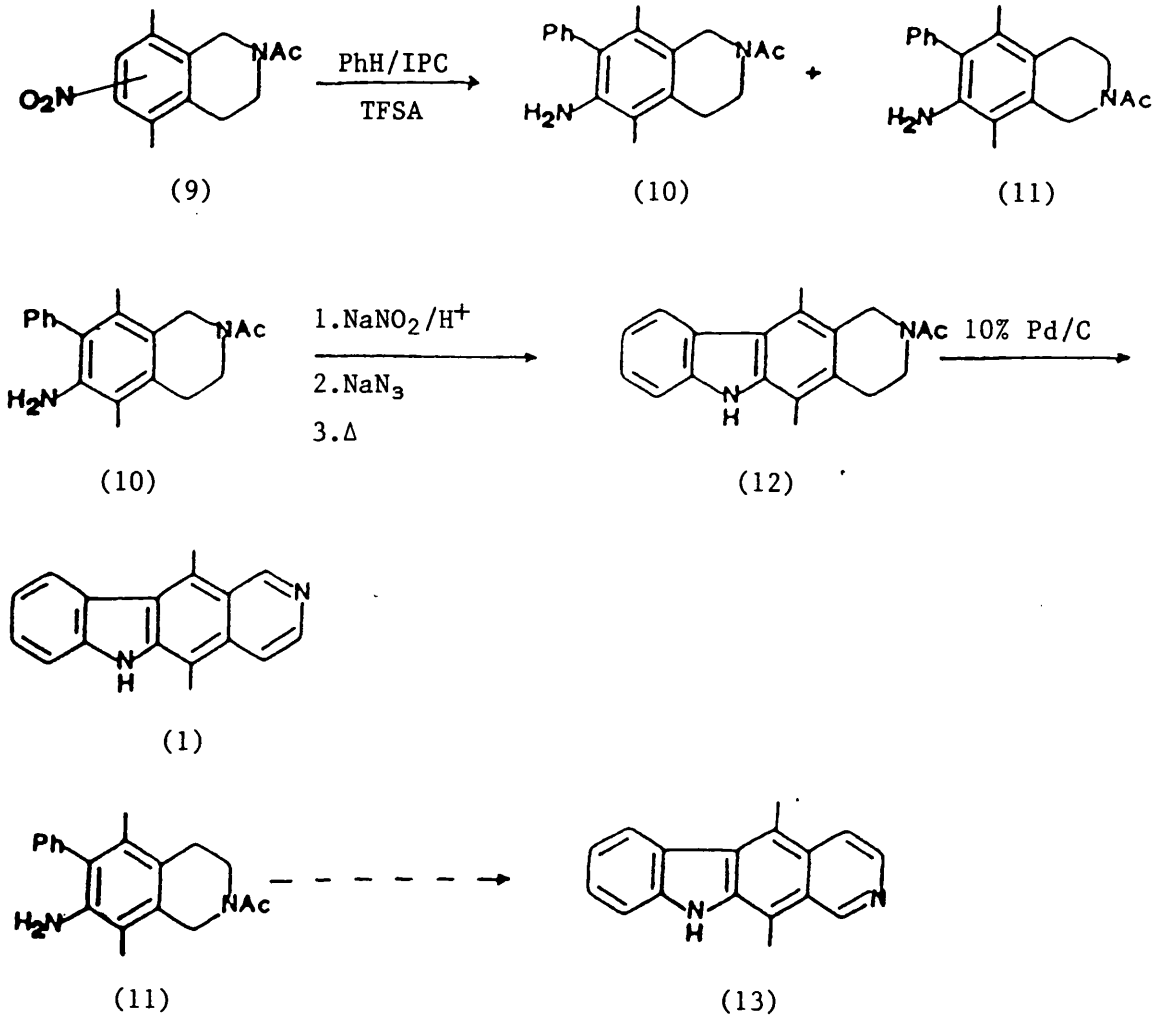
Miller *et al.*[17] have described an ellipticine synthesis based on earlier work by Bisagni and co-workers.[18] Here the ellipticine B ring (69%) is formed through the thermolysis (500 °C) of a N-isoquinolinylbenzotriazole (8). This compound being synthesised in 52% yield from the nitroaniline (6) and 6-bromo-5,8-dimethylisoquinoline (7) by a Goldberg coupling, followed by reduction and diazotisation. The sequence was also completed for 9-methoxyellipticine (2) in an overall yield of 31% (Scheme 1).



(6) (7) (8) (1)

Scheme 1

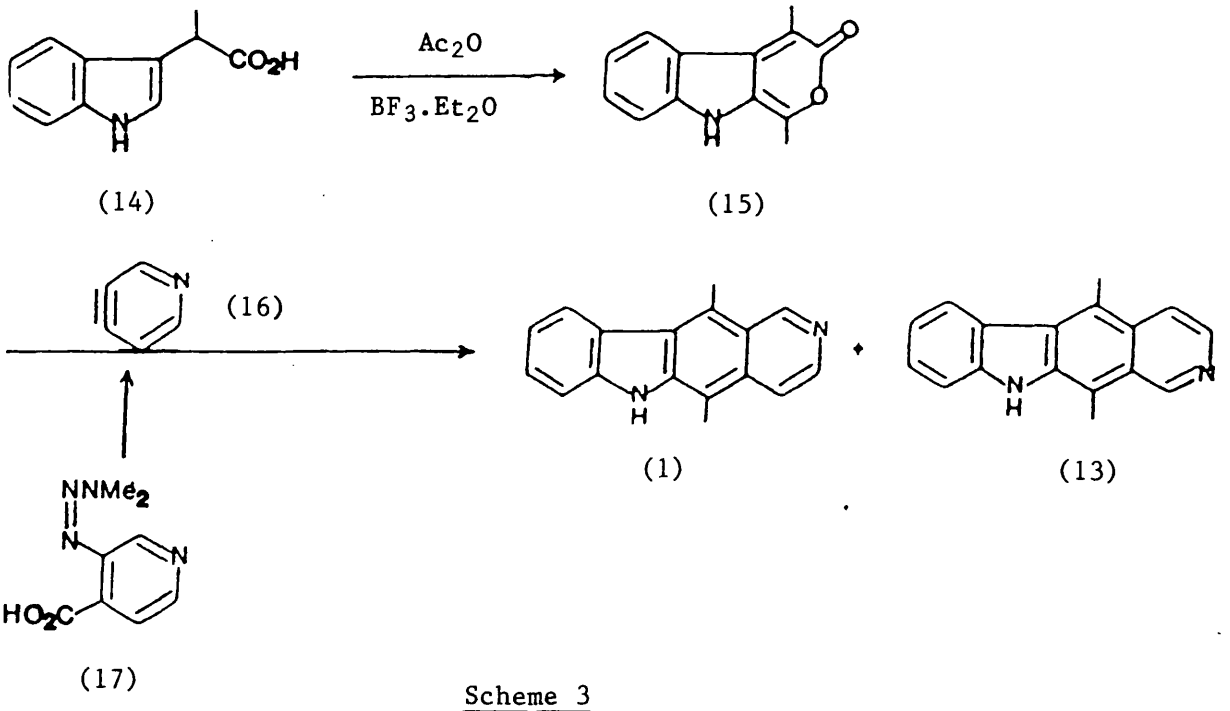
Shudo *et al.*[19] have recently developed a procedure for the reductive phenylation of nitroarenes and successively applied the method to the synthesis of ellipticine (1) and isoellipticine (13). Treatment of an inseparable 1:1 mixture of 2-acetyl-1,2,3,4-tetrahydro-5,8-dimethyl-6 and 7-nitroisoquinoline (9) in benzene and trifluoromethanesulphonic acid (TFSA) with iron pentacarbonyl (IPC) gave the phenylaminotetrahydroisoquinolines (10) (44%) and (11) (22%). Conversion of the primary amine in (10) to the azide moiety, followed by a thermal cyclisation furnished the cyclised carbazole (12) (43%), which was aromatised to ellipticine (46%) using 10% Pd/C. By a similar procedure, isoellipticine (13) was obtained from (11) (Scheme 2).



Scheme 2

C-type syntheses

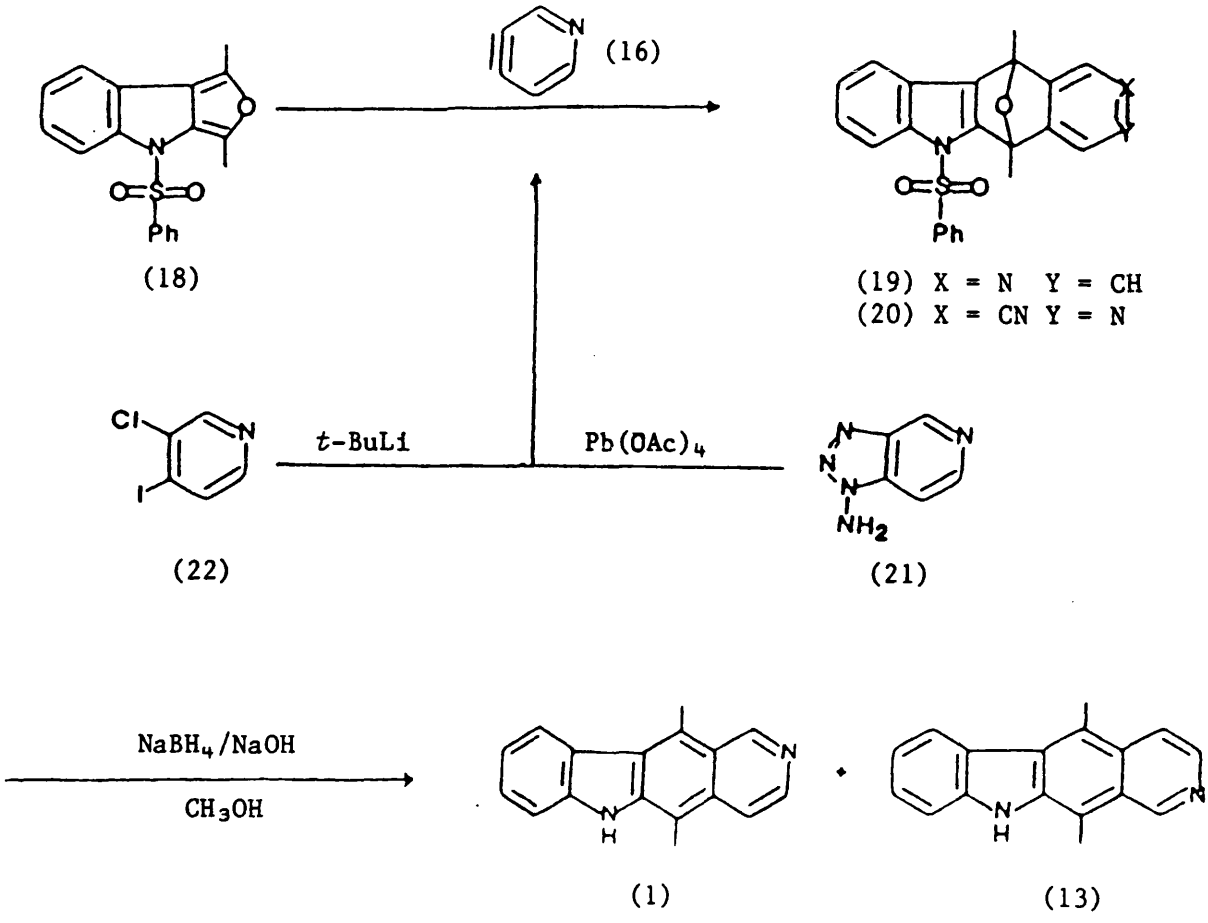
Moody *et al.*[20] have described a short synthesis of ellipticine based upon a Diels-Alder approach utilising the pyrano[4,3-*b*]indol-3-one (15) as the diene. This substrate was prepared by the reaction of α-methyl-indole-3-acetic acid (14) with acetic anhydride in the presence of boron trifluoride-etherate. The ene in this case was 3,4-pyridyne (16) which, when reacted with the diene gave after loss of carbon dioxide, ellipticine (1) (20%) and an equal amount of isoellipticine (13). The 3,4-pyridyne was generated *in situ* by the thermolysis of 3-(3,3-dimethyltriazen-1-yl)-pyridine-4-carboxylic acid (17) (Scheme 3).



Scheme 3

In a similar approach, Gribble *et al.*[21] have used the Diels-Alder reaction between 3,4-pyridyne and 1,3-dimethyl-4-(phenylsulphonyl)-4H-furo[3,4-*b*]indole (18). Oxygen bridge extrusion of the resultant Diels-Alder adducts (19) and (20) was achieved by treatment with sodium boro-hydride and also gave a mixture of ellipticine (1) (23%) and isoellipticine

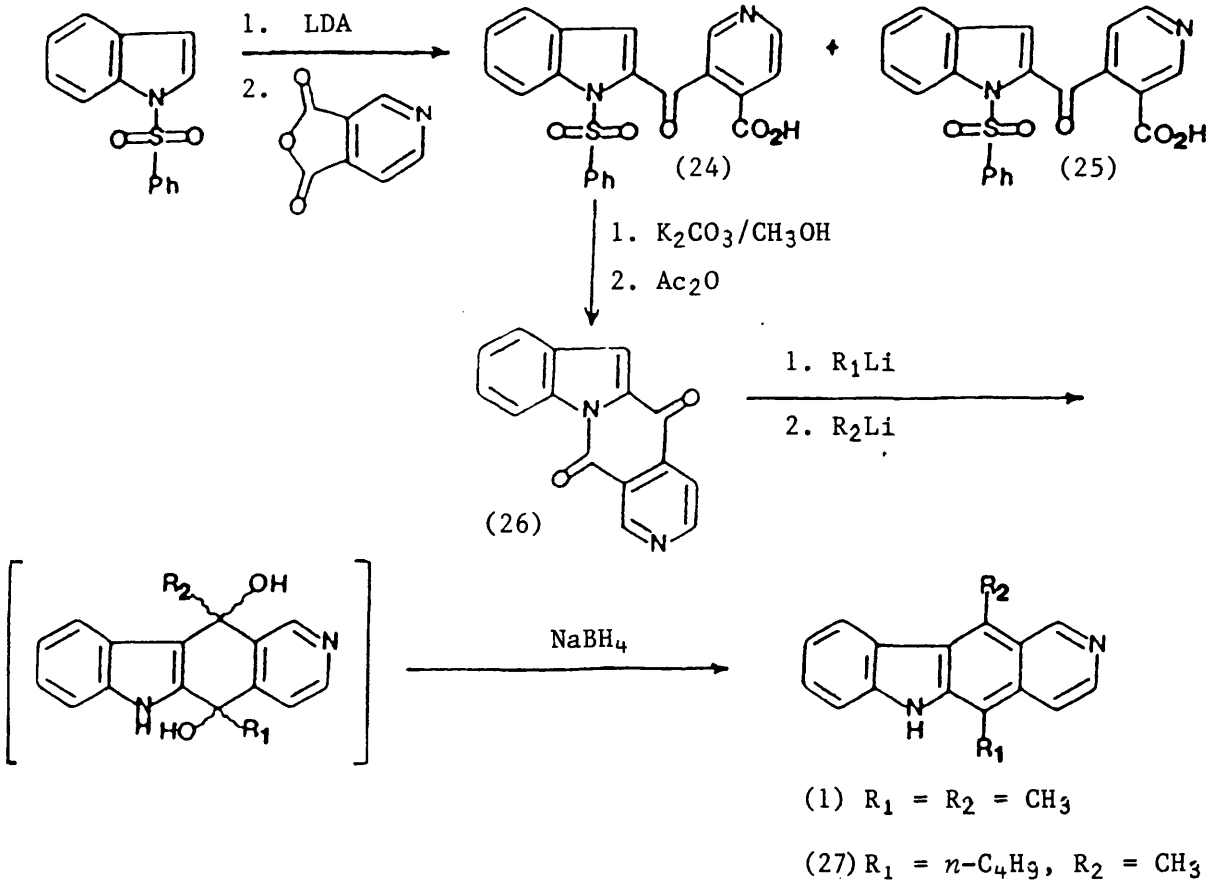
(13) (29%). Here, generation of 3,4-pyridyne (16) was accomplished either by lead tetraacetate oxidation of 1-aminotriazole[4,5-*c*]pyridine (21) or reaction of 3-chloro-4-iodopyridine (22) with *t*-butyllithium (Scheme 4).



Scheme 4

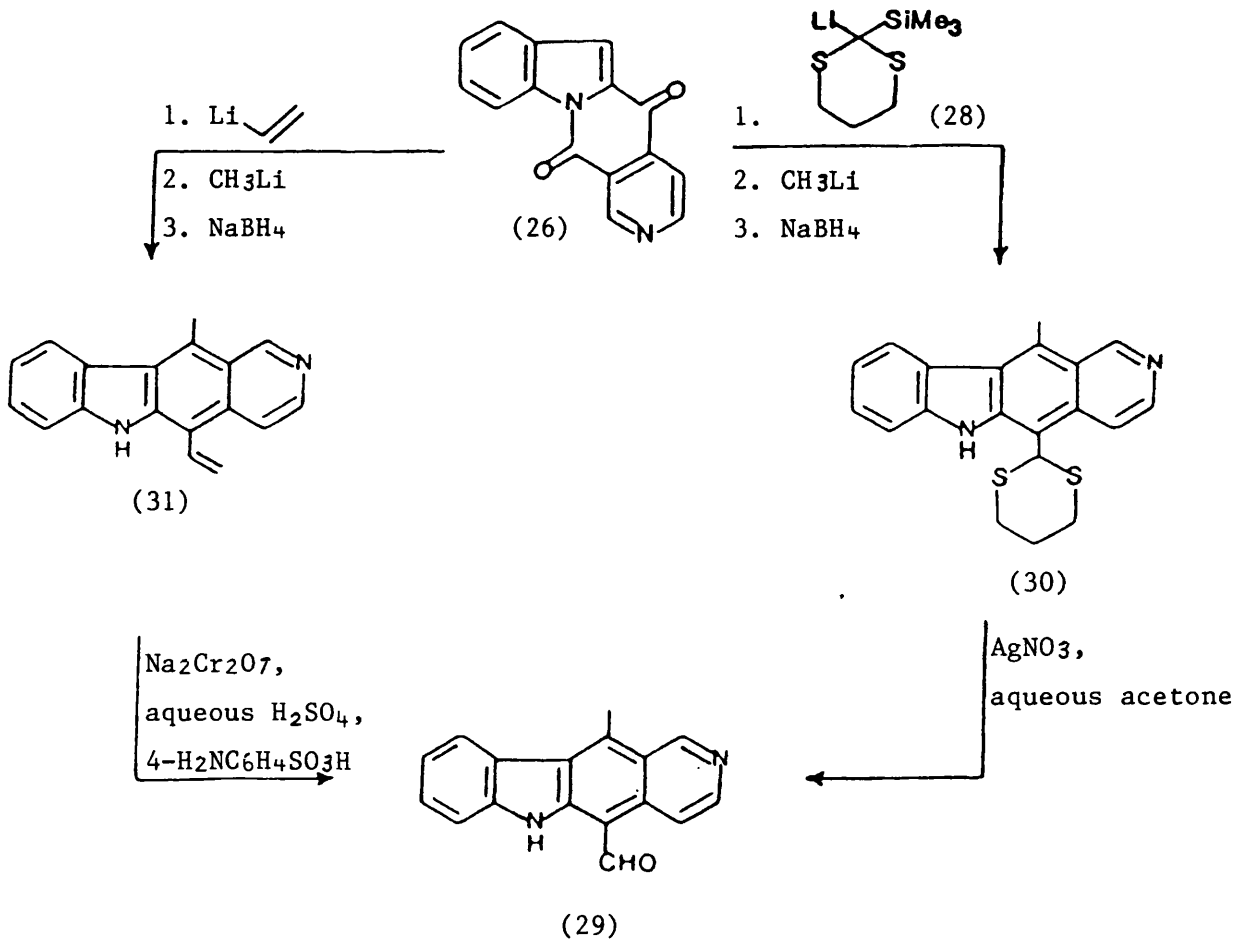
In 1982 Gribble and co-workers developed a new and high yielding synthesis of ellipticine based on the regioselective ring opening of cinchomeronic anhydride (23) by the anion of 1-(phenylsulphonyl)indole.[22] From the resulting mixture of keto acids (24) and (25) (92:8, 78%), the major isomer (24) on hydrolysis with potassium carbonate/methanol, followed by treatment with hot acetic anhydride furnished the keto lactam (26). Treatment of the keto lactam with methyllithium (two equivalents) at -100 °C gave a diastereomeric mixture of diols, which when treated with sodium borohydride afforded ellipticine (1) (82%). In identical fashion the

minor keto acid (25) has been converted to isoellipticine. When applied to 5-methoxyindole the same reaction sequence produced 9-methoxyellipticine in 47% overall yield. Recently, the same group have greatly extended the scope of this route by noting that the addition of the first molecular equivalent of an alkyllithium reagent to the keto lactam (26) is regioselective. As a result it has been possible to manipulate positions 5 and 11 of the ellipticine skeleton to construct unsymmetrical ellipticines by the sequential addition of different alkyllithiums. For instance, sequential treatment of the keto lactam with *n*-butyllithium, methyllithium and then sodium borohydride resulted in 5-*n*-butyl-11-methyl-6H-pyrido-[4,3-*b*]carbazole (27) (70%) along with the 5,11-di-*n*-butyl derivative (18%)[23] (Scheme 5).



Scheme 5

Furthermore, the use of 2-lithio-2-trimethylsilyl-1,3-dithiane (28) as a formyl anion synthetic equivalent in the reaction sequence, instead of *n*-butyllithium, led to the first total synthesis of 5-formyl-11-methyl-6H-pyrido[4,3-*b*]carbazole (29). Thus, stepwise treatment of the keto lactam (26) with (28), methyllithium and then with sodium borohydride gave 5-(1,3-dithian-2-yl)-11-methyl-6H-pyrido[4,3-*b*]carbazole (30), albeit in low yield (25%). Use of just 2-lithio-1,3-dithiane in the transformation led to an even lower yield of (30). Cleavage of the dithianyl function with aqueous silver nitrate gave the formylellipticine (29) (100%)[23] (Scheme 6).

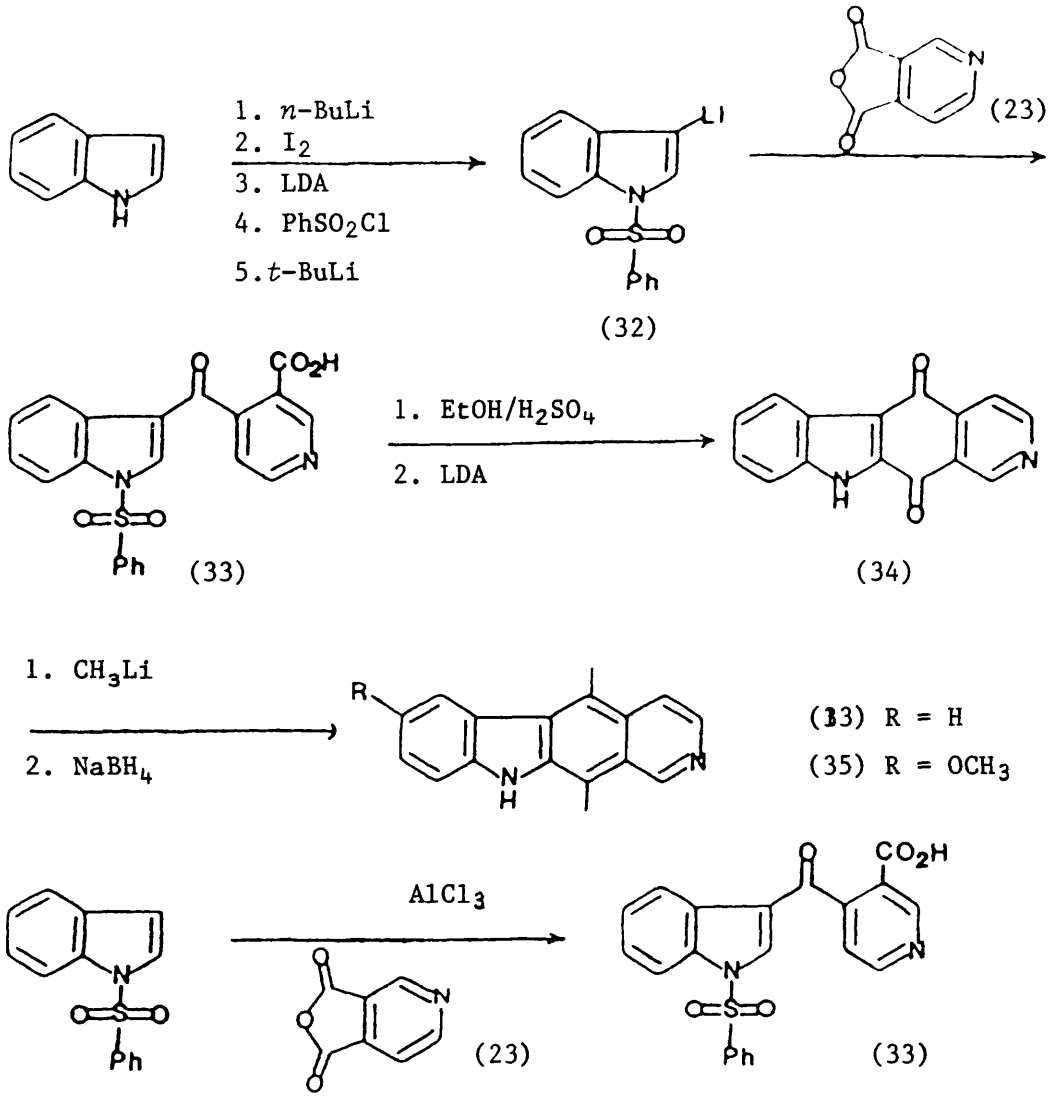


Scheme 6

This alkaloid, also known as 17-oxoellipticine (a consequence of its assumed biosynthesis from stemmadenine), was isolated and characterised by Koch *et al.* from the African tree *Strychnos dinklagei* in 1980.[24] Since its discovery and synthesis, the alkaloid has been isolated as a metabolite during the incubation of ellipticine (1) with cell cultures of several *Choisya ternata* strains.[25]

In an effort to improve the overall efficiency of the 17-oxoellipticine synthesis Gribble and co-workers have reported a second synthesis based on the same methodology, but utilising a vinyl group as a precursor to the formyl group. Thus, treatment of the keto lactam (26) with vinyllithium followed by methyllithium gave, after reduction of the intermediate diol, 5-vinyl-11-methyl-6H-pyrido[4,3-*b*]carbazole (31) (78%). Oxidation of (31) to 17-oxoellipticine (29) was achieved using chromic acid in the presence of a dispersing agent. Overall the alkaloid (29) was synthesised from indole in 7 steps and in 38% yield[26] (Scheme 6).

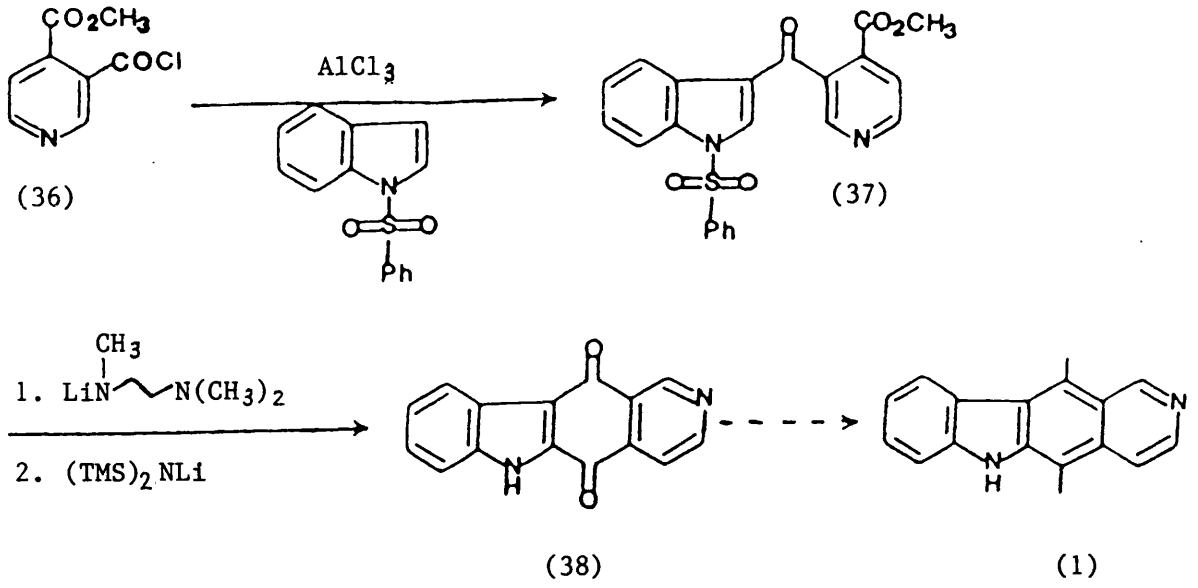
Gribble *et al.*[27] have also updated their synthesis of isoellipticine (13) by a more efficient alternative route. Regioselective acylation of the 3-lithioindole species (32) with cinchomeronic anhydride (23) yielded the keto acid (33) (57%). Conversion to the keto ester, followed by a strong base mediated cyclisation using lithium diisopropylamide furnished the quinone (34) (59%). Treatment with methyllithium, followed by sodium borohydride reduction gave isoellipticine (13) in 20% overall yield from indole. The same sequence when applied to 5-methoxyindole gave 7-methoxyisoellipticine (35) in 21% overall yield. It was found later that the keto acid (33) could be synthesised directly in 69% yield from a Friedal-Crafts acylation of 1-phenylsulphonylindole with cinchomeronic anhydride (23) and aluminium trichloride[28] (Scheme 7).



Scheme 7

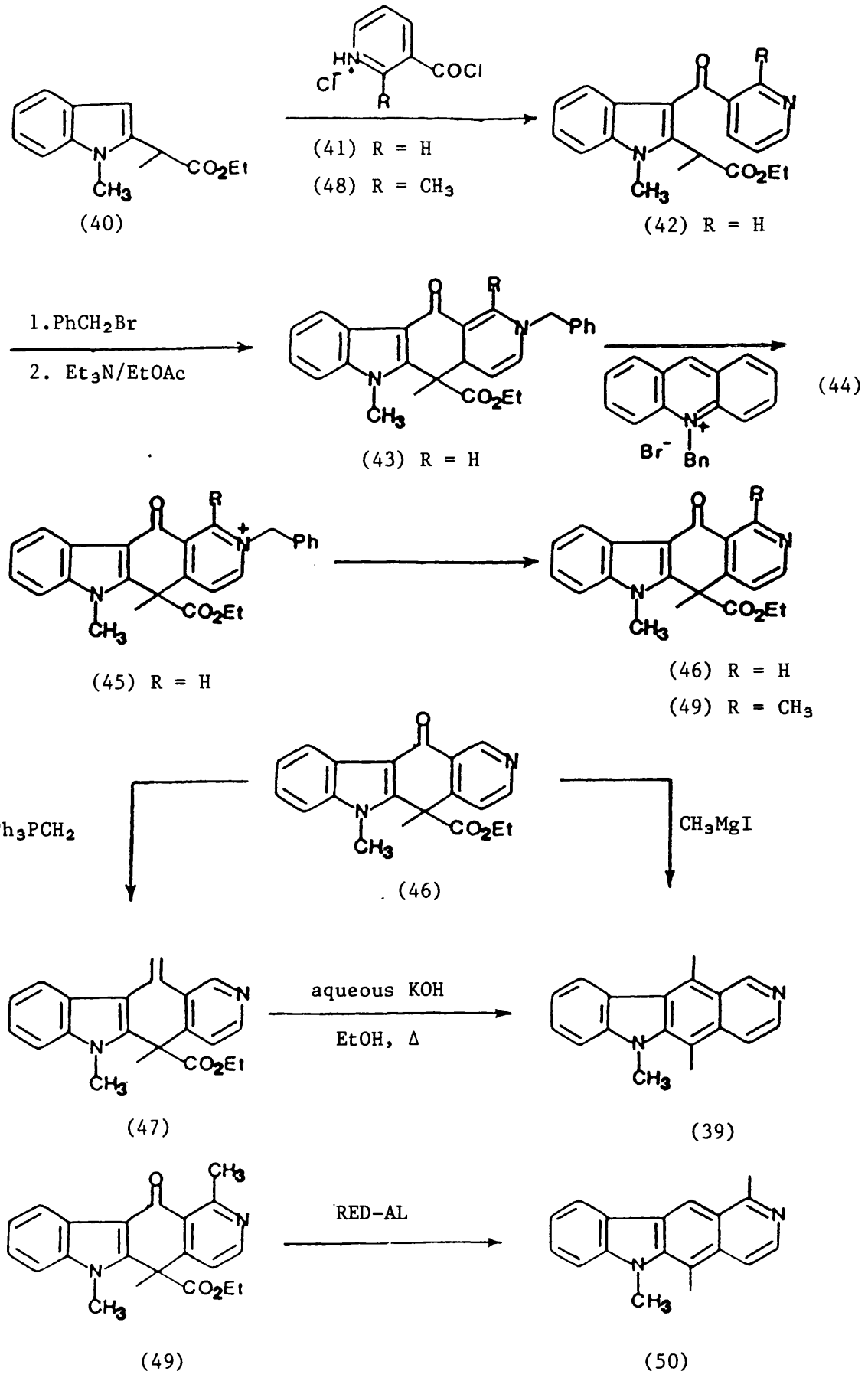
By applying a similar acylation reaction, but with the acid chloride (36), Gribble *et al.*[28] formulated yet another new synthesis of ellipticine. A base induced cyclisation reaction of the resulting keto ester (37) gave the quinone (38) [24% from (36)], which has previously been converted to ellipticine and several derivatives. The cyclisation step was best effected using the anion derived from N,N,N′-trimethylethylenediamine, followed by treatment with lithium bis(trimethylsilyl)amide, rather than lithium diisopropylamide used in generating the isoellipticine quinone

(34) (Scheme 8).



Scheme 8

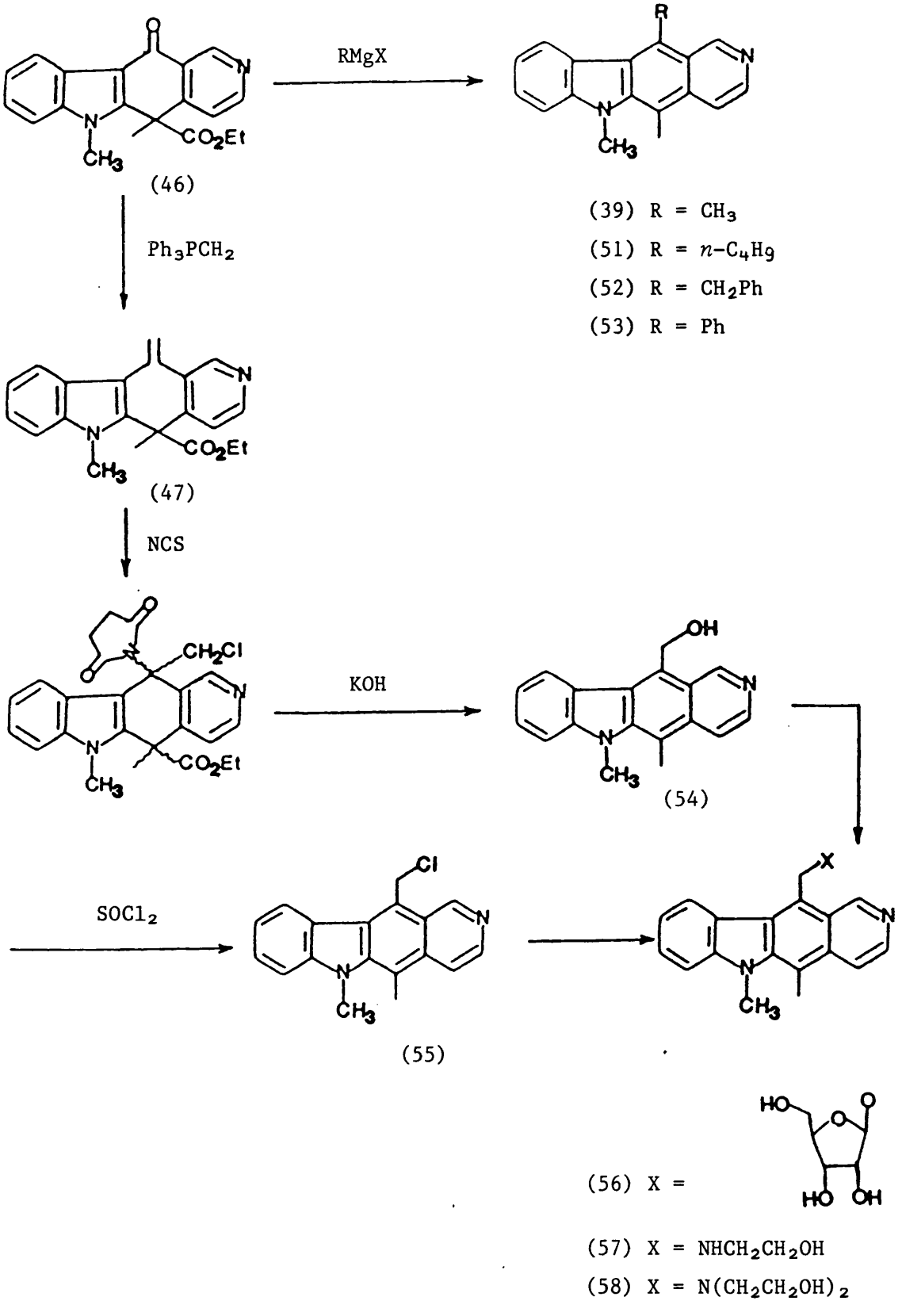
In 1982, Pandit *et al.*[29] reported the synthesis of 6-methylellipticine (39) utilising the following steps: acylation of the indole ester (40) with nicotinoyl chloride hydrochloride (41) gave the keto ester (42) (55%). Alkylation with benzyl bromide afforded the corresponding pyridinium salt, which was cyclised to give the dihydropyridine (43) as a mixture of diastereomers (1:1, 81%). Oxidation with N-benzylacridinium bromide (44) afforded the salt (45), which on reductive debenzylation gave the key keto ester (46) (72%). Conversion of (46) to 6-methylellipticine (39) was accomplished either by (a) excess methylmagnesium iodide (40%) or (b) Wittig reaction with methylenetriphenylphosphorane to give (47) (65%), followed by base hydrolysis and decarboxylation (60%). A similar sequence, but using 2-methylnicotinoyl chloride hydrochloride (48), led directly to the keto ester (49) [15% from (40)], which upon treatment with sodium bis(2-methoxyethoxy)aluminium hydride (RED-AL) was transformed to 6-methylolivacine (50) (57%) (Scheme 9).


(41) R = H
(48) R = CH3
(40)
(42) R = H
1.PhCH2Br
2. Et3N/EtOAc
(43) R = H
(44)
(45) R = H
(46) R = H
(49) R = CH3
Ph3PCH2
(46)
CH3MgI
aqueous KOH
EtOH, Δ
(47)
(39)
RED-AL
(49)
(50)

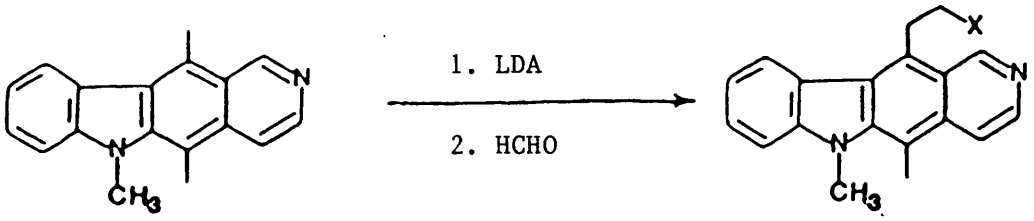
Scheme 9

Recently, the same group have exploited the presence of the 11-keto moiety in the intermediate (46) and synthesised a large number of 6-methyl-11- substituted ellipticine derivatives.[30] By analogy with the synthesis of 6-methylellipticine using excess methylmagnesium iodide [(40) to (39)], reaction of (46) with *n*-butylmagnesium bromide, benzylmagnesium bromide and phenylmagnesium iodide led to the corresponding 11-substituted derivatives (51) (25%), (52) (40%) and (53) (44%) respectively. Furthermore, treatment of (47) with N-chlorosuccinimide, followed by hydrolytic decarboxylation, furnished (54) [47% from (47)], which was converted on reaction with thionyl chloride to the corresponding chloro-derivative (55) (100%). Both compounds (54) and (55) served as useful intermediates for further side chain derivatisation, since the former corresponds to a nucleophilic reagent and the latter an electrophilic reagent. Thus reaction of the tribenzoate 1-β-acetate of ribose with tin tetrachloride and the alcohol (54) gave the acetal which was de-protected to the ribosyl derivative (56) (86%). Conversely, reaction of (55) with the nucleophilic amines $NH_2CH_2CH_2OH$ and $NH(CH_2CH_2OH)_2$ readily gave the derivatives (57) (70%) and (58) (60%) respectively (Scheme 10).

It was later found that 6-methylellipticine (39) underwent deprotonation with the base lithium diisopropylamide at the C-11 methyl group, thus leading to a means of direct functionalisation.[31] Quenching of the resulting anion with formaldehyde led to the hydroxymethyl derivative (59) (>50%), which was coupled in the presence of tin tetrachloride with the tribenzoate 1-β-acetate of ribose and the pentaacetates of glucose and galactose to yield the ellipticine sugar derivatives (60), (61) and (62) respectively (Scheme 11).

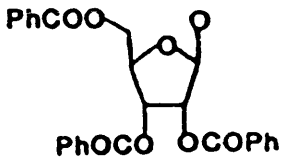

RMgX
(46)
(39) R = CH3
(51) R = n-C4H9
(52) R = CH2Ph
(53) R = Ph
Ph3PCH2
(47)
NCS
KOH
(54)
SOCl2
(55)
(56) X =
(57) X = NHCH2CH2OH
(58) X = N(CH2CH2OH)2

Scheme 10

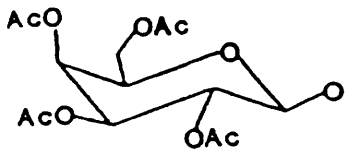


(39)

(59) X = OH

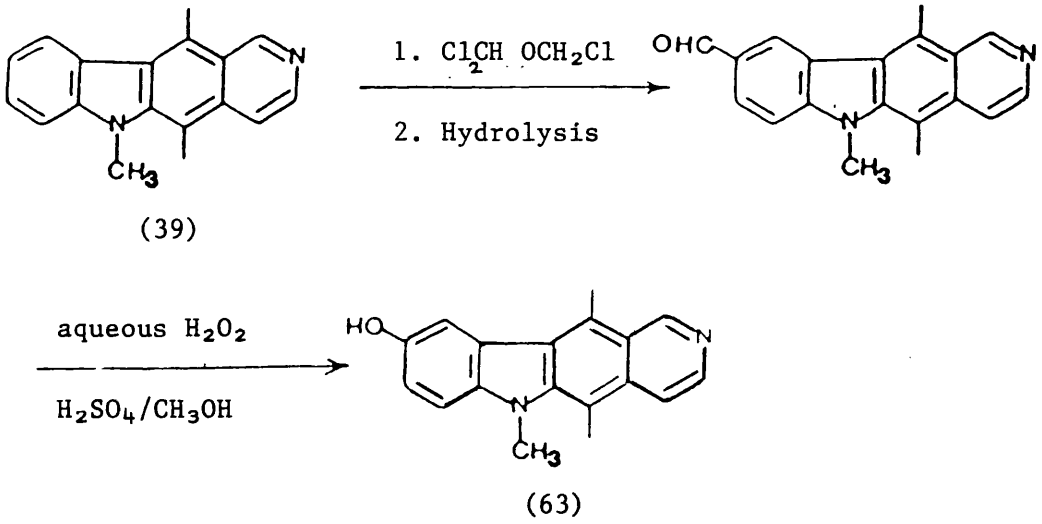
(60) X = 

(61) X = 

(62) X = 

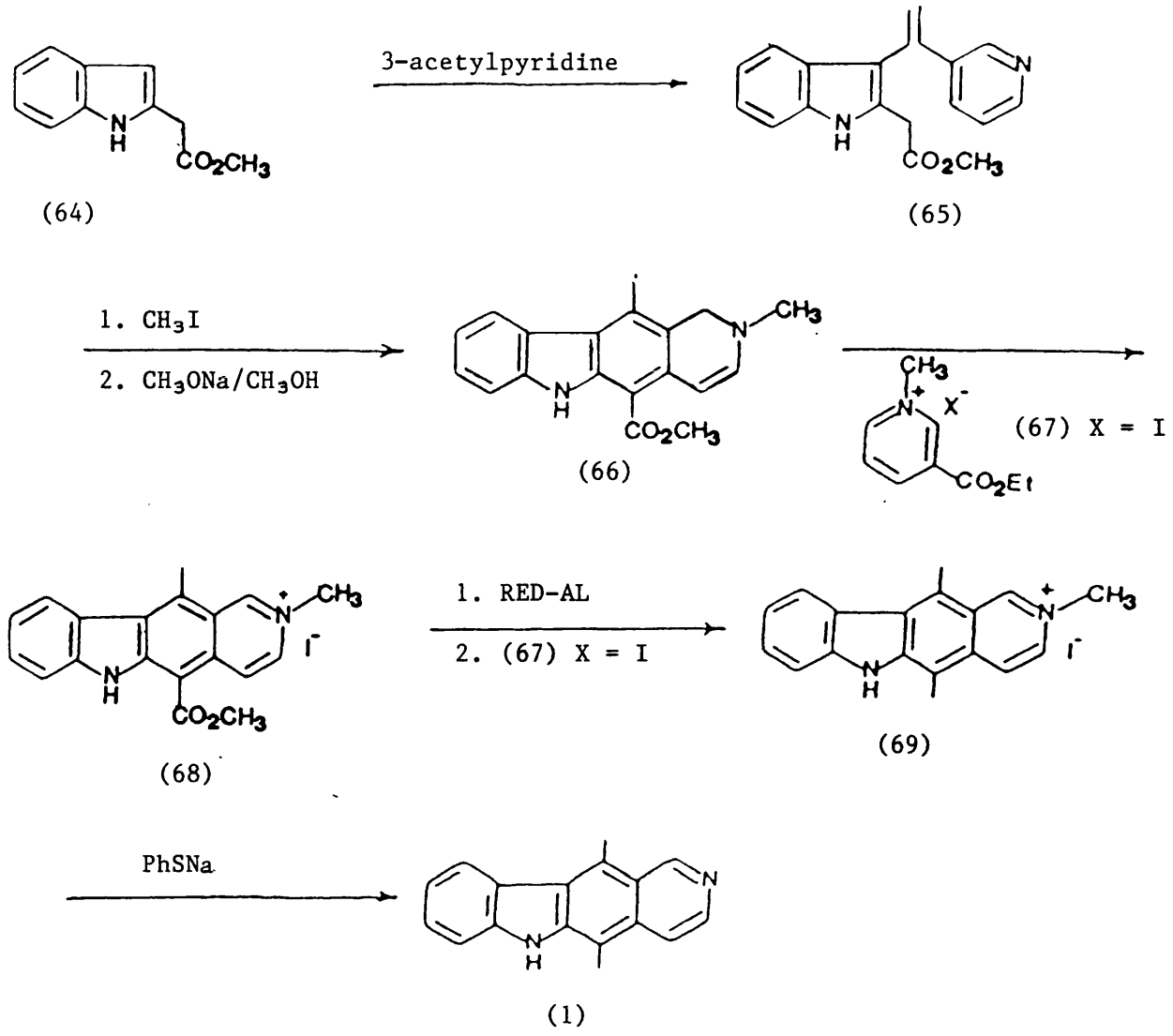
Scheme 11

Apart from one report describing the synthesis of 9-hydroxyellipticine (5), albeit in low yield directly from ellipticine (1),[32] introduction of a hydroxy group at position 9 of the ellipticine skeleton has been achieved by utilisation of an appropriate protected hydroxylated aromatic starting material. Recently, Pandit *et al.*[33] have developed a facile procedure to circumvent such a need. 6-Methylellipticine (39) was converted to the corresponding 9-hydroxy derivative (63) in high yield (85%) *via* a reaction sequence involving regioselective C-9 formylation, followed by a Baeyer-Villiger rearrangement (Scheme 12).



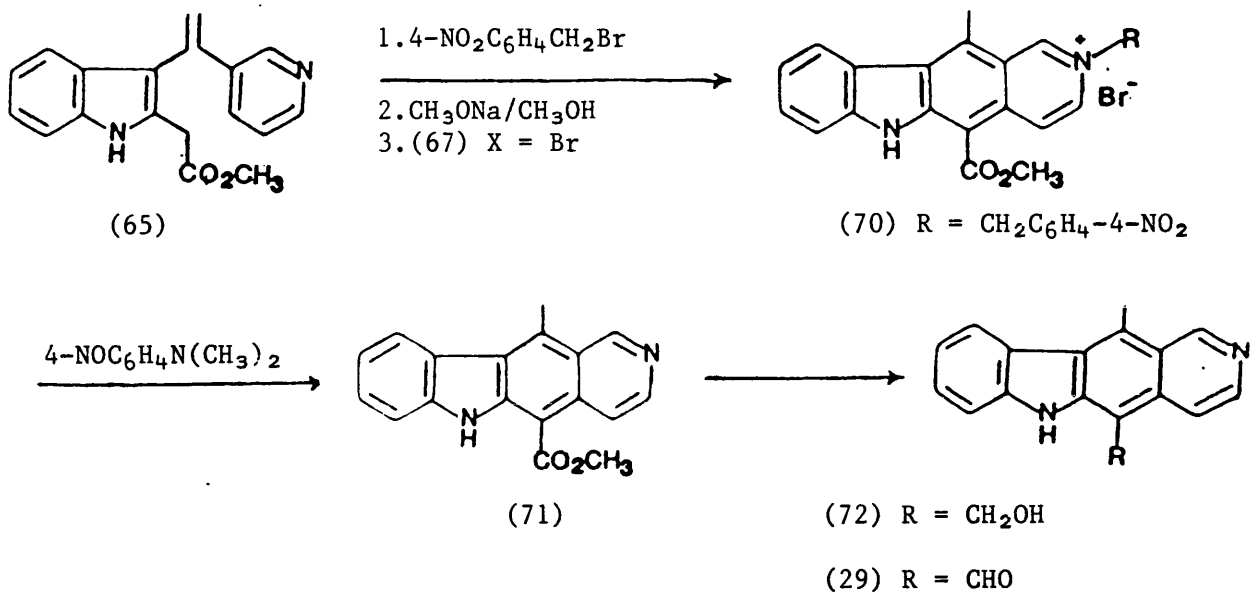
Scheme 12

Independently, Weller *et al.*[34] have recently extended Pandit's approach to the synthesis of ellipticine. Thus, condensation of 2-(methoxycarbonyl(methyl)indole (64) with 3-acetylpyridine, under conditions first used by Bergmann,[35] gave the adduct (65) (81%). Methylation and immediate exposure of the resulting pyridinium salt to methoxide generated the labile dihydropyridine (66) (62%), which on direct treatment with the oxidising agent 3-ethyl-nicotinatemethiodide (67) gave the quaternised salt (68) (78%). Sodium bis-(2-methoxyethoxy)aluminium hydride (RED-AL) reduction of the product (68), followed by oxidation with (67), furnished N-2-methylellipticinium iodide (69) (85%). Nucleophilic demethylation of (69) with sodium thiophenoxide gave ellipticine (1) (91%) (Scheme 13).



Scheme 13

Later, Archer *et al.*[36] attempted the demethylation of Weller's pyridinium salt (68) using sodium thiophenoxide, but found the conditions too drastic to prevent the methoxycarbonyl group surviving unchanged. By appropriate adaptation of Weller's synthesis of the salt (68),[34] the 4-nitrobenzyl analogue (70) was prepared from the adduct (65) (80%) and found to undergo debenzylation on treatment with 4-nitroso-N,N-dimethylaniline. Using the ester (71) [47% from (70)], Archer and co-workers reported a new synthesis of 17-oxoellipticine (29). Initial lithium aluminium hydride reduction of the ester (71) to the alcohol (72), followed by oxidation with manganese dioxide, furnished the alkaloid [44% from (70)],[36] (Scheme 14).



(65)

(70) R = $CH_2C_6H_4$-4-NO_2

(71)

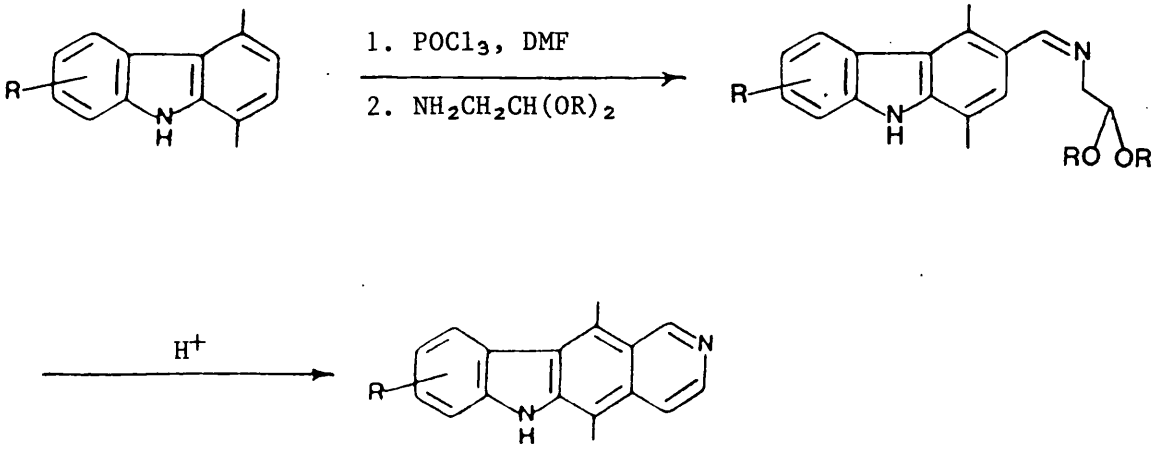
(72) R = CH_2OH

(29) R = CHO

Scheme 14

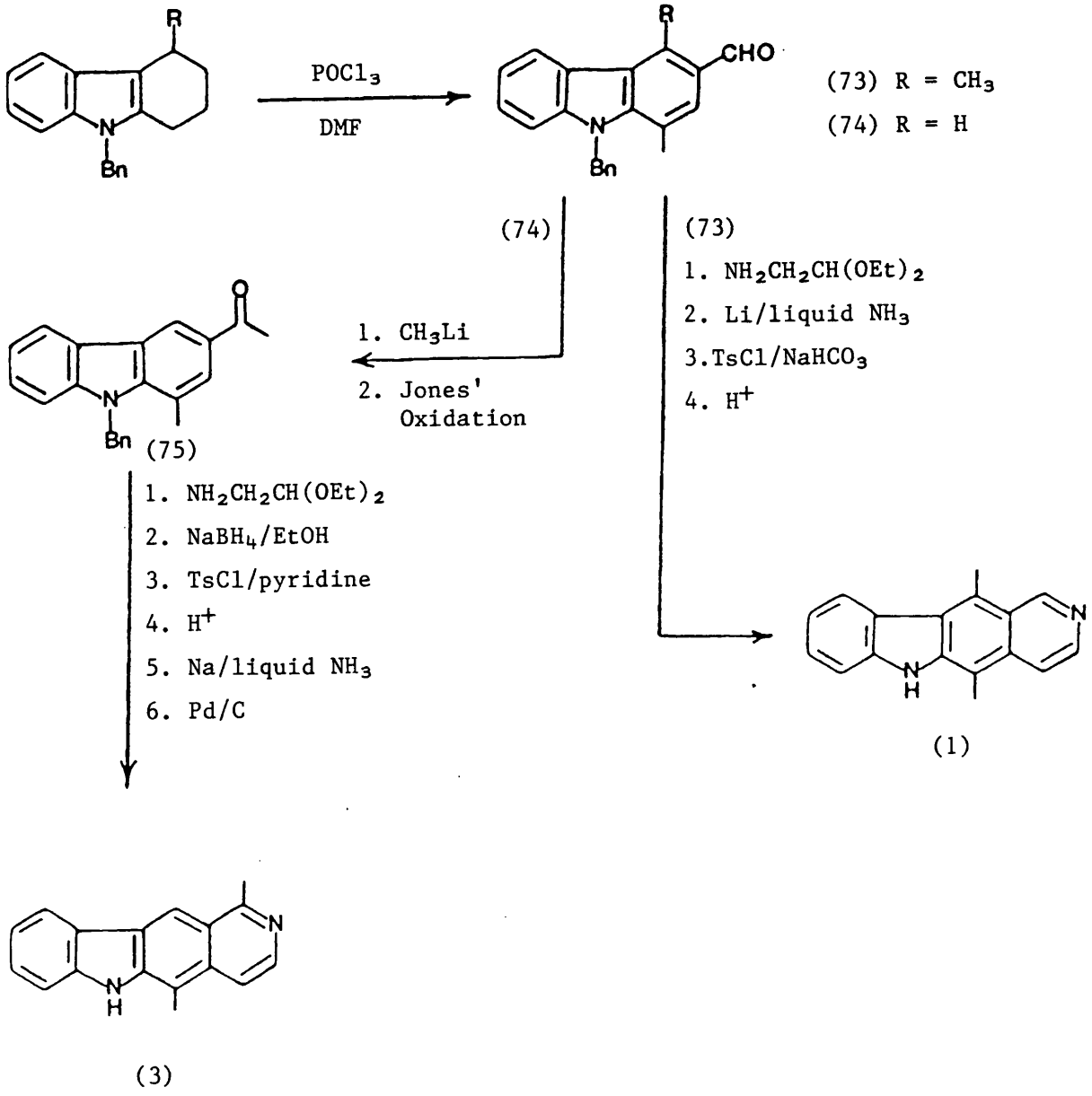
D-type syntheses

Since a versatile reaction sequence for D-ring construction, in the form of the prototype Cranwell-Saxton synthesis[37] (Scheme 15), is well established, many of the new syntheses in this class have been directed towards obtaining the appropriately substituted carbazoles.



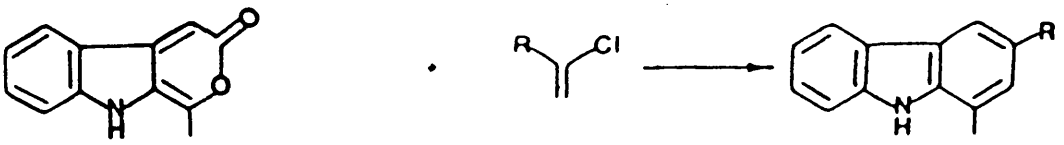
Scheme 15

Okuyama *et al.*[38] obtained the 3-formylcarbazoles (73) (39%) and (74) (45%) by a Vilsmeier-Haack reaction on the appropriately substituted 1,2,3,4-tetrahydro-N-benzylcarbazoles. Treatment of the substrate (74) with methyllithium, followed by Jones' oxidation, furnished the ketone (75) (61%). Ellipticine (1) (57%) and olivacine (3) (6%) were subsequently obtained from the carbazole derivatives (73) and (75) respectively by employing a modified Cranwell-Saxton synthesis[39] (Scheme 16).



Scheme 16

In an alternative route, Narsimham *et al.*[40] have developed a high yielding synthesis of 3-formyl- and 3-acetyl-carbazoles by employing a cycloaddition reaction of 1-methylpyrano[3,4-*b*]indol-3-one (76) with appropriately substituted haloalkenes. The resulting carbazoles (77) (76%) and (78) (78%) were converted to 11-desmethylellipticine (79) (72%) and olivacine (3) (66%) respectively, following the modified Cranwell-Saxton synthesis.[39] In the case of olivacine, an increase in the yield was noted by careful control of the amount of concentrated hydrochloric acid used (Scheme 17).



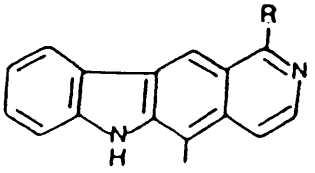
(76)

1. $NH_2CH_2CH(OMe)_2$

(77) R = CHO

(78) R = $COCH_3$

1. $NH_2CH_2CH(OMe)_2$
2. Reduction
3. $TsCl/K_2CO_3$
4. H^+

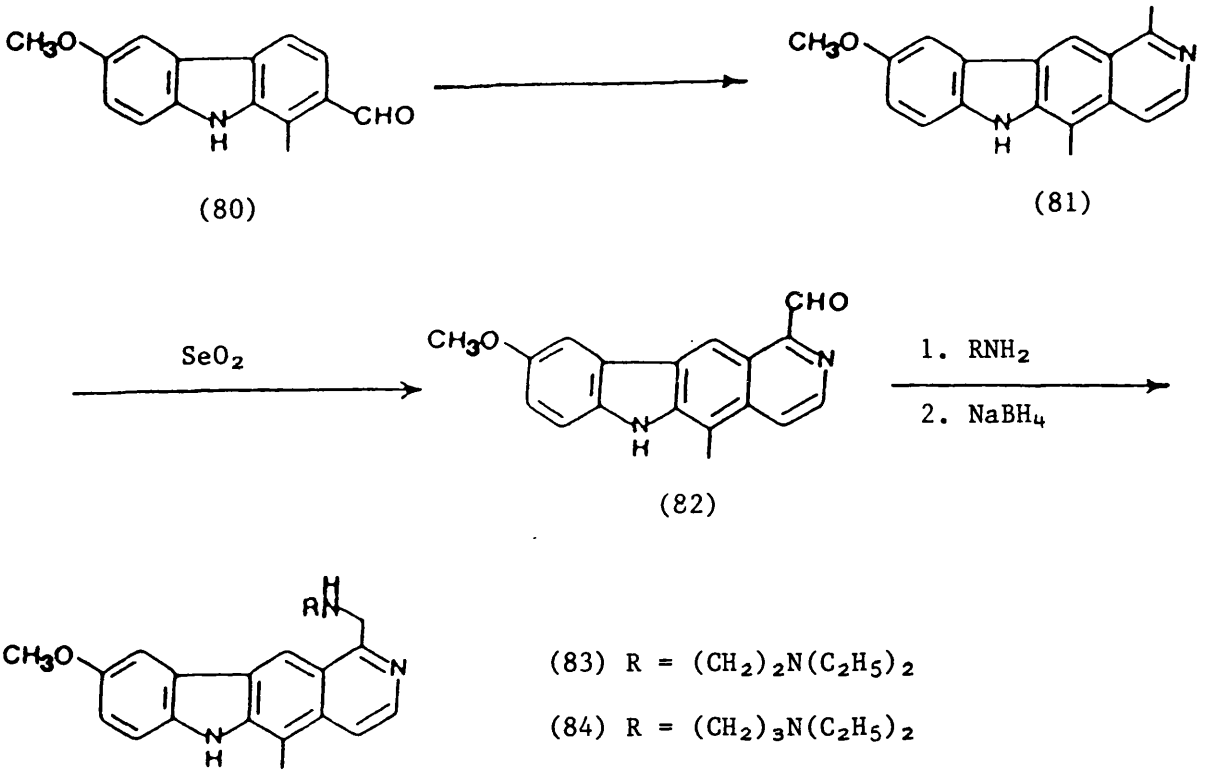


(3) R = CH_3

(79) R = H

Scheme 17

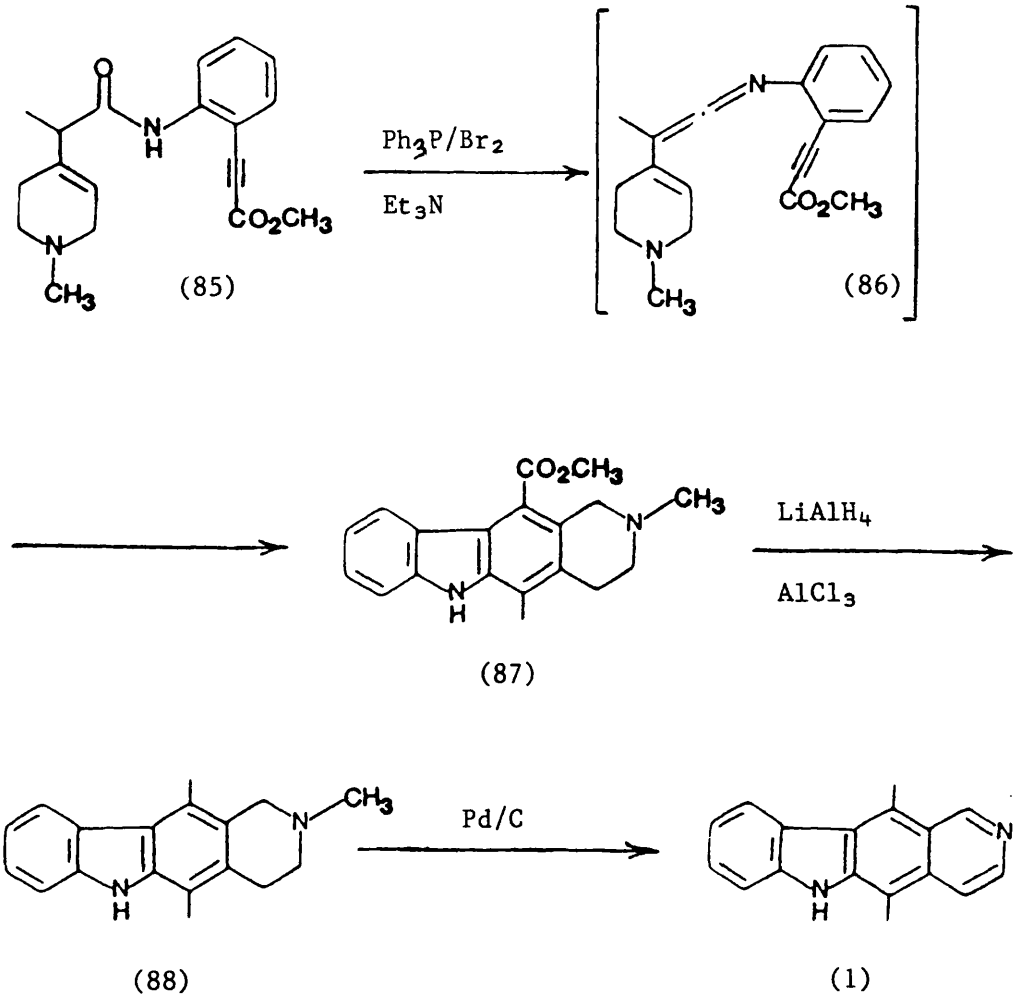
Starting from 1-methyl-2-formyl-6-methoxycarbazole (80), Bisagni *et al.*[41] have synthesised a number of 1-aminomethylpyrido[4,3-*b*]carbazole derivatives for pharmacological evaluation. Following classical procedures,[42] (80) was converted to 9-methoxyolivacine (81), which on oxidation with selenium dioxide gave 1-formyl-11-desmethylellipticine (82) (70%). Reductive amination of (82) with the amines $NH_2-(CH_2)_n-N(C_2H_5)_2$ (n = 2,3) and sodium borohydride afforded the corresponding amine derivatives (83) [n = 2, (67%)] and (84) [n = 3, (60%)] (Scheme 18).



Scheme 18

B+C-type syntheses

A highly convergent route towards ellipticine (1), involving simultaneous B and C ring construction, has recently been reported by Differding and Ghosez.[16] Heating the alkyne (85) with triphenylphosphine-bromine in the presence of triethylamine generates the vinylketenimine (86) *in situ*, which undergoes an intramolecular Diels-Alder cycloaddition to afford the carbazole (87) (50%). Mixed hydride reduction (lithium aluminium hydride and aluminium trichloride) of this product gives N-2-methyltetrahydroellipticine (88) (71%). This product, by dehydrogenation-dealkylation, has previously been converted to ellipticine (1)[43] (Scheme 19).



Scheme 19

(B) The Biochemical Properties of Ellipticine and its Derivatives

The range of antitumoural activity exhibited by 9-hydroxyellipticine (5) and 9-hydroxy-N-2-methylellipticinium acetate (4) against various experimental tumours in mice and rats has been considered by the pharmacological group of the European Organisation for Research and Treatment of Cancer,[4a] and these results are summarised in Table 1. Similarly, the activity of various ellipticine derivatives on leukaemia L1210 cells is presented in Table 2.[44]

Although much information regarding the physical effects of ellipticine and its derviatives on the structure of DNA is known, the mechanism of their cytotoxic action *in vivo* and *in vitro* is still not well understood. However, the ability of ellipticine to intercalate into DNA was initially established from viscosity, sedimentation and electric dichroism experiments,[7b] and later confirmed by ^{1}H n.m.r. studies of a DNA-ellipticine complex.[45] In addition, Jain *et al.*[46] co-crystallised ellipticine with 5-iodocytidylyl(3'-5')guanosine and were able to solve the three dimensional structure of the complex by *X*-ray analysis, so showing how intercalation of the drug with DNA at the molecular level might take place. Ellipticine and its derivatives are also known to bind to double stranded DNA with an affinity coefficient of 10^{-5} to 10^{-6} M,[7a] thereby destroying the kinoplastic[47] and base pairing activity of the nucleic acid.[48]

Ross *et al.*[49] observed a unique type of DNA strand break, termed a protein associated strand break, following exposure of ellipticine and adriamycin, a benzanthraquinone intercalating agent, to mouse leukaemia L1210 cells. Single strand DNA breaks also occurred, but only prior to DNA enzymatic deproteinisation. These protein associated strand breaks were further shown to have the protein tightly, if not covalently, bound to the DNA in a spatial and stoichiometric relationship, suggesting that

Table 1

Antitumoural activity of 9-hydroxyellipticine (5) and 9-hydroxy-N-2-methylellipticinium acetate (4) against various experimental tumours[4a]

Experimental animal	Experimental tumour	(5)[a]	(4)[a]
Mice	Leukaemia L1210	+	++
	Leukaemia P388	+	++
	Lewis lung carcinoma	±	±
	Myeloma	+	
	Osteosarcoma		-
Rats	Yoshida lymphosarcoma		++
	Gardner lymphosarcoma OG		++
	Squamous cell carcinoma		++

[a]Here + means active, ++ very active, - inactive, ± inconclusive result.

Table 2

Comparative effect of ellipticine derivatives on L1210 cells *in vivo* and *in vitro* and DNA binding affinity[44]

Compound	ID_{50}[a]		ILS (%)[b]			LD_0[c]	DNA
	(ng/ml)	(μm)	(1)	(1/2)	(1/5)	(mg/kg)	(affinity)[d]
(1)	242	0.99	68	40	12	50	1.5×10^5
(2)	164	0.60	70	-	-	70	1.0×10^5
(4)	13.8	0.05	62	53	28	5	1.3×10^6
(5)	3.9	0.015	53	58	28	50	2.0×10^6

[a]Dose which reduced the cell growth by 50% after 48 h, as compared to control.

[b]ILS (Increase in Life Span) over controls.

[c]LD_0 = highest non lethal dose (IP treatment).

[d]Measurement carried out at 25 °C in 0.1 M NaCl - 0.1 M tris-HCl (pH = 7.4).

the protein is bound at or near the break site.

Similar qualitative effects have been noted with a wide variety of other intercalating agents including actinomycin D, daunomycin, lucanthone[50] and 4'-(9-acridinylamino)methanesulphon-3-anisidide (*m*-AMSA).[51] At one time it appeared that these lesions were solely related to this class of drug. Ross and co-workers[50] showed that protein associated DNA breaks were not observed for non-intercalating DNA binding compounds, or agents that inhibited macromolecular synthesis without binding to DNA. From the data, the investigators hypothesised that the protein associated strand breaks may have resulted from local perturbations imposed on the DNA by the intercalating agent. These lead to weakness and/or vulnerability and hence strand scission by a nuclease which ultimately remains bound to one terminus of the break site.

Subsequently, the formation of protein associated DNA breaks were reported for the interaction of the antibiotic nalidixic acid with DNA gyrase.[52] Gyrase is a form of topoisomerase(II) found only in bacterial systems and is important in the mediation of supercoiling of doubly stranded DNA. DNA topoisomerases are enzymes which are important in the regulation of DNA topology and thus facilitate such processes as DNA replication, transcription, transposition and viral integration.[52b,53] Two types of topoisomerases are known, type I and type II. The biological role of type I topoisomerase is poorly understood and as yet, there is little known about it which could be used to aid the improvement of the design of anticancer drugs. However, a great deal is known regarding type II topoisomerase. This enzyme possesses the ability transiently to break and reseal both DNA strands simultaneously, and also by virtue of a phosphotyrosine linkage, bind to free DNA ends during the breakage-reunion process. In the gyrase study nalidixic acid caused the formation of a so-called "cleavage complex", which upon exposure to a protein denaturant,

resulted in DNA-double strand breaks with the enzyme covalently bound at the 5'-terminus of the break site (Figure 1).[52] Similar effects are caused by DNA exposure to intercalating agents of the ellipticine, adriamycin and actinomycin D types. Thus Liu *et al.*,[54] using highly purified topoisomerase(II) from calf thymus, proved that stimulated site specific DNA cleavage by the enzyme occurred in the presence of a variety of intercalating agents, including 9-hydroxy-N-2-methylellipticinium acetate (4). Moreover, they showed that the enzyme remained bound at

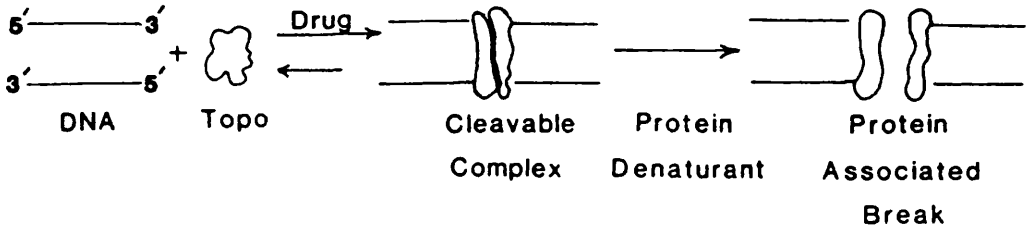


Figure 1 Reaction mechanism for DNA cleavage by topoisomerase (II). The reaction is shifted to the right in the presence of certain intercalating agents or epipodophyllotoxins.

the 5'- end of the cleavage site *via* a phosphotyrosine bond. However, that stimulated DNA cleavage by topoisomerase did not require a topologically restrained substrate, thus suggesting that drug intercalation may not be an essential requirement. Liu and co-workers confirmed this point later by demonstrating that the non-intercalative epipodophyllotoxins, etoposide (VP-16) and teniposide (VM-26), also induced protein linked DNA breaks both *in vivo* and *in vitro*.[54b] As an alternative, the same group has suggested that drug intercalation into DNA enables the

drugs to interact specifically with topoisomerase(II), and thus interfere with topoisomerase(II) resealing action by stabilisation of the cleavable complex.[54]

The relationship between these unique topoisomerase mediated DNA breaks and the cytotoxicity of the drugs has been more difficult to substantiate. Several early papers suggested no relationship existed. For example, ellipticine has been shown to generate a much greater frequency of DNA protein associated strand breaks than adriamycin, even though it is the less potent drug in cytotoxic assays.[55] However, this probably results from the fact that ellipticine induced breaks are repaired much more rapidly than those of adriamycin.[56] Consequently, in many of the early cases, direct comparisons between the activities of drugs of vastly different structures were unfair in view of the possibility of competition between factors other than DNA cleavage.

When structurally similar groups of congeners have been studied, a much better relationship has been demonstrated between DNA cleavage and cytotoxicity. For instance, although both the *ortho* and *meta* isomers of 4'-(9-acridinylamino)methanesulphonanisidide (AMSA) are known to intercalate to approximately the same degree into DNA, *in vitro* studies have revealed that only the more potent *meta* isomer induces topoisomerase mediated DNA cleavage.[51,54a] Similarly, 9-hydroxy-N-2-methylellipticinium acetate (4) is more cytotoxic than ellipticine and has been found to be much more efficient in stimulating DNA protein associated breaks *in vitro*.[54b] Perhaps hydrogen bonding from the hydroxy group leads to stabilisation of the drug-DNA-topoisomerase complex and this may be the reason for enhancement of cytotoxicity. Long *et al.*[57] examined a large series of epipodophyllotoxin congeners against a human lung adenocarcinoma cell line and found a close relationship between DNA breakage

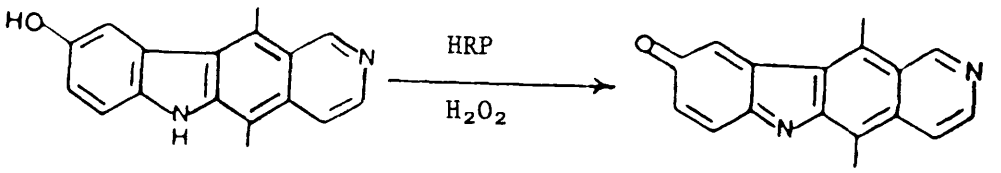
intracellularly and cytotoxicity. However, no direct proof exists which correlates DNA cleavage to cytotoxicity, but a most likely relationship will be established in mutant cell lines which are resistant to topoisomerase mediated drugs. For instance, Grupta[58] has isolated a Vpm-5 Chinese hamster ovary line which has exhibited significant cross-resistance to several topoisomerase-mediated drugs. Preliminary evidence has suggested a reduction in the DNA cleavage activity, but whether this represents a qualitative or quantitative relationship to cytotoxicity remains undefined.[59]

There is growing evidence that if cytotoxicity of the drug is related to topoisomerase-mediated DNA cleavage, then it is formation of the cleavable complex, and not subsequent loss of topoisomerase activity, which is responsible for lethality.[60] Additional support comes from the fact that the weakly cytotoxic *ortho* isomer of 4'-(9-acridinylamino)methane sulphonanisidide (AMSA) has been shown to be an effective inhibitor of the topoisomerase catalytic function.[51,54] However, there is little information in mammalian cells regarding the mechanism by which the cleavable complex results in cell death. Clearly further studies are necessary in order to unravel the role of topoisomerase(II) in mediating antitumour activity in cancer therapy.[60]

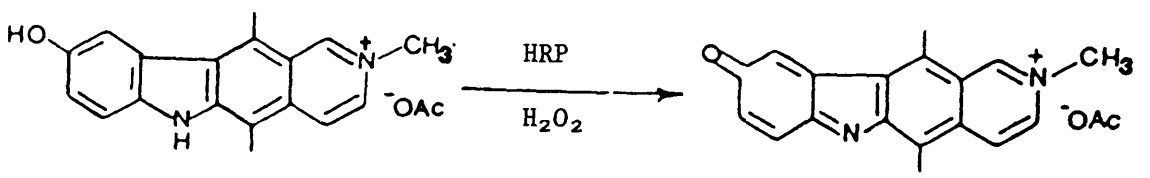
Although slightly different modes of drug-DNA-topoisomerase interactions may account for the increased cytotoxicity and antitumour activity *in vitro* of 9-hydroxyellipticines as opposed to ellipticine, oxidative studies of 9-hydroxyellipticines *in vitro* have suggested that apart from classical drug intercalation into DNA, 9-hydroxyellipticines may have a totally different type of biological effect. For example Auclair *et al.*[8a] showed using horse radish peroxidase (HRP) and human myeloperoxidase (MPO) in the presence of hydrogen peroxide, that 9-hydroxyellipticine (5) and

9-hydroxy-N-2-methylellipticinium acetate (4) generated phenoxy radicals *in vitro* that preferentially decayed by a dismutation process to yield the quinone-imines (89) (95%) and (90) respectively. Furthermore, only ellipticine derivatives in which there exist a hydroxy group *para* to the indolic NH act as substrates for the peroxidases. Peroxidases are enzymes known to catalyse the oxidation of various organic compounds, such as arylamines and phenols, in the presence of hydrogen peroxide. Not surprisingly the chemical reactivity of the product quinone-imines depends upon the charge of the nitrogen atom in ring D. Compound (89) has been obtained as a well-defined stable solid, but (90) has not yet been isolated and fully characterised due to its high reactivity in solution. Auclair and co-workers attributed this to the electrophilic property of the nitrogen in the pyridinium ion strongly facilitating the quaternised quinone-imine to nucleophilic addition.[8a] Subsequently Meunier *et al.*[8b] isolated the stable orthoquinone (91) [71% from (4)] on further reaction of the quaternised quinone-imine (90) with the peroxidase-hydrogen peroxide system at pH 8, and further showed at this pH that the product resulted from C-10 nucleophilic addition of hydrogen peroxide on the quinone-imine. Protonation of the orthoquinone (91) led to the aromatised analogue (92).

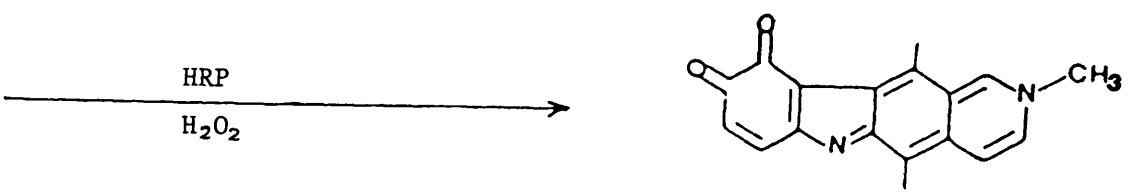
The unusual feature of this reaction is that nucleophilic addition has occurred regiospecifically at C-10, which is more sterically hindered than C-8, but this fact was confirmed later when Meunier and co-workers generated the quaternised quinone-imine in the presence of pyridine and several sulphur nucleophiles (93a-c).[61] They were then able to isolate the adducts (94) [38% from (4)] and (95a-c) [30-40% from (4)] respectively. The same group also reported the formation of the monomethoxy quinone-imine adduct (96) (75%) from the oxidation of 9-hydroxy-N-2-methyl-


HO
N
H
HRP
H_2O_2
N
N

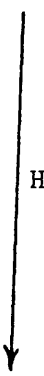
(5) (89)

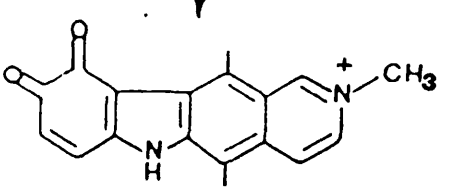

HO
N
H
CH_3
^-OAc
HRP
H_2O_2
N
CH_3
^-OAc

(4) (90)

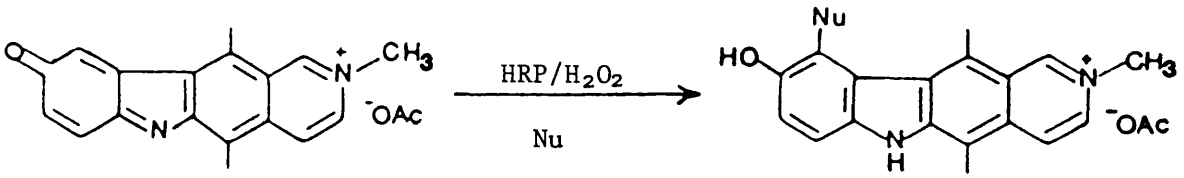

HRP
H_2O_2
N
CH_3

(91)


H^+

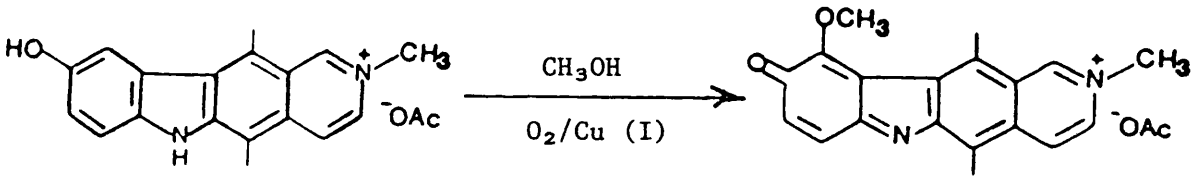

N
H
CH_3

(92)



(90)

Nucleophile (Nu)	Adduct
Pyridine	94
Cysteine $HSCH_2CH(COOH)NH_2$ 93a	95a
N-acetylcysteine $HSCH_2CHNHCOCH_3$ (COOH) 93b	95b
glutathione $HSCH_2CHNHCOCH_2CH_2CHCOOH$ ($CONHCH_2COOH$, NH_2) 93c	95c



(4) (96)

ellipticinium acetate (4) by molecular oxygen in the presence of copper(I) compounds in methanol.[61]

All these products, including the orthoquinone (92) have been tested *in vitro* on leukaemia L1210 cells and found to be less cytotoxic than the starting compound (4).[8b,61] Meunier and co-workers argued that the biological activity of the adducts were in line with the possibility that the quinone-imines represented the activated forms of the 9-hydroxyellipticines, and that they were subsequently alkylated by biological nucleophiles. Such a "bioxidative alkylation" process is analogous to the quinonemethide intermediates proposed by Moore in his "bioreductive alkylation" model for various anticancer agents.[62]

The stimulation of oxygen consumption by the orthoquinone (92) was noted using rat liver microsomes-NADPH and xanthine oxidase-NADH systems.[8b] Such an effect has been described by Bachur *et al.* for a large number of benzanthraquinones and N-heterocyclic quinone anticancer drugs.[63] Indeed, evidence has accumulated that suggests one possible mode of action in which these quinones impart their cytotoxicity is their reduction to transitory semiquinone free radicals, and that re-oxidation to the quinones is concomitant with reduction of molecular oxygen to superoxide ions (Figure 2).[63] The reaction of superoxide ions with water could furnish highly reactive hydroxy radicals which may damage DNA, and also initiate lipid peroxidation and subsequent cell damage.[64]

Although Auclair *et al.*[8a] showed that the phenoxy radicals generated *in vitro* by peroxidase-hydrogen peroxide treatment undergo preferential decay by a dismutation process to yield the quinone-imines, at cellular level a second route from the phenoxy radicals to the quinone-imines may parallel the behaviour of the quinone anticancer agents, which leads to the reduction of molecular oxygen to superoxide ions.

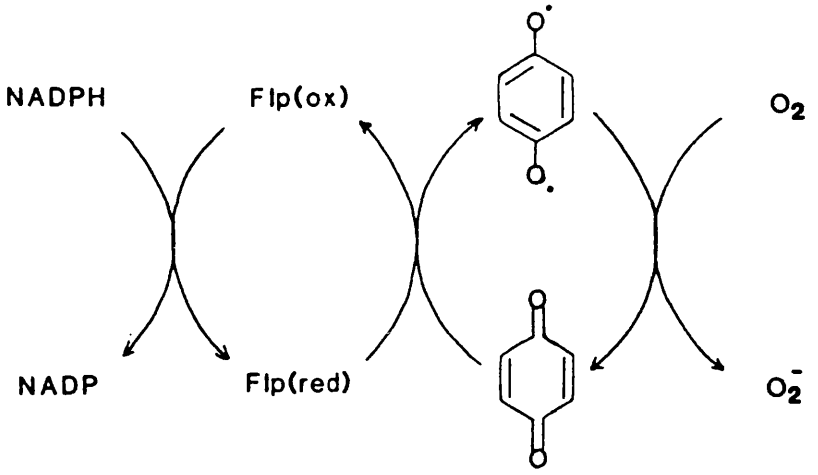
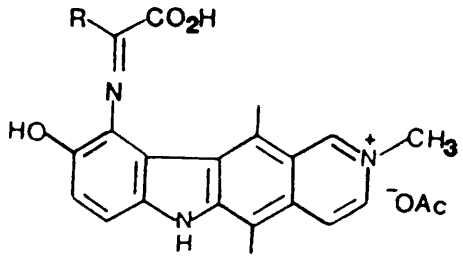


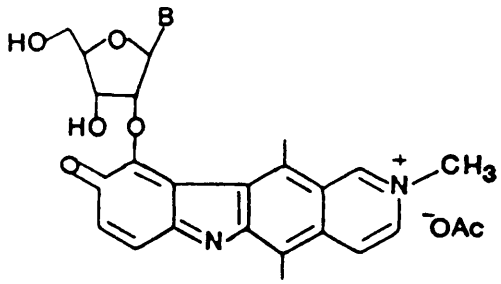
Figure 2 Model for catalysed oxygen consumption by quinone derivatives

Meunier *et al.*[65] have provided indirect evidence for the formation of quinone-imines *in vivo* since the glutathione (93c), N-acetylcysteine (93b) and cysteine (93a) conjugates of 9-hydroxy-N-2-methylellipticinium acetate have been isolated as excretory metabolites of both humans and rats.

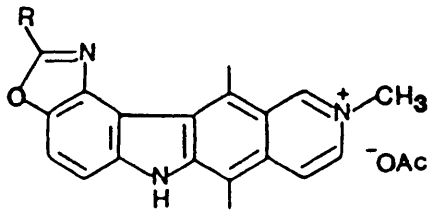
Further adducts of the quaternised quinone-imine (90) with amino acids (97)[66] and ribonucleosides (98)[67] were noted by Meunier and co-workers. Unfortunately, the constitutions of these and the methanol adduct (96)[61] were incorrectly assigned and have recently been revised by Potier *et al.* as structures (99), (100) and the dimethoxy adduct (101) respectively.[68] The same group of chemists has also shown that the quaternised quinone-imine (90) reacts with ribonucleotides to produce the adduct (102)[69] and extended the study to include an investigation of the bioxidative alkylation of 9-hydroxyellipticinium acetate (103), 9-hydroxyolivacinium acetate (104) and 9-hydroxy-N-2-methylolivacinium acetate (105).[70]



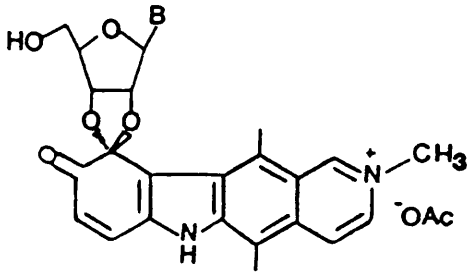
(97)



(98)



(99)



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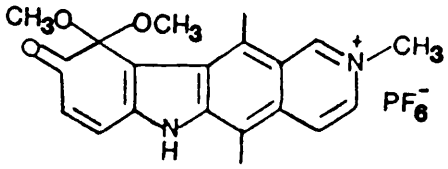
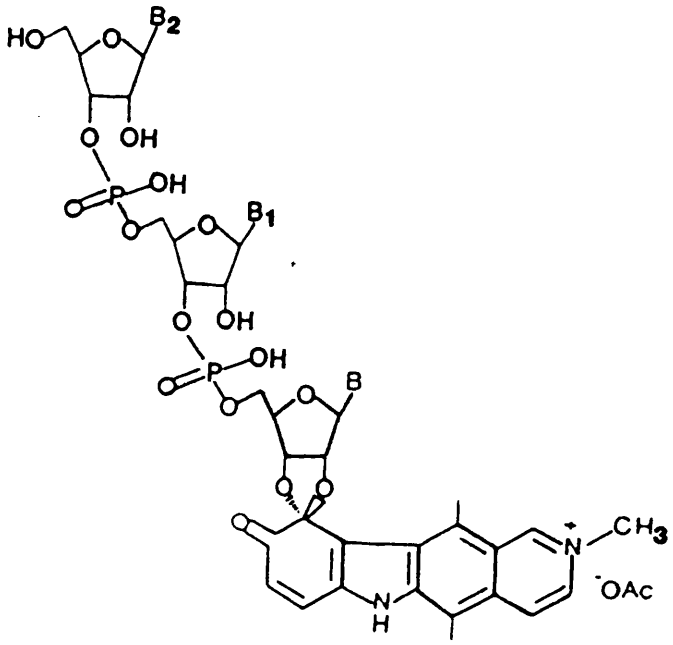
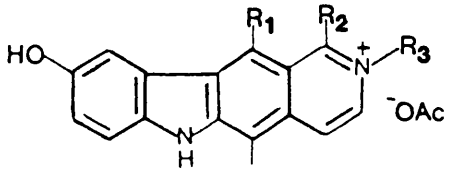


(101)



(102)



(103) R_1 = CH_3, R_2 = R_3 = H

(104) R_1 = R_3 = H, R_2 = CH_3

(105) R_1 = H, R_2 = R_3 = CH_3

Several interesting features were observed during the reaction of ribo-nucleosides and -nucleotides with 9-hydroxy-N-2-methylellipticinium acetate (4) under oxidative conditions:[69,70,71]

(1) With purine and pyrimidine ribo-nucleosides and -nucleotides the first electrophilic addition always occurs at the 2'-oxygen of ribose, but more slowly for pyrimidine than for purine bases; in a second step cyclisation of the re-oxidised product leads to a spiro derivative, with only one stereoisomer detected for purine ribonucleosides [absolute configuration represented in (100).] With all ribonucleotides and pyrimidine ribo-nucleosides a second stereoisomer is formed as a minor product (10-20%). This has been attributed to different stacking interactions encountered by the quaternised quinone-imine on reduction of base size from purine (two rings) to pyrimidine (one ring), or by introduction of a phosphate residue at 5'-OH as in ribonucleotides.

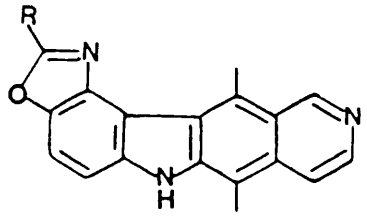
(2) *cis*-2'- And 3'-hydroxy groups in the ribo-nucleosides and -nucleotides are essential for stable adduct formation. Reaction of 2'-deoxyadenosine under oxidative conditions exclusively results in the formation of the orthoquinone (92).

(3) For sulphur-containing bases such as 6-thioguanine and 6-thioguanosine, alkylation occurs on the sulphur in preference to ribose bonding.

(4) All the spiro adducts isolated are less cytotoxic than the parent compound.

It has been suggested that the biological consequences of these observations are that 9-hydroxyellipticines may exert some of their anti-tumour activity through alkylation of RNA's, leading to inhibition of protein synthesis. For example, alkylation at the terminal end of t-RNA may stop the formation of aminoacyl t-RNA or at the level of the "cap" present at the 5'- end of m-RNA or poly-A-tail present in m-RNA.[69-71]

Potier *et al.*[72] have shown that the quinone-imine of 9-hydroxy-ellipticine reacts with α-substituted primary amines to give rise to adducts of type (106). In the light of this and formation of a similar oxazole ring system (99) on reaction of the quinone-imine of 9-hydroxy-N-2-methylellipticinium acetate with various amino acids,[68b] it has also been hypothesised that the oxidised forms of 9-hydroxyellipticines could covalently bind to biogenic amines, amino acids, peptides and proteins and may therefore act as inhibitors of protein synthesis and of enzyme action.[72,73] Thus, *in vitro* experiments have demonstrated that bovine serum albumin (BSA) and other proteins bind to 9-hydroxy-N-2-methyl-ellipticinium acetate (4) irreversibly under bioxidative conditions.[73]



(106)

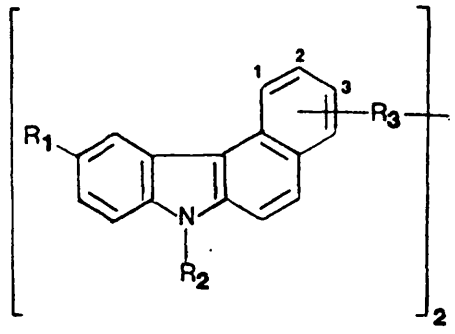
In summary, it is still not certain exactly what the role(s) of the ellipticine drugs are. Three possible biological properties have been identified for these compounds: (i) intercalation and involvement with topoisomerase, (ii) the intracellular formation of radicals and (iii) oxidation to potential alkylating agents.

(C) Bis-intercalators

The phenomenon of bifunctional bis-intercalation into nucleic acids was orignally discovered with the antibiotic echinomycin[74] and has also been observed with "dimeric" derivatives of 9-aminoacridine,[75] ethidium bromide[76] and substituted acridines and quinolines.[77] Recently, this series has been extended by Roques *et al.*[78] to include several 7H-pyrido[4,3-*c*]carbazole (107 a — h), 7H-pyrido[3,2-*c*]carbazole (108a-c) and 6H-pyrido[4,3-*b*]carbazole (ellipticine) (109a-b) dimers.

The antitumour activity of these dimers against L1210 murine leukaemia was found to be strongly dependent on the position of attachment, nature and rigidity of the linking chain. The three most highly active dimers were obtained in the series of 7H-pyrido[4,3-*c*]carbazole dimers with rigid bis(ethylpiperidinyl) chains (107a-c). Apart from a considerable increase in the antitumour activity induced by the dimerisation process, the DNA binding affinity of these dimers was also shown to be 100-1000 times higher than that of the monomers. In contrast, the two 6H-pyrido[4,3-*b*]carbazole (ellipticine) dimers (109a-b) were found to be completely inactive.[78]

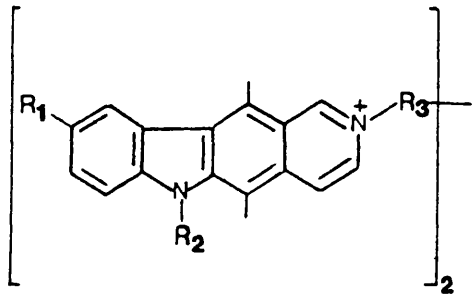
Preliminary investigations on the *in vitro* effects of the 7H-pyrido[4,3-*c*]carbazole dimers (107a-b)[79] on leukaemia L1210 and Chinese hamster lung cells have indicated a cytotoxic action through a mechanism markedly different from that of the monomers, or other bifunctional intercalators such as the quinoxaline antibiotics and diacridine derivatives. Although little is known about the actual mode of action, the 7H-pyrido[4,3-*c*]carbazole dimers may represent a new class of antitumour drugs.



(107)	R_1	R_2	R_3
a	OCH_3	H	2-N^+ $(CH_2)_2$-1-piperidyl, Cl^-
b	OCH_3	CH_3	2-N^+ $(CH_2)_2$-1-piperidyl, Cl^-
c	OH	H	2-N^+ $(CH_2)_2$-1-piperidyl, Cl^-
d	OCH_3	H	2-N^+ $(CH_2)_2$-1-piperidyl-$(CH_2)_{1,5}$, Cl^-
e	OCH_3	H	2-N^+ $(CH_2)_5$, Br^-
f	OCH_3	H	2-N^+ $(CH_2)_2N(CH_3)(CH_2)_{2.5}$, AcO^-
g	OCH_3	H	2-N^+ $CH_2CH(OH)CH_2$-1-piperidyl, AcO^-
h	H	H	2-N^+ $(CH_2)_2$-1-piperidyl, Cl^-

(108)

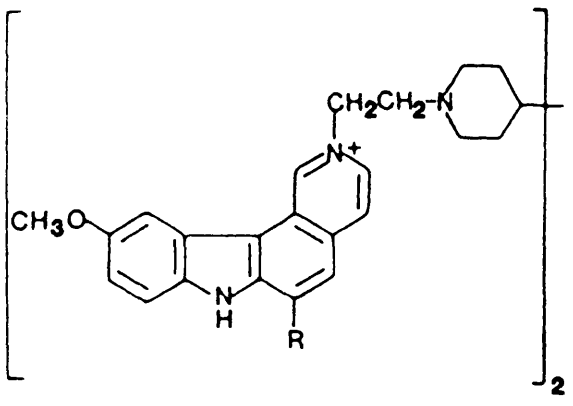
a	OCH_3	H	3-N^+ $(CH_2)_2$-1-piperidyl, Cl^-
b	OCH_3	CH_3	3-N^+ $(CH_2)_2$-1-piperidyl, Cl^-
c	OH	H	3-N^+ $(CH_2)_2$-1-piperidyl, Cl^-



(109)	R_1	R_2	R_3
a	OCH_3	H	-$(CH_2)_2$-1-piperidyl, Cl^-
b	OH	H	-$(CH_2)_2$-1-piperidyl, Cl^-

Roques and co-workers have extended the nature of the 7H-pyrido-[4,3-*c*]carbazole dimers to include the 6-alkyl substituted dimers (110a-b) without significant loss of cytotoxic properties.[80] Interestingly, introduction of a methyl group in other positions led to a strong decrease in the antitumour effect.

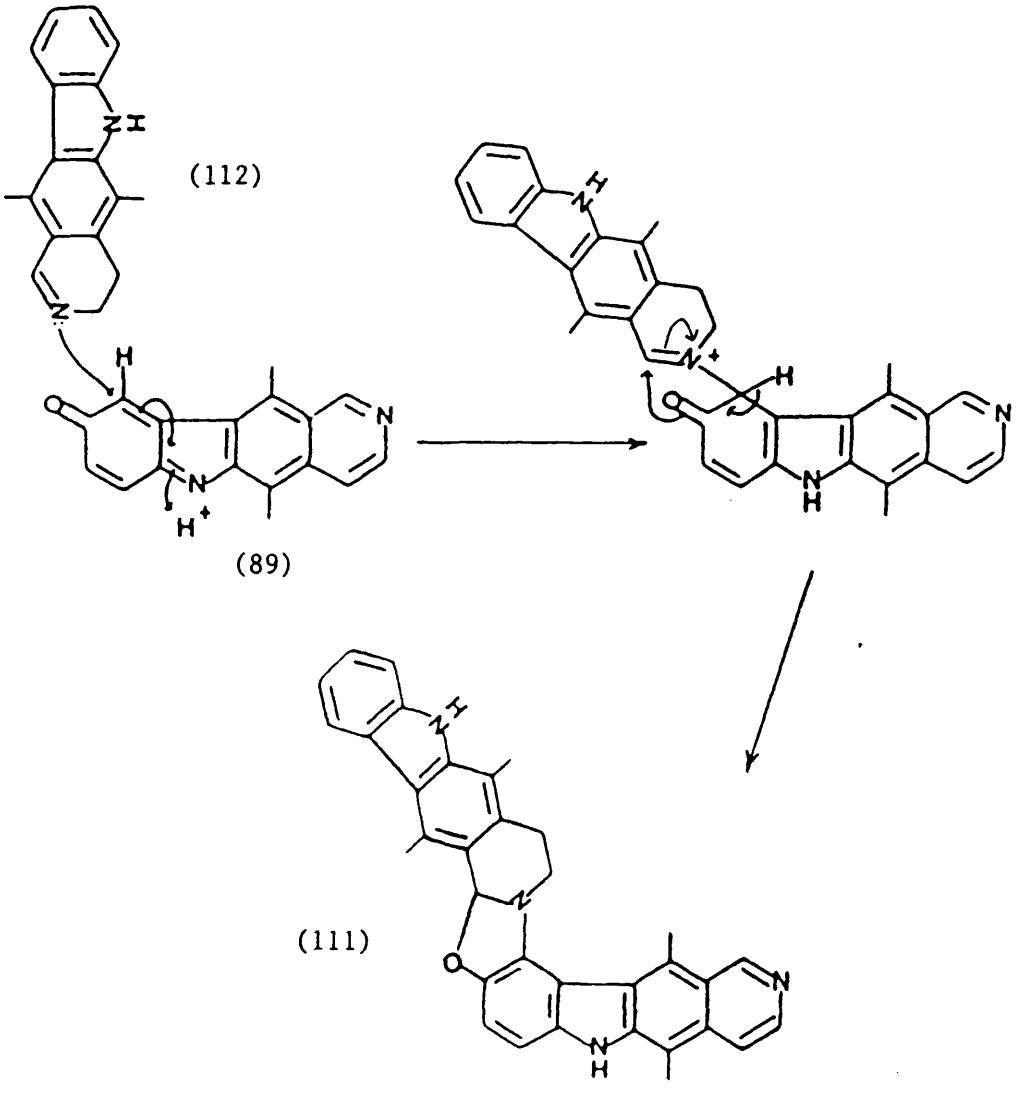
It is appropriate in this section to mention the very recent and first example of a natural dimer alkaloid of the ellipticine type. Thus strellidimine (111) has been isolated by Koch *et al.*[81] from the stem bark of *Strychnos dinklagei* Gilg. The discoverers suggest that the alkaloid arises from the addition of the dihydroellipticine (112) to the iminoquinone (89), itself generated by the oxidation of 9-hydroxyellipticine (5), followed by cyclisation of this adduct to the fused oxazole system (Scheme 20). Such a sequence is similar to that previously implied from the alkylation of nucleosides by ellipticine derivatives.



(110)

a R = CH_3

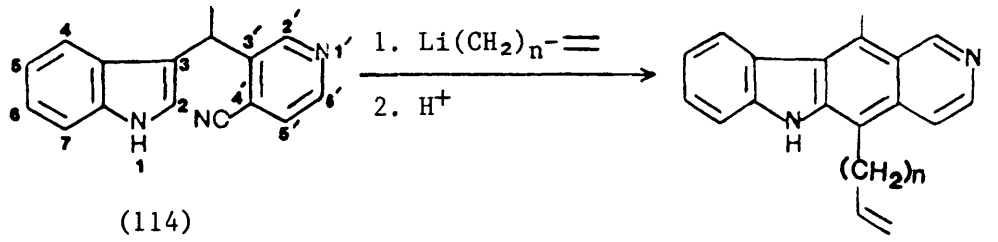
b R = C_2H_5



Scheme 20

DISCUSSION AND RESULTS

A previous worker in this Department attempted unsuccessfully to prepare some ellipticine dimers linked through positions 1 and 3.[82] However, in the latter stages of this work, a series of computer graphics simulations were carried out which indicated that these were not the positions of choice.[13] Indeed, had dimers of this type been synthesised, it seems probable that they would not have intercalated because of severe non-bonding interactions within the DNA receptor pocket. The conclusion of the modelling experiments was that sites on the lower face of the molecule at C-5, C-7 and C-8 are more suitable anchorage points, with C-5 being the least likely to hinder docking with the nucleic acid. Subsequently, therefore, our efforts have been directed towards linking ellipticine units through position 5, while other researchers in the group are examining C-7 and C-8 analogues. In a preliminary investigation, the two monomeric 5-alkenylellipticines (113a—b) were prepared, so that the reactivity of their olefinic side chains could be investigated.[82] The route chosen followed an adaptation of the 'nitrile' synthesis, previously developed at Bath for the preparation of a number of A-ring substituted ellipticines and ellipticines bearing extended alkyl chains at position 5.[83] Thus, reaction of the carbonitrile (114) with the appropriate alkenyllithium, followed by acid hydrolysis of the product imines (not isolated), resulted in the tetracycles (113a) and (113b) in yields of 28% and 40% respectively.[82] However, functionalisation of the double bonds in order to allow subsequent dimerisation presented difficulties. For instance, attempted cleavage of the olefinic bond in either compound to afford the corresponding aldehydes resulted in formation of the carbinolamine derivatives (115a-b).[82]

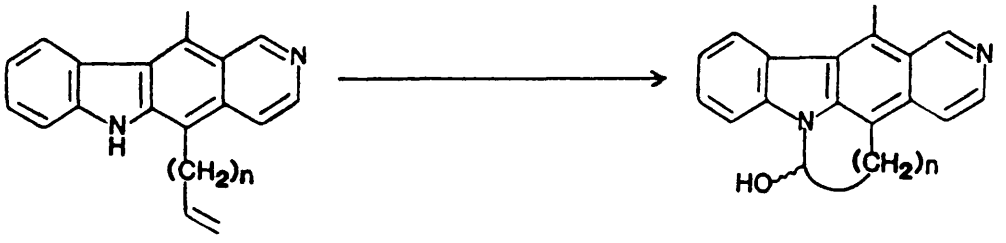


(114)

(113)

a n = 2

b n = 3



(113)

a n = 2

b n = 3

(115)

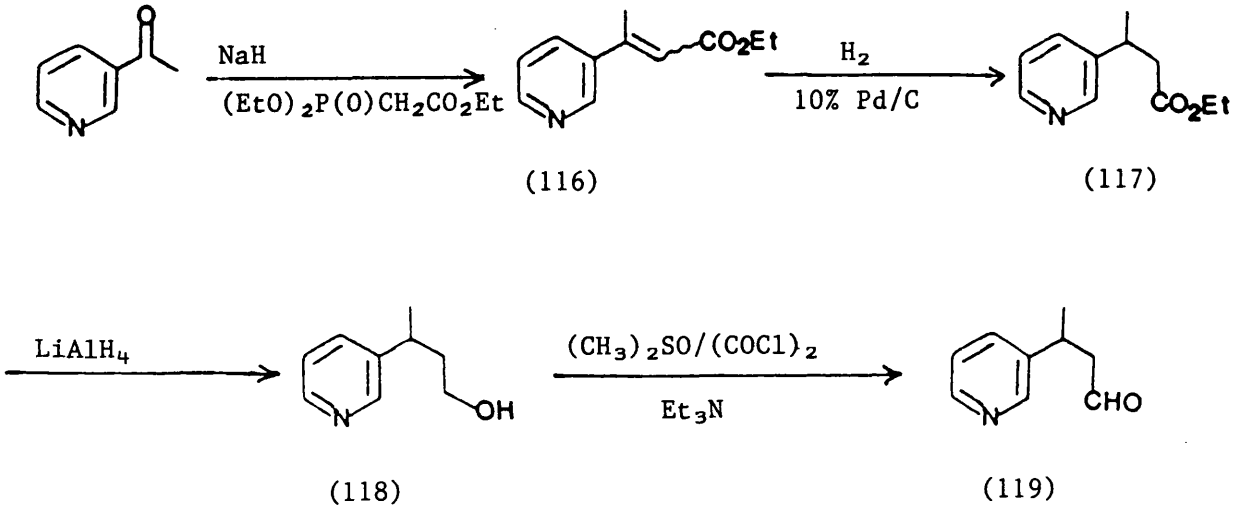
a n = 2

b n = 3

The objective given to the author of this thesis was to continue the studies directed towards the synthesis of dimeric ellipticines linked *via* position 5, but, in view of the relatively moderate yields obtained in the formation of the 5-alkenylellipticines from the carbonitrile (114) and the difficulties encountered with their further functionalisation, it was decided to synthesise other derivatives already bearing groups useful for the linking process.

The carbonitrile (114) was synthesised as previously described by Sainsbury and co-workers at Bath.[82,83a,117] Thus, an Emmons-

Wadsworth reaction between 3-acetylpyridine and the sodium salt of triethyl phosphonoacetate afforded the unsaturated ester (116) in 93% yield as a mixture of *E*- and *Z*-isomers (4:1). Hydrogenation of the unsaturated esters at 125 psi over 10% Pd/C gave the saturated analogue (117) in 88% yield. Lithium aluminium hydride reduction of the ester (117) resulted in formation of the corresponding alcohol (118), which under Swern oxidative conditions (dimethyl sulphoxide/oxalyl chloride) was converted to the aldehyde (119) in an overall yield of 71% for the two step process (Scheme 21).

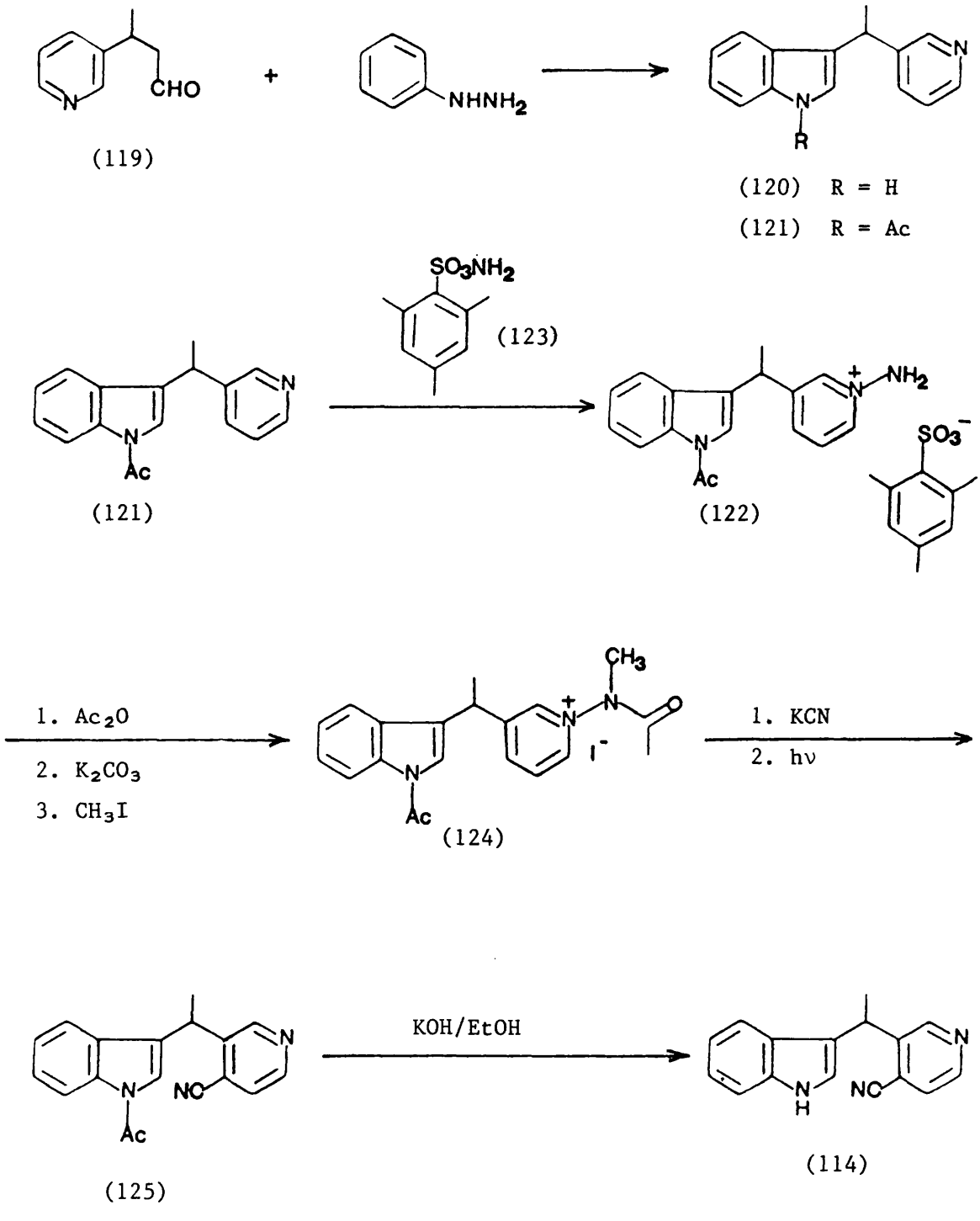


(116) (117)

(118) (119)

Scheme 21

A Fischer indolisation reaction between phenylhydrazine hydrochloride and the aldehyde (119) gave the required pyridylethylindole (120) in 50% yield, which was acetylated to (121) in 72% yield by heating in acetic anhydride and triethylamine. To prepare the desired carbonitrile (114), it was necessary to activate the γ-position of the pyridine ring towards nucleophilic attack, while, at the same time, blocking the two α-positions. This was achieved as previously reported[83a,117] by

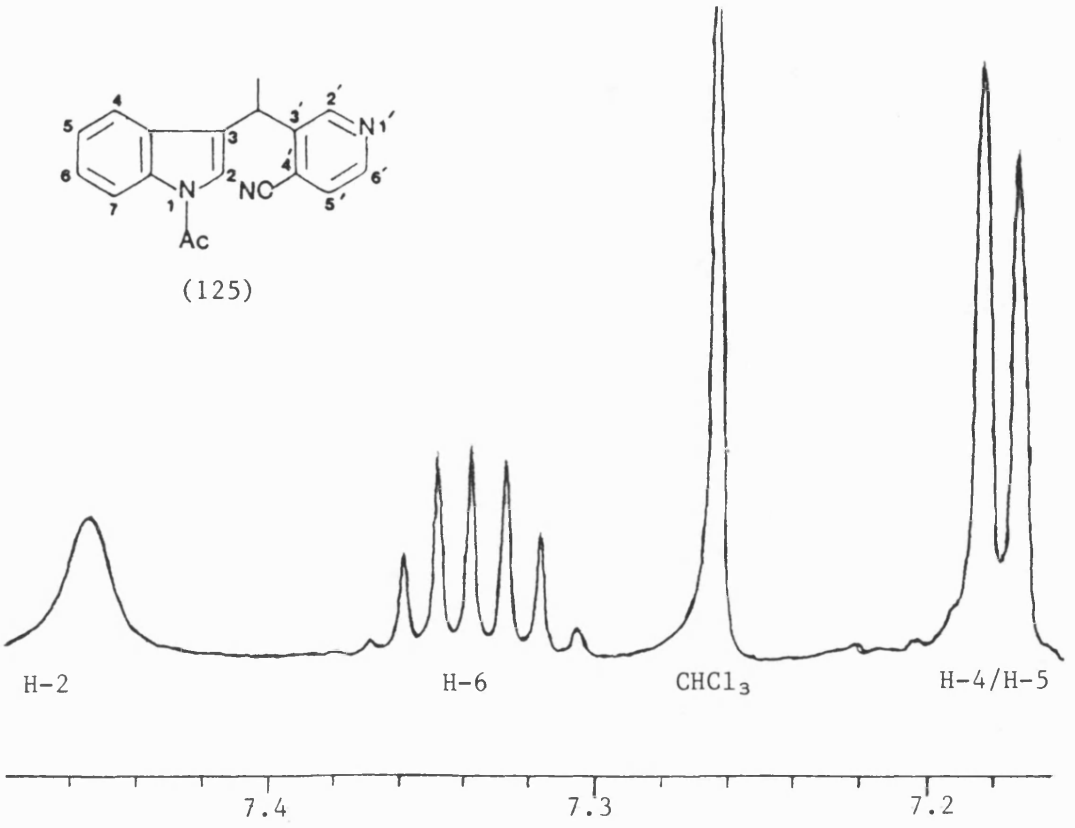

CHO
NHNH2
(119)
(120) R = H
(121) R = Ac
SO3NH2
(123)
Ac
(121)
NH2
SO3-
(122)
1. Ac2O
2. K2CO3
3. CH3I
CH3
I-
(124)
1. KCN
2. hν
NC
(125)
KOH/EtOH
NC
H
(114)

Scheme 22

amination of the pyridine nitrogen atom in the acetyl derivative (121) to afford the salt (122) using O-mesityl sulphonylhydroxylamine (MSH) (123).[84] This reaction was followed by acetylation and iodomethylation of the product to give the N-methylacetamido salt (124) in 67% overall yield for the three step process (on a 2.6 g scale). This compound, bearing a large, but easily cleavable N-substituent, was treated with an aqueous solution of potassium cyanide to afford the corresponding 4-cyano-1,4-dihydropyridine adduct. This was extracted from the aqueous phase and irradiated with u.v. light to give the N-acetylcarbonitrile (125) in 46% yield. Deacetylation to the carbonitrile (114) was effected in quantitative yield by reaction with potassium hydroxide in ethanol. By repeating this route several times, a stock of the carbonitrile (114) was built up. The complete reaction sequence is summarised in Scheme 22.

Although all these compounds have been previously synthesised, only 60-100 MHz ^{1}H n.m.r. spectra were recorded during their characterisation. In the indole derivatives especially, such n.m.r. spectra are inadequate in attempting total assignment of the protons. Consequently, for several of the compounds we obtained high field ^{1}H n.m.r. spectra, and where appropriate a series of decoupling experiments were conducted, as in the case of the N-amino salt (122) (see Experimental). For the N-methylacetamido salt (124), the high field ^{1}H n.m.r. spectrum recorded at room temperature exhibits broad and poorly resolved resonances for protons in the pyridine ring and N-methylacetamido group. Better signal definition was observed by recording the spectrum at high temperature, thus suggesting at room temperature there is appreciable restricted rotation about the nitrogen-nitrogen bond. In addition, there is the possibility that the amide group promotes the existence of *E*- and

Z-isomerides at low temperature. The 400 MHz ^{1}H n.m.r. spectrum of the N-acetylcarbonitrile (125) is interesting in that it shows a non-first order splitting pattern for multiplet due to the resonance of proton H-6 at δ 7.34 and a doublet for the signals of protons H-4 and H-5 at δ 7.18 (J = 4 Hz) (Figure 3). ^{1}H n.m.r. decoupling experiments



(125)

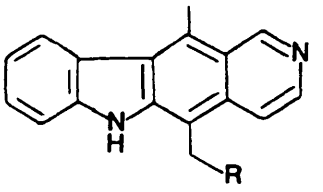
Figure 3 400 MHz ^{1}H n.m.r. spectrum of the N-acetylcarbonitrile (125) in the region δ 7.16-7.48

indicate that the unusual splitting pattern can be explained by the accidental equivalence of the resonances of protons H-4 and H-5, the resulting 4 Hz coupling constant being an average of the *ortho* and *meta* couplings. Interestingly, removal of the acetyl group to give the carbonitrile (114) returns the observed aromatic splitting pattern in the ^{1}H n.m.r. spectrum to that of an approximately first order relationship. In this particular spectrum, the aromatic assignments are supported by the evidence from a COSY experiment.

The N-amination reaction can be successfully performed on larger quantities of the N-acetylpyridylethylindole (121) (5 g), but subsequent acetylation and iodomethylation results in the formation of a yellow foam containing the salt (124) and substantial quantities of other pyridinium salts. We are unable to assign the exact structures of these products and attempts to separate them proved unsuccessful. Nevertheless, it was hoped that these products would behave in a similar manner to the simple N-methylacetamido salt in directing the cyanide ion to the γ-position. Consequently, the yellow foam was treated as before with potassium cyanide, and the product was irradiated with u.v. light. N-Acetylcarbonitrile formation occurred, but in much lower yield than previously obtained using the purified salt.

In later work directed towards the synthesis of the 5-methoxy-carbonitrile analogue, this problem was re-addressed. It was found that the acetylation step is relatively slow and occurs in low yield on large scale reactions. Since this step was carried out in an aqueous medium, a probable cause may be competing hydrolysis of the acetic anhydride. This seems likely for good yields of the 5-methoxy-N-methylacetamido salt were subsequently obtained when the acetylation step was performed using neat acetic anhydride.

Initially, we chose to examine the reaction of the carbonitrile (114) with the anion of nitromethane, in the hope of obtaining 5-nitromethylellipticine (126), which could then be reduced to 5-amino-methylellipticine (127). However, no reaction occurred under the various conditions outlined below [(i)-(iii)] and starting material was returned in each case:



(126) R = NO_2

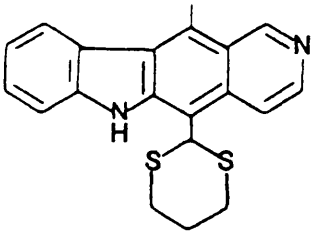
(127) R = NH_2

(i) four molecular equivalents of the lithium salt of nitromethane in ether, -75 °C to +25 °C;

(ii) five molecular equivalents of the lithium salt of nitromethane in nitromethane, in the presence of a few drops of 12-crown-4 ether, -15 °C to boiling point;

(iii) five molecular equivalents of the lithium salt of nitromethane in methanol, in the presence of a few drops of 12-crown-4 ether, -15 °C to +25 °C.

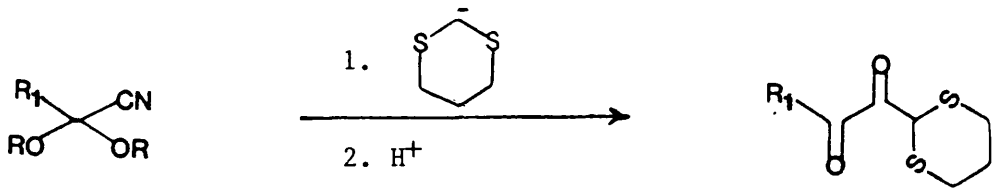
We next attempted an addition of the carbonitrile (114) and the 1,3-dithiane anion. If successful, this would lead to the thioacetal (30), previously synthesised in low yield by Gribble and co-workers, and used in their synthesis of the alkaloid 17-oxoellipticine (29),[23] (see Scheme 6). Reaction of the 1,3-dithiane anion with nitriles



(30)

has been previously reported; for instance, in the syntheses of a number of functionally substituted cyclopentenones. Thus, Kawamoto *et al.*[85] have combined a number of 2,2-dialkoxynitriles (128a-c) with

the 1,3-dithiane anion to form, after acid hydrolysis, a series of α—oxoalkanoyl-1,3-dithianes (129a-c) in yields of 52-79%. In a related

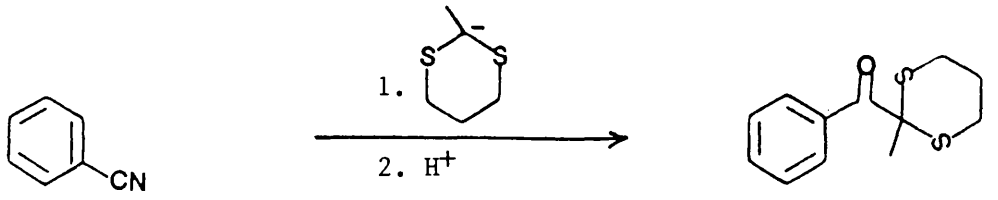


(128) (129)

a $R_1 = CH_3$, $R = C_2H_5$
b $R_1 = C_2H_5$, $R = C_2H_5$
c $R_1 = n\text{-}C_5H_{11}$, $R = CH_3$

a $R_1 = CH_3$
b $R_1 = C_2H_5$
c $R_1 = n\text{-}C_5H_{11}$

case, Corey and Seebach reacted the anion of 2-methyl-1,3-dithiane with benzonitrile and obtained the ketone (130) in 78% overall yield.[86]



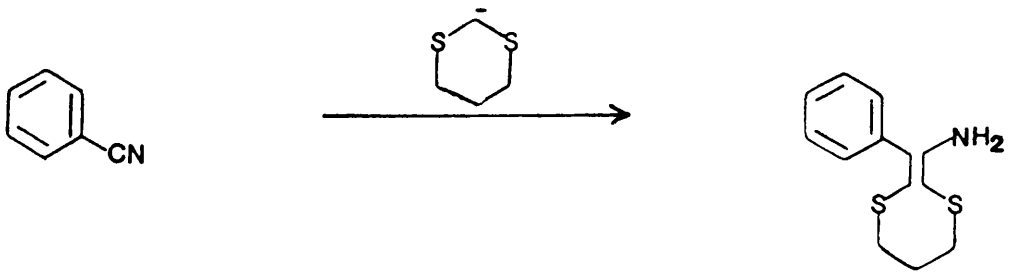
(130)

Initially, reaction of a four-fold excess of 2-lithio-1,3-dithiane with the carbonitrile (114) in dry tetrahydrofuran for 6 days, followed by acid hydrolysis, resulted only in a 24% yield of the thioacetal (30) after purification. However, a substantial amount of starting material was recovered, allowing a corrected yield of 52%. In a separate experiment, the extent of metallation of the 1,3-dithiane ring generated

under similar conditions was determined by deuteration and subsequent ^{1}H n.m.r. analysis. The result showed a very low extent of deuterium incorporation into the 1,3-dithiane ring, suggesting anion generation was very sensitive and all traces of water must be eliminated prior to base treatment. Under more stringent experimental conditions in which 4 Å molecular sieves were employed, >95% metallation was achieved. A reaction of a seven-fold excess of 2-lithio-1,3-dithiane generated in this manner with the carbonitrile (114) (506 mg) for 16 hours (-78 °C to +25 °C), followed by acid hydrolysis and column chromatography, afforded a 93% yield of the thioacetal (30), with no trace of starting material. The product so obtained gave spectroscopic data (u.v., ^{1}H n.m.r., mass spectroscopy) identical to those recorded by Gribble and co-workers, but the compounds differed in melting point (294-296 °C, lit., [23] 236-240 °C). Nevertheless, even with this discrepancy, we were satisfied that we had the correct product. A conclusion confirmed by data from its ^{13}C n.m.r. spectrum, which contains carbon resonances entirely consistent with the structure of (30). However, the presence of only four aliphatic carbon resonances, one of which is due to the C-11 methyl group, suggests that the ellipticine chromophore lies in the mirror plane of the 1,3-dithiane ring, bonding through the equatorial position of the sulphur bearing heterocycle at C-2. Such an orientation would lead to the equivalance of the carbon atoms at C-4 and C-6 in the latter ring. Furthermore, the 1,3-dithiane ring protons in the 400 MHz ^{1}H n.m.r. spectrum showed a certain degree of first order character. Based on a conformationally rigid ring model, assignment of the signals followed from the splitting patterns and known effects of ring heteroatoms on proton chemical shifts. The appearance of large or small vicinal couplings distinguish axial from equatorial protons.[87]

A large scale reaction of the carbonitrile (114) (3.41 g) with 2-lithio-1,3-dithiane under analogous conditions to those used previously, afforded only a 64% yield of the thioacetal (30). This lower yield may stem from difficulties encountered in the isolation and purification of compound (30) on a larger scale. The product appears to be stable as a solid (over several months), whereas solutions tend to darken with time (over several weeks) and show signs of considerable decomposition (by t.l.c. analysis).

During this stage of our research, Page *et al.*[88] reported the formation of primary aminoketene thioacetals from the reaction of the 1,3-dithiane anion with simple nitriles. Thus, reaction of benzonitrile with 2-lithio-1,3-dithiane generated the primary aminoketene thioacetal (131) in 90% yield. Page has suggested that the reaction probably

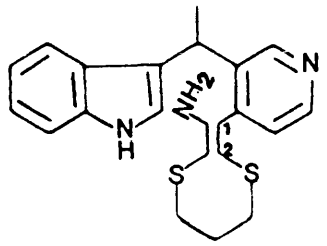


(131)

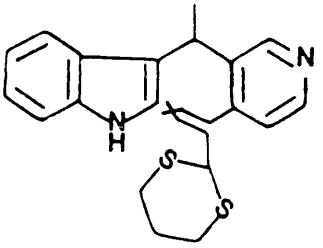
forms the corresponding imine first, but this is rapidly followed by an inter-or intra-molecular proton transfer to give the more conjugated tautomer.

In the formation of the ketones (129a-c), little attention was paid to the nature of the intermediates prior to acid hydrolysis, although it was likely that imines were assumed to be formed. However, in view of the new work, it was of interest to us to discover whether

initial attack of the 1,3-dithiane anion on the nitrile function in (114) provided the primary aminoketene thioacetal (132) or the tautomeric imine (133). Consequently, prior to acid hydrolysis in the large



(132)



(133) X = NH

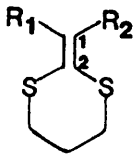
(134) X = O

scale formation of the thioacetal (30), a small amount of the intermediate was isolated and purified by chromatography on silica eluting with ethyl acetate. The electron impact mass spectrum shows a molecular ion at *m/z* 367, consistent with either the aminoketene thioacetal (132), or the tautomeric imine (133). However, since the hydrolysis of imines containing the C=NH moiety is known to be facile, passage of the imine (133) through silica would have been expected to form the ketone (134), requiring a molecular ion of *m/z* 368. The ^{1}H n.m.r. and ^{13}C n.m.r. of the product were recorded, in order to substantiate the aminoketene thioacetal structure. Although our product corresponded to one single component by t.l.c. analysis on silica plates eluted with various solvent systems, both spectra appeared sufficiently complex to suggest the presence of diastereomers. This is particularly obvious in the fully decoupled ^{13}C n.m.r. spectrum, where each resonance is doubled. The diastereomers may possibly result from restricted rotation about the chiral carbon bridgehead. Nevertheless, the spectra are consistent with the aminoketene thioacetal structure (132). Of the more important

features in the 270 MHz ^{1}H n.m.r. spectrum, are the appearance of two broad singlets at δ 4.26 and 4.10, of overall integration two protons, but relative abundancies 6:7 respectively. Both disappear on deuterium exchange and these resonances can be assigned to the primary amino proton signals of individual diastereomers. Of interest in the ^{13}C n.m.r. spectrum of (132), are two resonances at δ 92.5 and 92.3, which can be assigned to the signals of the C-2 atoms of the primary aminoketene thioacetal groups of these isomers. This allocation is based on the following arguments:-

(i) We are certain from our extensive knowledge of the ^{13}C chemical shifts in related molecules, such as the carbonitrile (114)[89] and thioacetal (30), that the new resonances must be associated with the C-5 substituent.

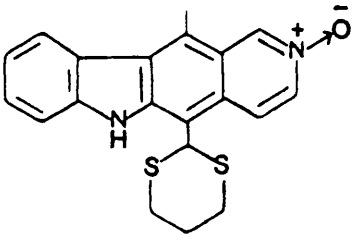
(ii) The only ^{13}C n.m.r. shift data available for ketene thioacetals are those of the structural type (135), in which R_1 and R_2 are alkyl substitu ents.[90]



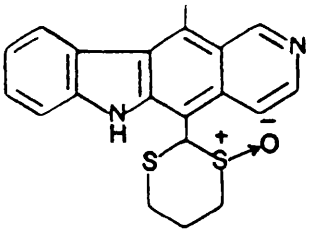
(135)

In these cases, the carbon atom resonances belonging to C-1 and C-2 are reported to appear in the regions δ 150-135 and 125-110 respectively. However, the positions of the sp^2 carbon resonances depend heavily on the electron distribution within the double bond, and if groups are present, such as an amino group, an upfield shift of C-2 might be expected. Even a 4-pyridyl group at C-1 should not offset this effect.

Having successfully prepared the thioacetal (30), we now turned our attention to modification of the 1,3-dithiane ring. Gribble and co-workers have already reported an efficient conversion of the thioacetal (30) to the alkaloid 17-oxoellipticine (29) using silver nitrate, but only disclosed brief experimental details (two equivalents, aqueous acetone, +25 °C, 48 hours, 100%).[23] It seemed appropriate to repeat this reaction, since the aldehyde (29) could serve as a pivot for a wide variety of reactions. However, in our hands, when a mixture of silver nitrate (two equivalents) and compound (30) in a water/acetone mixture (1:18 v/v) was stirred for 48 hours at room temperature, no cleavage of the 1,3-dithiane ring occurred. Instead, the N-oxide derivative (136) was produced in 44% yield. Although this compound



(136)



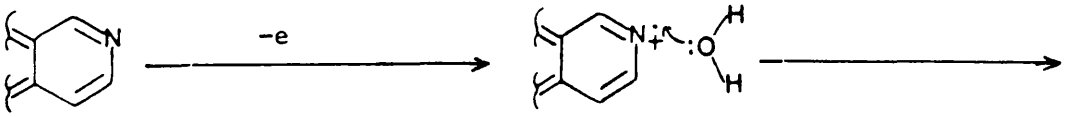
(137)

possessed the same R_F in several t.l.c. solvent systems and solid supports as the starting thioacetal (30), it was characterised spectroscopically and shown by ^1H n.m.r. to be homogeneous. The electron impact mass spectrum shows a molecular ion at *m/z* 366, which is compatible with a mono-oxygenated derivative of the thioacetal (30) (molecular mass 350). After an initial loss of 16 mass units, the fragmentation pattern of the compound is the same as that of the parent (30). Such a mass spectral breakdown is characteristic of N-oxides,[91] and the lack of a band in the 1800-1610 cm^{-1} region of the i.r. spectrum or a shift of the u.v. absorption in alkaline solution confirms that the oxygen

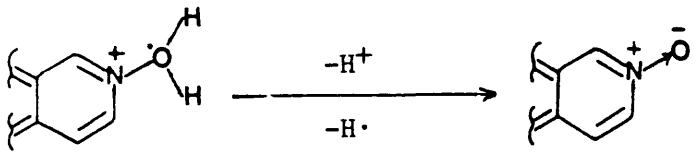
is not involved in a carbonyl group or phenolic hydroxy group. The occurrence of oxidation on the pyridine nitrogen atom in preference to that on sulphur, which would have led to the isomeric S-oxide derivative (137), is inferred from the 400 MHz ^{1}H n.m.r. spectrum. Although the H-1 and H-3 proton resonances of the pyridine ring are observed as very broad signals, their positions at δ 8.64 and 8.00 respecitvely are consistent with the N-oxide structure (136). Further support for (136) is the appearance in the spectrum of aliphatic proton resonances of almost exact multiplicity and chemical shifts to those found in the ^{1}H n.m.r. spectrum of the thioacetal (30). If a sulphoxide had formed, chemical shift changes and dissymmetric effects should have been observed.

Interestingly, from the bark of the trees *Ochrosia vieillardii* and *Aspidosperma nigricans*, the N-oxides of ellipticine and olivacine respectively have been isolated.[92] Both compounds exhibit similar spectroscopic behaviour (u.v., ^{1}H n.m.r., mass spectroscopy) to their respective parent heterocycles as we observed for our compounds. Recently, Pandit and co-workers have synthesised the N-oxide of 6-methylellipticine as part of studies directed towards side chain substitution at the C-11 methyl group, but in this case there was no choice between the sites of oxygenation.[31]

A possible mechanism for the formation of the N-oxide (136) may involve the one electron oxidation of the pyridine nitrogen atom by a silver(I) species to afford the corresponding radical cation (138). This may then undergo attack by an oxygen-bearing nucleophile, possibly water, followed by breakdown to the N-oxide.



(138)



(136)

The cleavage of the 1,3-dithiane ring by silver nitrate has been reported previously in the synthesis of the natural terpene alcohol 2-methyl-6-methylene-2,7-octadien-4-ol.[93] Interestingly, attempts have been made to cleave ethylenethioacetal groups in a number of isoquinoline derivatives with mercury(II) chloride, but these were unsuccessful because of the formation of insoluble donor-acceptor complexes between the pyridine nitrogen atom and mercury(II) chloride.[94] In view of the general similarity between silver(I) and mercury(II) salts towards heteroatoms, we suspect that complexation by silver ions with the pyridine nitrogen atom of our compound (30) is a facile event, and may promote oxidation at this site, rather than scission of the 1,3-dithiane ring.

In an attempt to overcome this problem, the reaction between silver nitrate and the thioacetal (30) was conducted in an acidic medium, in which it was hoped that protonation of the pyridine ring would reduce the probability of N-oxide formation. Thus, reaction of silver nitrate (two equivalents) with the thioacetal (30) (144 mg) in a warm mixture of tetrahydrofuran and 2 M nitric acid under an atmosphere of nitrogen

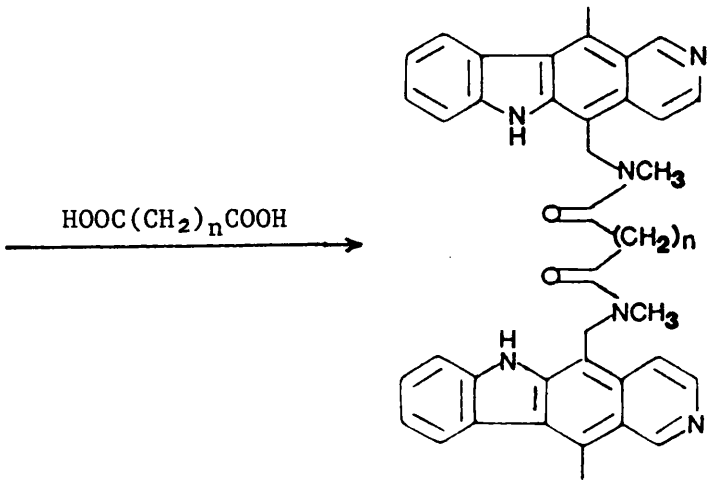
for 22 hours resulted in a clean conversion to the alkaloid 17-oxo-ellipticine (29) in 67% yield, after work-up and column chromatography. The product so obtained gave physical data (u.v., i.r., ^{1}H n.m.r., m.p., mass spectroscopy) identical to that of the natural product,[24] and to the synthetic compound prepared by Gribble and co-workers.[23,26]

A large scale reaction of the thioacetal (30) (1.10 g) under analogous conditions afforded a 53% yield of 17-oxoellipticine, after column chromatography. Unfortunately, repetition of this reaction on the same scale gave disappointingly low yields (34% and 18%), even though t.l.c. analysis indicated total consumption of the starting material. This result suggests that cleavage of the 1,3-dithiane ring *via* this method is very sensitive to the reaction conditions employed, and although the optimum reaction conditions for the deprotection have not been investigated, subsequent reaction runs [≈0.5 g of (30)] were conducted with nitrogen bubbling through the stirred reaction mixture to ensure elimination of all traces of oxygen. Work-up and column chromatography then resulted in the isolation of 17-oxoellipticine (29) in consistent yields of 52-63%.

As a potential route to dimeric ellipticines linked *via* the 5-position, we envisaged that conversion of 17-oxoellipticine (29) to the amine (139) would provide suitable functionality in the 5-substituent to enable coupling with dicarboxylic acids and the generation of ellipticine dimers (140) (Scheme 23).

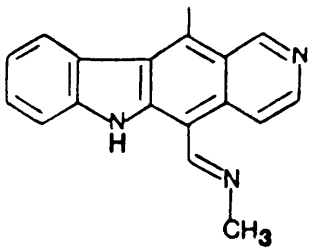
Gaseous methylamine was bubbled through a stirred suspension of 17-oxoellipticine (29) in dry benzene containing activated 3 Å molecular sieves at room temperature to give a 93% yield of the imine (141).

(29) (139)



(140)

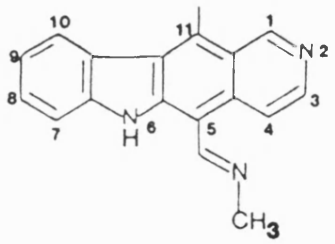
Scheme 23



(141)

The reaction could not be followed by t.l.c., since the imine appeared to have the same R_F on silica supports as the starting aldehyde in a

number of solvent systems. This is a probable consequence of the acidic nature of the silica leading to hydrolysis of the imine. Both basic and neutral alumina supports proved similarly unsatisfactory and solution i.r. spectroscopy proved to be the most reliable means of monitoring the reaction, which was stopped when the carbonyl stretching band at 1645 cm^{-1} in the starting aldehyde had completely disappeared. At this point, a new absorption at 1630 cm^{-1} attributable to the imine moiety had become fully established. The 250 MHz ^1H n.m.r. spectrum confirmed lack of starting material and showed the presence of finely split resonances at δ 9.36 (1H) and 3.78 (3H), which may be assigned to the signals of the methine proton and those of the N-methyl group of the imine system $CH=NCH_3$ respectively. The possibility that the observed splitting patterns for these resonances could result from *E*- and *Z*-isomerism about the carbon-nitrogen double bond was dismissed from decoupling experiments, which showed that the methine proton is weakly coupled through the π system to the N-methyl group (J = 1.5 Hz). Consequently, steric restraints probably ensure that the imine (141) exists entirely in the *E*—configuration as shown. Of particular interest is the observation of a similar phenomenon to that noted in the ^1H n.m.r. spectrum of the N-acetylcarbonitrile (125), namely a non-first order splitting pattern for multiplet due to the resonance of proton H-9 at δ 7.36 and a doublet at δ 7.56 (J = 4 Hz) for the signals of proton H-7 and H-8 (Figure 4). As demonstrated for the N-acetylcarbonitrile, ^1H n.m.r. decoupling experiments suggest that this unusual pattern can be explained by the accidental equivalence of two resonances. In this case, protons H-7 and H-8, the resulting 4 Hz coupling constant being an average of the *ortho* and *meta* couplings.



(141)

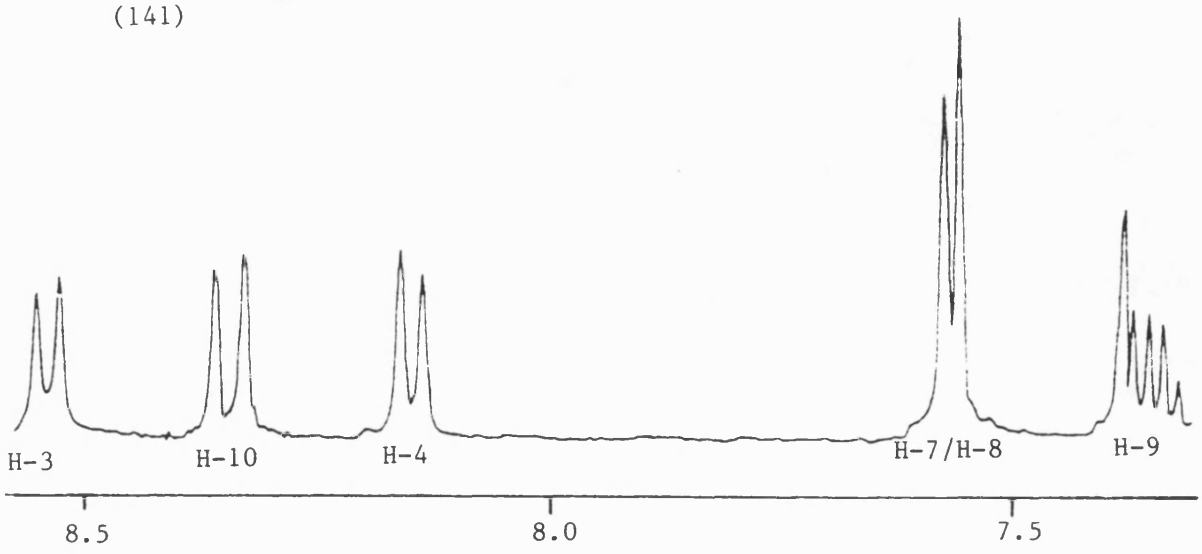


Figure 4 250 MHz ^{1}H n.m.r. spectrum of the imine (141) in the region δ 7.36-8.56

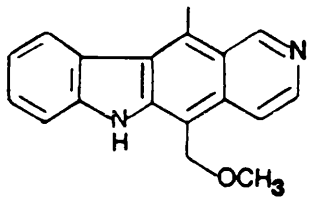
Treatment of the imine (141) in dry methanol at 0 °C with sodium borohydride gave, after work-up, a complex mixture from which the amine (139) was isolated in 60% yield by column chromatography. The structure of this material is supported by its electron impact mass spectrum, which shows a molecular ion at *m/z* 275 [(139) requires M^+ 275] and a major fragmentation ion at *m/z* 244 (M^+-CH_3NH_2). The presence of singlets at δ 4.54 (2H) and 2.58 (3H) in the 270 MHz ^{1}H n.m.r. spectrum are consistent with the resonances of the benzylic methylene and N-methyl protons respectively. However, no resonances are observed for the indolic and secondary amino protons. Their absence may be due to rapid relaxation processes. Indeed, NH stretching bands in the i.r. spectra (recorded either as Nujol mull or in solution) of both the imine (141) and the amine (139) appear very broad and poorly resolved. This is in stark contrast to the indole NH bands in the spectra of the thioacetal (30) and aldehyde (29), which appear sharp and relatively strong. Such a difference may reflect favourable hydrogen

bonding opportunities present in both of these two last compounds.

Although there are many methods available for peptide bond formation, diphenylphosphoryl azide has found prominent use in effecting the direct coupling of acylamino acids or peptides with amino acids or peptide esters.[95] This became our method of choice in coupling the amine (139) with adipic acid (n = 4, Scheme 23); thus, a mixture of the amine (139) (two equivalents), adipic acid (one equivalent), diphenylphosphoryl azide (four equivalents) and triethylamine (eight equivalents) in dimethylformamide were stirred in the cold under an inert atmosphere. Analysis of the mixture after several hours by t.l.c., during which time a yellow precipitate had formed, revealed a complex mixture of products, but with no starting material remaining. The precipitate was collected by filtration and shown to be one major component. This was purified by column chromatography and gave spectral data in accord with the 6H-pyrido[4,3-*b*]carbazole dimer [(140), n = 4]. Work-up of the filtrate yielded a further quantity of the dimer after column chromatography, and an overall yield for the coupling reaction of 37%.

A reductive amination procedure using sodium cyanoborohydride[96] was attempted in an earlier approach to the amine (139) from 17-oxoellipticine (29). Thus, a methanolic solution of 17-oxoellipticine with ethanolic methylamine (ten equivalents) in the presence of sodium cyanoborohydride (one and a half equivalents) at pH 6 (methanolic hydrogen chloride) was stirred at room temperature. After 23 hours, t.l.c. analysis revealed a mixture of starting material and one major product. Consequently, further portions of sodium cyanoborohydride (two equivalents), ethanolic methylamine (ten equivalents) and methanolic hydrogen chloride (to pH 6) were added. A re-examination of the mixture after a further 25 hours showed only traces of starting material. The major product

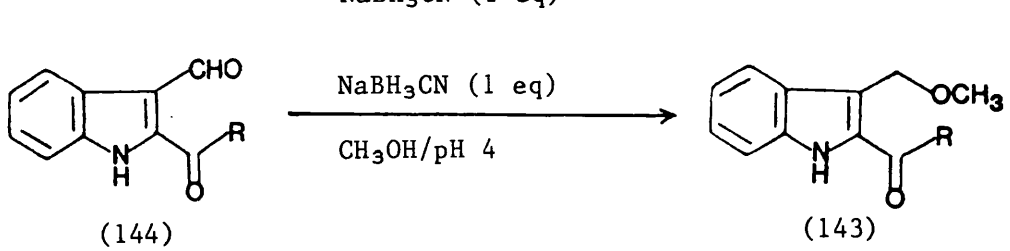
isolated, after work-up and purification, corresponded to the methyl ether (142) in 45% yield. Spectral data confirmed the nature of the



(142)

product; in particular, the 250 MHz ^1H n.m.r. spectrum which showed the presence of methoxy and benzylic methylene proton singlets at δ 3.43 and 5.20 respectively. In the mass spectrum, a molecular ion is observed at *m/z* 276, which is also in accord with the proposed structure, and this is further supported by major cleavage products at *m/z* 245 (M^+-OCH_3) and 244 (M^+-CH_3OH).

Djurić *et al.*[97] have reported the formation of the methyl ethers (143a-b) by the reduction of the corresponding 2-acyl-3-formylindoles (144a-b) with sodium cyanoborohydride (one equivalent) in methanol at pH 4. Yields were extremely high (90-92%), but the mechanisms of the reactions were not discussed.

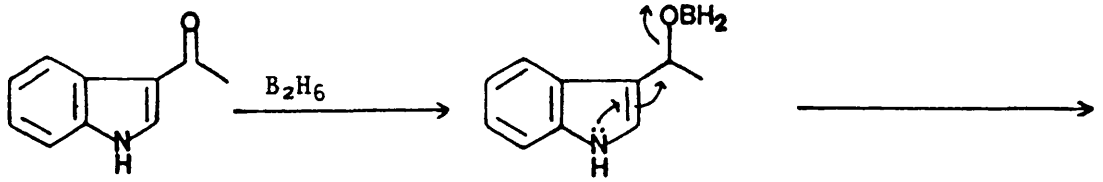


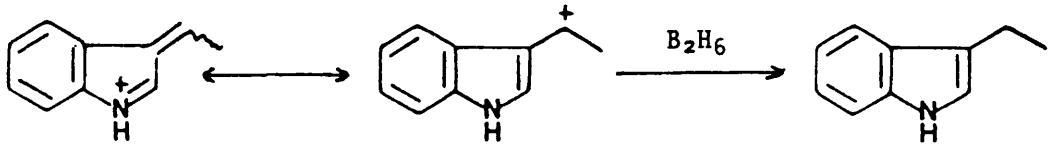
(144) (143)

a R = CH_3
b R = Ph

a R = CH_3
b R = Ph

However, having observed the reduction of 3-acetylindole to 3-ethylindole using excess diborane, Jackson *et al.*[98] have postulated that an elimination addition sequence is involved. Thus, the initially formed alkoxy borane complex undergoes a 1,4-elimination to give the methylene intermediate (145a), or the equivalent carbonium ion (145b), which then undergo reduction by diborane.

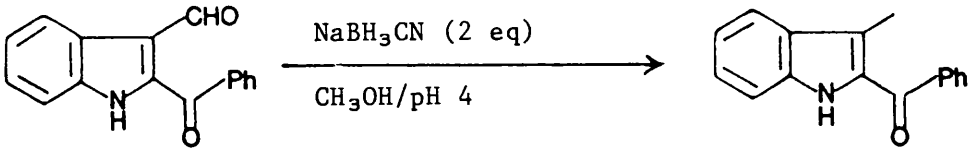




(145a) (145b)

A similar mechanism would account for Djurić *et al.* products, except instead of reduction the methylene or carbonium species then traps methanol. Interestingly, when these authors reduced the acylaldehyde (144b) with excess sodium cyanoborohydride (two equivalents) in methanol at pH 4, skatole (146) was formed in 84% yield. Now it seems that hydride capture is favoured over methanol addition.

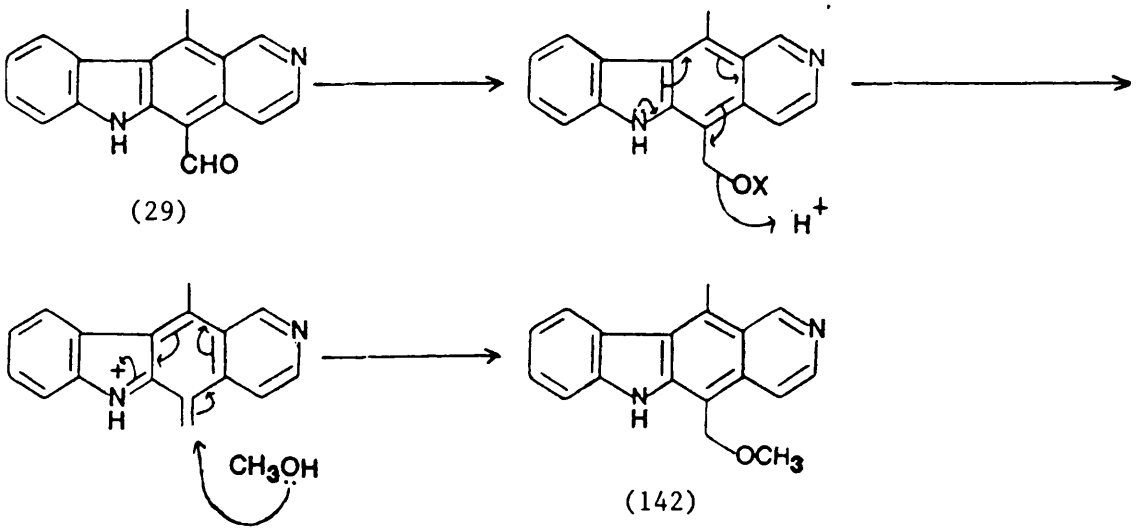
Since in our particular case excess sodium cyanoborohydride was used and no ellipticine (1) isolated, we feel it is unlikely that elimination of the reduced carbonyl group in (29) occurs prior to formation of the methyl ether (142). In accord with this, it is known that



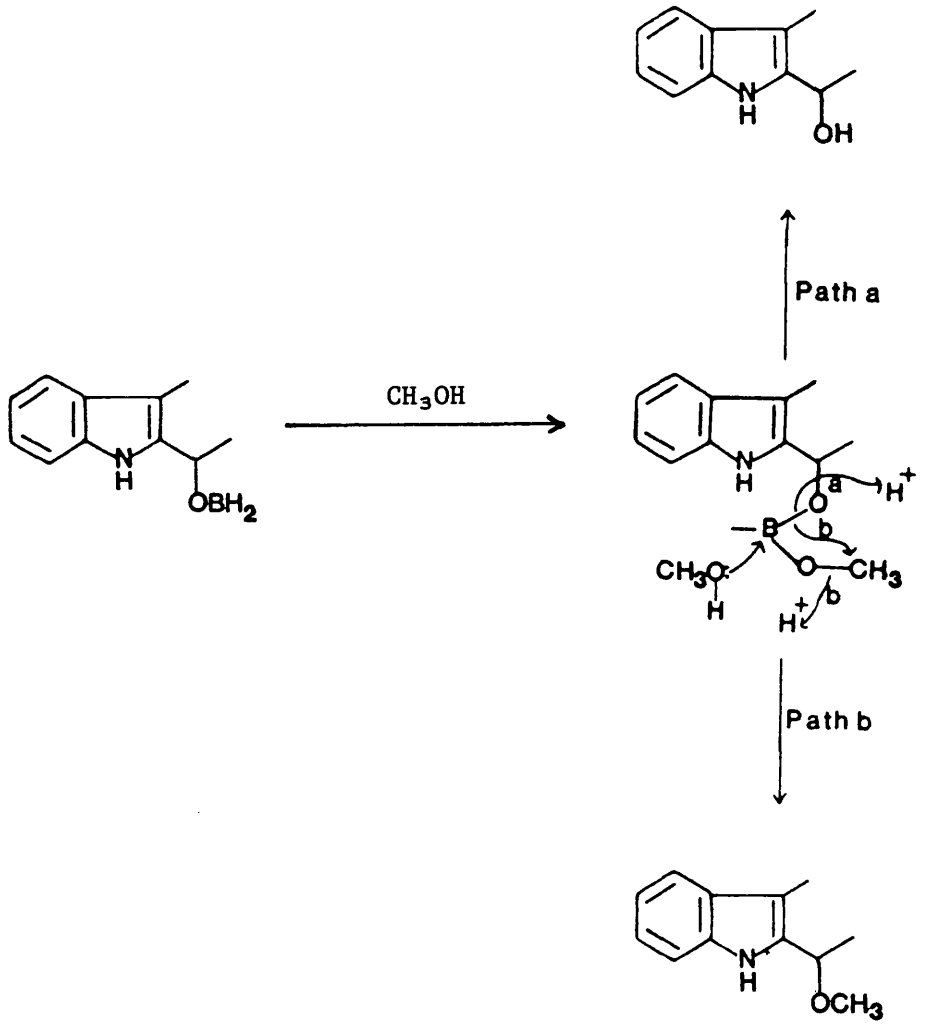
(144b) (146)

under neutral or slightly acidic conditions (pH 6) that negligible reduction of the carbonyl group by sodium cyanoborohydride is observed.[96] Indeed, it is this fundamental property that allows successful reductive amination reactions of carbonyl compounds using this reagent.

Reduction of 2-acetyl-3-methylindole with excess diborane gives 2-ethyl-3-methylindole in 65% yield, but in addition the 2-hydroxyethyl- and 2-methoxyethyl-indoles were obtained in yields of 14% and 4% respectively.[98] Jackson *et al.* have suggested that in this case, the intermediate alkoxy borane shows less tendency to dissociate to the methylene intermediate because of an unfavourable disturbance of the π-electrons in the benzene ring, a process which we could use to explain the observed reductive methylation of 11-oxoellipticine. In Jackson's



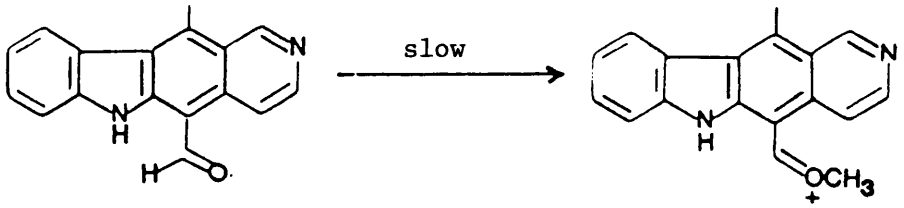
argument the 2-methoxyethylindole derivative is thought to arise from methanol used in the work-up to decompose excess diborane and alkoxy boranes.[98] Since the alcohol is presumably liberated by methanolysis, it may suggest that the methyl ether derivative results from methyl transfer from within a methoxy boron complex. However, Jackson *et al.*[99]



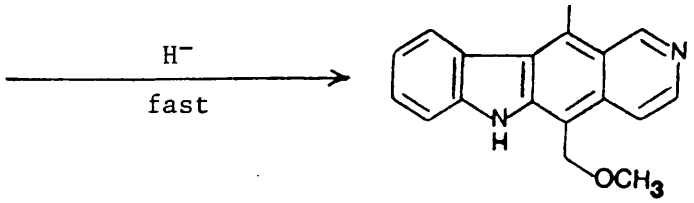
have used a methanol work-up in the diborane reduction of several other carbonyl compounds and found in the cases where alcohols have formed, little evidence for the corresponding methyl ether derivatives. It would appear that the nature of the carbonyl group in 2-acetyl-3-methyl-indole governs methyl ether formation. In this context, it is noteworthy that in this series the indolic nitrogen is appropriately placed for

co-ordination to the boron atom of the reagent.

In aqueous solutions, hydrolysis of the cyanoborohydride anion is very slow. However, the rate is increased by addition of a small amount of acid.[100] A similar situation may be envisaged in methanolic solutions in which methoxy-borohydride complexes are formed as intermediates. We suggest that formation of the methyl ether (142) may parallel the diborane reduction of 2-acetyl-3-methylindole, but that O-methylation occurs *via* a complex resulting from methanolysis of the cyanoborohydride anion. We have circumstantial evidence (see later) that O-methylation of the aldehyde, rather than its derived alcohol, is the first step, and that the oxonium ion (147) produced is rapidly reduced, even at pH 6, to the methyl ether (142). As in the case of



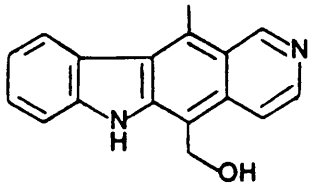
(29) (147)



(142)

2-acetyl-3-methylindole, it is probable that the special nature of the aldehyde influences the course of the reaction.

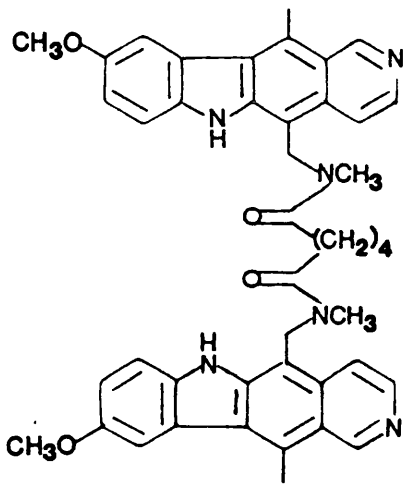
In seeking further support for this mechanism, it was of interest to investigate the reduction of 17-oxoellipticine using excess sodium cyanoborohydride in methanol at pH 3 (methanolic hydrogen chloride), conditions under which the aldehyde would be expected to form the alcohol (72).[96] After thirty minutes, t.l.c. analysis revealed that no starting



(72)

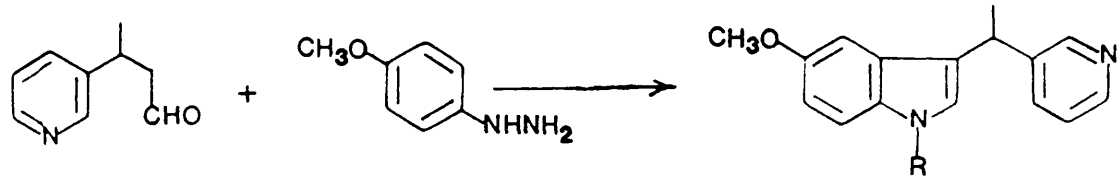
aldehyde remained in the reaction mixture, and two products, one corresponding in R_F to the methyl ether (142) had formed. Thereafter the product composition did not change. Five days later, the two products were separated by column chromatography and were shown to be the methyl ether (142) (23%) as expected, and the other the alcohol (72) (26%), previously described by Archer and co-workers.[36] The survival of the alcohol (72) adds support to the view that the methyl ether (142) is unlikely to occur *via* a methylation process, or an elimination addition sequence, involving the alcohol, but more appropriately results from a direct methylation process on the aldehyde. Furthermore, the overall rate of formation of the methyl ether (142) at pH 6 (2 days) is much slower than that at pH 3 (30 minutes) which may suggest that the rate determining step in the reaction is the methanolysis of the cyanoborohydride anion.

Since it is known that the presence of an alkoxy or hydroxy group at position 9 in the ellipticine ring usually leads to an enhancement in the antitumour activity,[6] we decided to repeat our synthetic route in order to obtain the bis-9-methoxy analogue (148) of the parent dimer (140, n = 4).



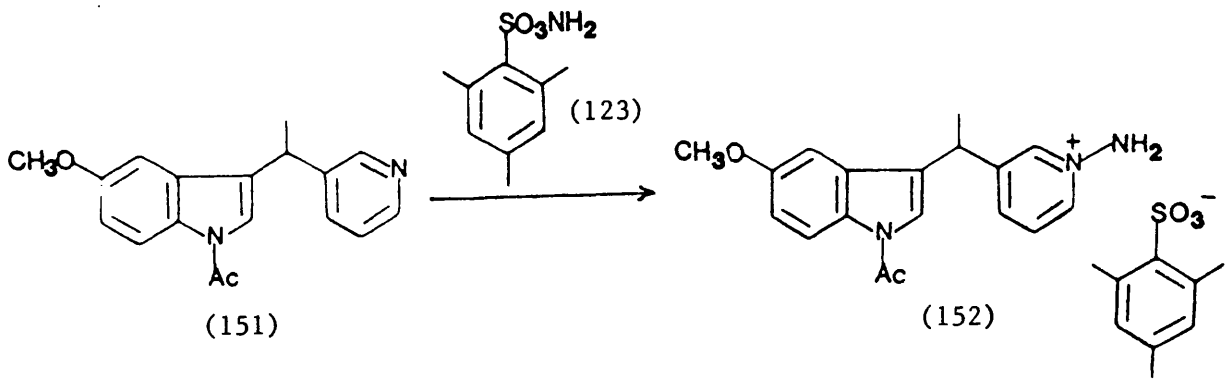
(148)

Accordingly, the 5-methoxycarbonitrile (149) was synthesised;[82,83a] thus a Fischer indolisation reaction betweer *para*-methoxyphenylhydrazine hydrochloride and the aldehyde (119) gave the required 5-methoxypyridyl-ethylindole (150) in 40% yield. This product was acetylated with acetic anhydride and triethylamine to afford the N-acetyl derivative (151) in 92% yield. Amination of the pyridine nitrogen atom of this compound using O-mesityl sulphonylhydroxylamine (MSH) (123) gave the N-amino salt (152). This salt is somewhat hydroscopic and prolonged exposure of the solid to air resulted in the formation of a gum. Nevertheless, subsequent acetylation and iodomethylation of either the pure salt or the gum afforded the 5-methoxy-N-methylacetamido salt (153) in 69% yield for the three step process (on a 8.3 g scale). The ^{1}H n.m.r. spectrum of this salt recorded at room temperature exhibits similar broad and poorly resolved resonances for protons in the pyridine ring and N-methylacetamido group as previously noted in the desmethoxy

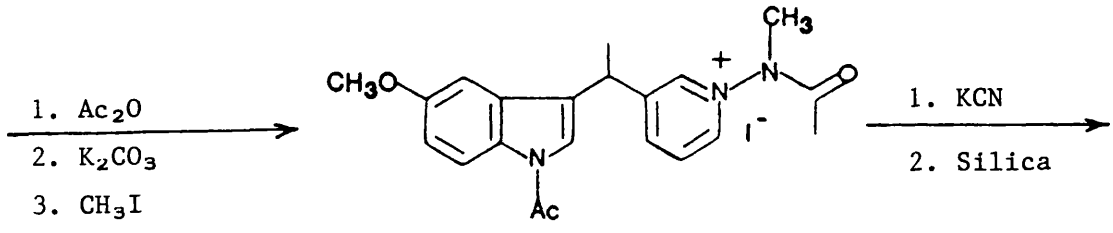


(119) (150) R = H

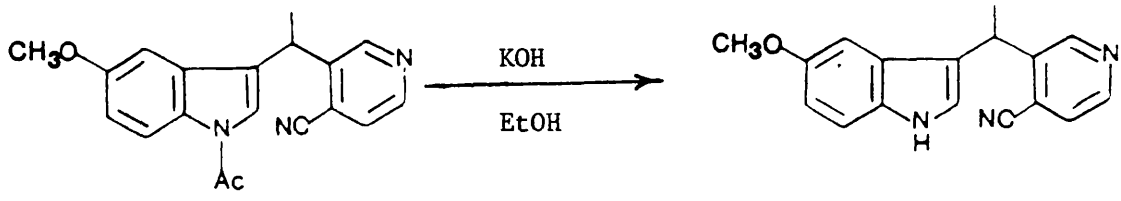
(151) R = Ac



(151) (152)



(153)

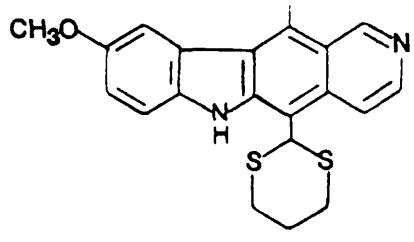


(154) (149)

Scheme 24

N-methylacetamido salt (124) (see page 47). However, by carrying out the acetylation step in neat acetic anhydride, it was discovered that the whole reaction sequence could be performed successfully on a much larger scale than previously used for the synthesis of the des-methoxy N-methylacetamido salt (124), where the acetylation step was conducted in an aqueous medium (see page 49). Reaction of the 5-methoxy-N-methylacetamido salt (153) with an aqueous solution of potassium cyanide gave the corresponding 4-cyano-1,4-dihydropyridine intermediate, which re-aromatised to N-acetyl-5-methoxycarbonitrile (154) in 60% yield simply by passing it down a silica column. Such a procedure circumvented the need for u.v. light, while at the same time leading to purification of the compound. Finally, deacetylation was effected by treatment with ethanolic potasium hydroxide leading to 5-methoxycarbonitrile (149) in 86% yield (Scheme 24).

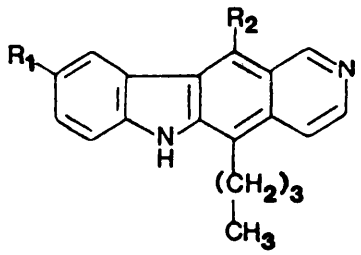
Reaction of a four-fold excess of 2-lithio-1,3-dithiane, generated under the stringent conditions previously employed, with the 5-methoxy-carbonitrile (149) (845 mg) in dry tetrahydrofuran (-78 °C to +25 °C, 17 hours), followed by acid hydrolysis and column chromatography gave a 82% yield of the thioacetal (155). Spectral data for the 1,3-dithiane



(155)

ring in this material are as previously observed for the parent thio-acetal (30). In particular, the ^{13}C n.m.r. suggests a similar orientation of the ellipticine chromophore to the 1,3-dithiane ring (see page 52).

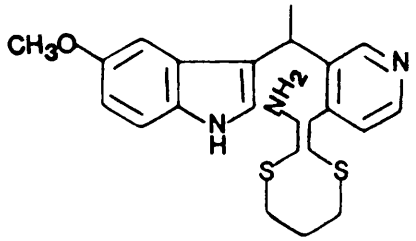
From this particular reaction, however, a second ellipticine product was isolated and characterised as 5-*n*-butyl-9-methoxy-11—methyl-6H-pyrido[4,3-*b*]carbazole (156). Since Sainsbury *et al.*[83b] have previously



(156) $R_1 = OCH_3$, $R_2 = CH_3$, (157) $R_1 = R_2 = H$
(27) $R_1 = H$, $R_2 = CH_3$

synthesised the desmethoxy analogue (27) and 5-*n*-butyl-6H-pyrido[4,3-*b*]-carbazole (157) by treatment of the appropriate carbonitrile with *n*-butyllithium, this 9-methoxy analogue, which amounted to 5% yield, must have resulted from a slight excess of *n*-butyllithium used in generating the 2-lithio-1,3-dithiane. In subsequent reaction runs, a slight excess of 1,3-dithiane was ensured to prevent formation of this novel, but unwanted by-product.

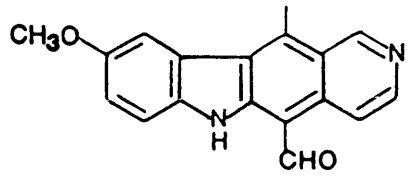
With this precaution in hand, a large scale reaction of the 5-methoxycarbonitrile (149) (4.4 g) with 2-lithio-1,3-dithiane (four-fold excess) gave a 62% yield of the thioacetal (155), with no traces of the 5-*n*-butyl derivative (156). A small amount of the intermediate prior to acid hydrolysis was isolated and purified by chromatography. This material gave spectral data (^1H n.m.r., ^{13}C n.m.r., mass spectroscopy) consistent with the primary aminoketene thioacetal (158), a conclusion which was based on our previous experience (see page 54). In this case, as noted earlier for the desmethoxy analogue (132), the n.m.r. spectra are sufficiently complex to suggest the presence of diastereomers. Notably, the ^1H n.m.r. spectrum where, apart from two broad singlets corresponding to the signals of the primary amino



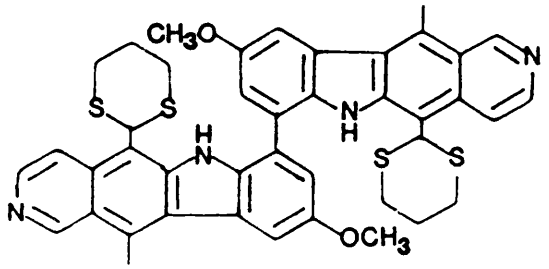
(158)

protons, two sets of resonances are observed for the methoxy and methyl protons. Thus, instead of a singlet and a doublet respectively, these signals are exhibited as a pair of singlets and two doublets. When the spectrum was re-run at higher temperature (+127 °C), each set of these signals merged together to provide the spectrum predicted for the simple model of the aminoketene thioacetal. In addition, a simplification of the aromatic proton splitting patterns occurred. This evidence confirmed that diastereomers resulting from restricted rotation about the chiral carbon bridgehead were present in the product, and indeed, when the sample was cooled to room temperature and the spectrum re-examined the original pattern of signals was restored.

Surprisingly, treatment of the thioacetal (155) under the reagent conditions previously found to cleave the 1,3-dithiane ring, namely silver nitrate (two equivalents) in a warm mixture of tetrahydrofuran and 2 M nitric acid under an oxygen-free atmosphere, did not afford the aldehyde (159), but led, instead, to the formation of the dehydrodimer (160) in 68% yield. The structure of this dimer is consistent with its 270 MHz ^{1}H n.m.r. spectrum where the absence of any signal attributable to the proton H-7, and the resonances of the protons H-10 and H-8 as doublets at δ 8.23 and 7.94 respectively (each of coupling



(159)



(160)

constant 2.5 Hz), can only be reconciled with a coupling *via* C-7 of the ellipticine ring. Assignment of the lower field doublet to the signal of proton H-10 is based on the chemical shift differences of these resonances relative to protons H-10 (δ 7.87) and H-8 (δ 7.19) in the ^{1}H n.m.r. spectrum of the thioacetal (155). Similar chemical shift differences are noted for dimerisation of carbazole compounds.[101] Further comparison between the aliphatic proton resonances in the dehydrodimer and thioacetal spectra establish that the 1,3-dithiane ring had remained intact during the dimerisation process. Unfortunately, mass spectrometric studies, using either ionisation (electron or chemical)

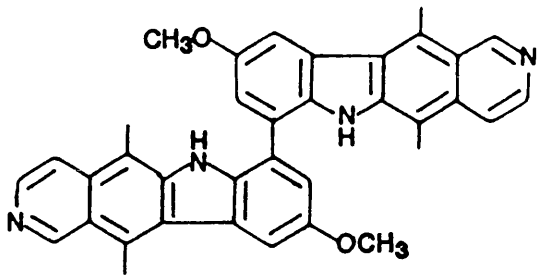
or fast atom bombardment techniques, showed no molecular ion corresponding to the dehydrodimer (160). However, elemental analysis confirmed that the empirical formula is $C_{21}H_{19}N_2OS_2$.

Oxidative coupling of carbazoles to dehydrodimers is well documented and can be accomplished electrochemically or by a variety of chemical oxidising agents.[102] It is postulated that these couplings proceed by way of radical cations, although whether the coupling mechanism involves dimerisation of two cation radicals followed by loss of two protons, or reaction of a cation radical with its neutral precursor followed by loss of an electron and two protons, is unknown. We assume that a similar reaction occurs in this case and that a radical cation is formed, even though the pyridine ring of the substrate may be protonated. Presumably, silver ions act as one electron oxidants, although nitric acid may function in a similar way. In any event, it is clear that the methoxy group enhances the electron density of the starting material and facilitates the coupling process, for no sign of the corresponding dehydrodimer was observed in the desmethoxy series.

Previous studies concerning the electrochemical oxidation of substituted carbazoles have shown that the site of coupling is highly dependent on the structure of the substrate and nature of the oxidising medium,[102a] but in our case, the choice of the carbon-carbon coupling site is interesting in that the positions *ortho* to the methoxy group are not selected. Instead, the reaction occurs at C-7, which is conjugated with the indolic nitrogen atom.

We were interested in the generality of this oxidative coupling reaction and subsequently 9-methoxyellipticine (2) and 9-hydroxyellipticine (5) were treated with silver nitrate under the same reaction conditions

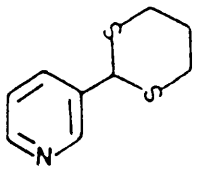
as before. However, only 9-methoxyellipticine gave the corresponding dehydrodimer (161), albeit in 5% yield. In the case of 9-hydroxy-



(161)

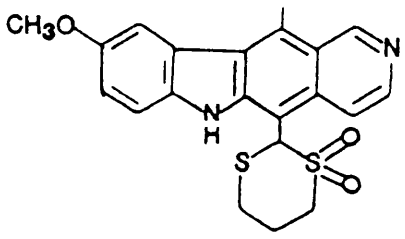
ellipticine all the substrate was consumed and a complex mixture resulted (by t.l.c. analysis). From this, we were unable to isolate any pure components. Interestingly, differences between 9-methoxyellipticine and 9-hydroxyellipticine have been noted by Moiroux *et al.*[103] in a study of the electro-oxidative behaviour of the two compounds. These workers suggest that under aqueous acidic conditions, 9-hydroxyellipticine undergoes dimerisation with C-9-O-C-10 bonding, while 9-methoxyellipticine undergoes a one electron oxidation in acetonitrile solution to form the dehydrodimer with coupling *via* the indolic nitrogen atom. However, in both cases, no spectral data were offered to support these claims.

Meerwein's reagent has been used previously to cleave thioacetals,[94,104] but in our case we considered that methylation of the pyridine nitrogen atom would take precedence, thus we turned next to the reagent combination of N-chlorosuccinimide and silver nitrate,[87] as recommended by Seebach[105] for the deprotection of the pyridine derivative (162). Addition of our thioacetal (155) (283 mg) to an aqueous acetonitrile solution (40% water) of N-chlorosuccinimide (four equivalents) and silver nitrate



(162)

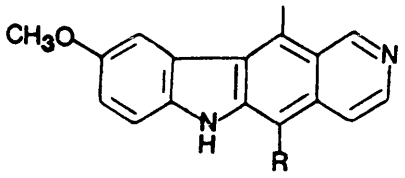
(≈five equivalents) led to the rapid formation of two new products. These products were separated by column chromatography and the less polar compound shown to have spectral data consistent with the desired aldehyde (159) (71%). The second much more polar product was demonstrated to be the sulphone (163c) (10%) as follows. The mass spectrum of the



(163c)

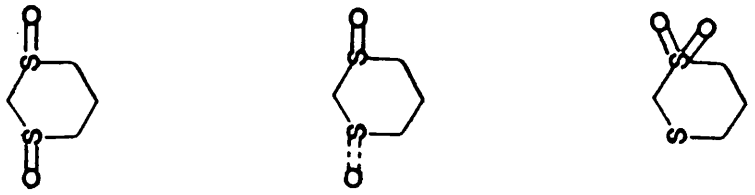
by-product gave a molecular ion at *m/z* 412 consistent with the incorporation of two extra oxygen atoms in the starting thioacetal (molecular mass 380). A comparison between the ^{1}H n.m.r. spectra of the starting thioacetal and the by-product show that the chemical shifts and spin-spin patterns of the aromatic proton resonances are virtually identical, but the signals of the 1,3-dithiane ring protons resonate at different chemical shifts, and in the case of the by-product, no longer with any first order character. This supports the view that it is this unit that has undergone oxidation. Although three structural isomers are possible for our product (163a-c), the ^{1}H n.m.r. spectrum reveals the presence

of only one isomer, by virtue of a single resonance at δ 6.29 belonging to the proton between the two sulphur atoms. Unfortunately, the nature



(163) a b c

R =

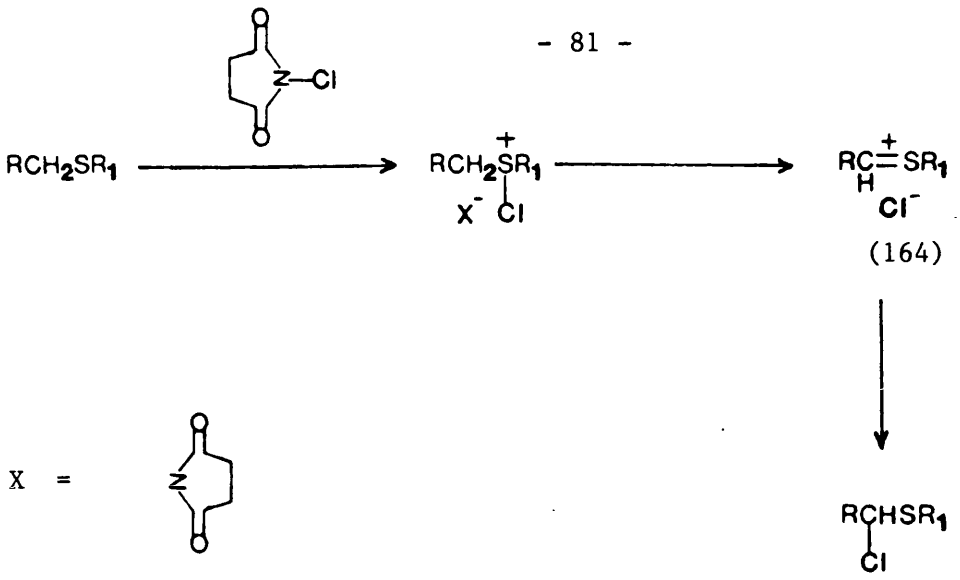


of the remaining 1,3-dithiane ring protons proved too complex to establish which isomer had formed. However, it has been demonstrated in a series of 2-substituted derivatives of 1,3-dithiane 1-oxide that the ^{13}C n.m.r. resonances of the carbon atoms at C-2 and C-6 are deshielded on oxidation of the sulphide to the sulphoxide (range δ 44.9-76.8).[106] In the case of C-6, the extent of the downfield shift is highly dependent on the orientation of oxygen on sulphur (equatorial δ 55.2-55.4, axial δ 44.9-47.9).[106] Although such a study has not been extended to any dioxide derivatives of 1,3-dithiane, in principle, each of the isomers (163a-c) should give rise to a characteristic set of aliphatic carbon resonances for the 1,3-dithiane ring, the number and chemical shifts

of which relate to the position of oxygen on sulphur(s). Accordingly, the ^{13}C n.m.r. of the by-product was recorded and showed the presence of six aliphatic carbon resonances at δ 16.2, 31.2, 31.7, 56.0, 59.3 and 64.8. By comparison with the unoxidised thioacetal (155), the resonances at δ 16.2 and 59.3 correspond to the C-11 methyl and methoxy groups respectively, and the remaining resonances must belong to the signals of the 5—substituent. If the sulphone structure (163c) is correct, then only two of these signals (those due to C-2 and C-6) should show significant downfield chemical shifts compared to those of the starting thioacetal (155). This is what we observe. The two other alternative structures, the *cis*- and *trans*-sulphoxides (163a and 163b), can be dismissed by a similar argument. Thus, in the case of the *cis*-isomer, the spectrum should show a degree of symmetry, and only three aliphatic carbon resonances should be anticipated (C-4≡C-6). In the *trans*-isomer, this symmetry is lost, but three carbon resonances should be shifted downfield since C-2, C-4 and C-6 of the 1,3-dithiane ring are adjacent to sulphoxide groups.

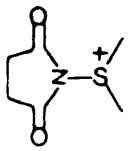
Further support for the sulphone (163c) is evident from its mass spectrum, where the major fragmentation peak at *m/z* 348 (M^+-64) corresponds to the extrusion of sulphur dioxide from the molecular ion. In addition, the i.r. spectrum shows stretching bands at 1290 and between 1100-1200 cm^{-1}, characteristic of the sulphone function.

Much of the early work attempting to explain the mechanism by which aliphatic sulphides undergo α-chlorination with N-chlorosuccinimide assumes that an initial chlorosulphonium salt is produced, which *via* a Pummerer-type rearrangement gives a sulphocarbonium ion (164). This species is then quenched by attack of chloride ion at the α-carbon.[107] However, when dimethyl sulphide is treated with the reagent saccinimidyl-



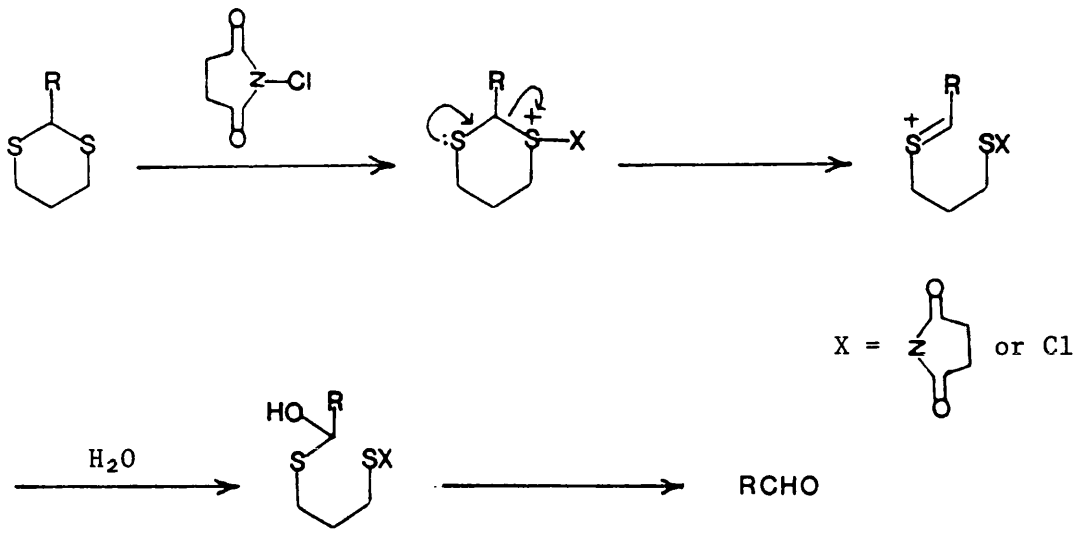
(164)

dimethyl-sulphonium chloride (165) can be isolated,[108] implying that this type of salt may be the reactive species in the α-chlorination sequence.[109] To date, the true nature of the reactive species has not been unequivocally demonstrated. Nevertheless, it is presumably



(165)

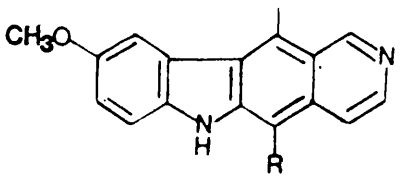
the aqueous reaction conditions which ensures carbon-sulphur bond cleavage and hydrolysis of the 1,3-dithiane ring in the thioacetal (155) is dominant over the Pummerer-type rearrangement. The use of silver nitrate as co-reagent is recommended, perhaps to suppress α-chlorination further by acting as a scavenger for chloride ion. Although the exact mechanism of hydrolysis is unknown, cleavage of the initially formed salt (chlorosulphonium or succinimidylsulphonium) is promoted by the ability of the second sulphur atom to participate in the carbon-sulphur bond breaking step. Since it is known that under the appropriate choice of conditions, sulphides can be oxidised to sulphoxides or sulphones



through the intermediacy of halosulphonium salts,[109,110] it is not surprising that oxidation of the 1,3-dithiane ring in the thioacetal (155) is a side reaction to carbon-sulphur bond cleavage.

A large scale hydrolysis of the 1,3-dithiane ring in the thioacetal (155) (1.01 g) under analogous conditions afforded a 64% yield of the corresponding aldehyde (159), together with a 8% yield of the sulphone by-product (163c).

Formation of the imine (166) and amine (167) were accomplished using methods similar to those previously described in the desmethoxy series and both gave spectral data of a similar nature as that obtained for their parent compounds. Thus, treatment of the aldehyde (159) in



(166) R = $CH{=}NCH_3$

(167) R = CH_2NHCH_3

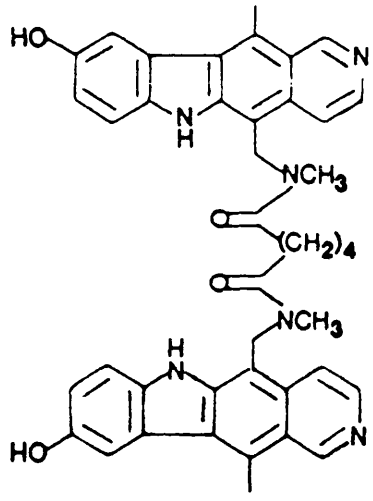
dry benzene with gaseous methylamine afforded the imine (166) in 99% yield as one stereoisomer (*E*-configuration, ^{1}H n.m.r. decoupling experiments). Reduction of the imine (166) in dry ethanol with sodium borohydride gave the amine (167) in 49% yield, after column chromatography.

As an alternative to the reduction of the imine (166) to amine (167) using sodium borohydride, an attempt was made to hydrogenate the carbon-nitrogen double bond at atmospheric pressure over 10% Pd/C as catalyst in dry ethyl acetate. This reaction failed and the imine was recovered unchanged. We did not pursue this reaction further, although it is probable that suitable hydrogenation conditions might have been discovered with a little more effort.

Originally, acylation of the amine (139) with adipic acid to form the 6H-pyrido[4,3-*b*]carbazole dimer (140, n = 4) was carried out using diphenylphosphoryl azide and triethylamine as reagents. The yield was a moderate 37%. In an attempt to increase the productivity, acylation of the amine (167) (two equivalents) with adipic acid (one equivalent) was performed as previously described, but now in the presence of 4-dimethylaminopyridine (four equivalents) and 1-hydroxybenzotriazole (four equivalents). These two last reagents have been shown to increase dramatically coupling yields in difficult cases.[111] With our new compound, however, the resulting overall yield of the bis-9-methoxy dimer (148) was 45%, which represents only a slight improvement on our earlier experience with the parent and diphenylphosphoryl azide alone.

A general method that has proved satisfactory for the O-demethylation of ellipticine derivatives has been the use of hot pyridine hydrochloride.[15a] Consequently, in an attempt to obtain the bis-9-hydroxy dimer (168) from our product (148), the compound was heated

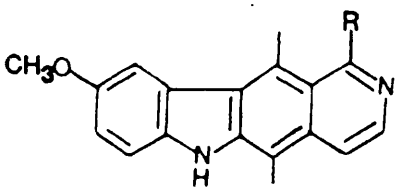
to 220 °C with a large excess of pyridine hydrochloride under anhydrous conditions. Starting material soon disappeared in favour of a more polar



(168)

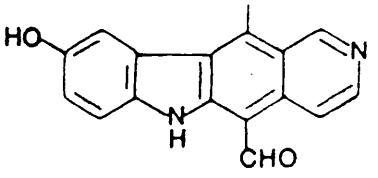
component, which after work-up was isolated by preparative plate chromatography eluting with dichloromethane/methanol/triethylamine 100:8:1. Compared to the starting bis-9-methoxy dimer (148) the ^1H n.m.r. of this compound showed an extra single proton resonance in the aromatic region at δ 9.33. This coupled with the absence of any signal due to the methoxy protons suggest that the resonance is attributable to the phenolic hydroxy proton and O-demethylation had occurred. A fact which was confirmed by u.v. studies, in which a bathochromic shift of the major absorption is observed on passing from neutral to alkaline solution. However, several proton resonances characteristic of the amide linkage are absent in this hydroxylated material, including the signal due to the benzylic methylene protons, suggesting that the amide linkage had not survived the harsh reaction conditions. Unfortunately, the ^1H n.m.r. spectrum is very complex and we are unable to be certain of the purity or the

structure of this product. Furthermore, both i.r. and mass spectrometric studies also failed to yield any useful information. A similar result of O-demethylation accompanied by cleavage of a side chain has been noted by Bisagni and co-workers on treatment of the ellipticine derivative (169) with hot pyridine hydrochloride.[112] A good deal of the available



(169) R = $NH(CH_2)_3N(C_2H_5)_2$

bis-9-methoxy dimer (148) was lost in the above reaction and since the remainder was reserved for biological testing, we sought to evaluate the O-demethylation of the more abundant aldehyde (159) to the hydroxy-aldehyde (170), with the view to synthesising the bis-9-hydroxy dimer (168) from it by the sequence previously developed.



(170)

A clean and high yielding O-demethylation of the aldehyde (159) was accomplished using boron tribromide as a reagent.[113] Addition of this reagent to a dry dichloromethane solution of the aldehyde (159) resulted in the instantaneous formation of a deep-red colour. The reaction mixture was monitored by t.l.c. and, after stirring at room

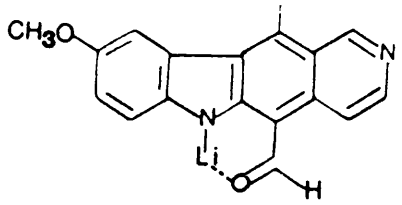
temperature for 24 hours, this showed a small amount of starting aldehyde still present together with a much more polar component in larger quantity. After an acid-base work-up these components were separated by column chromatography. The yield of starting material returned corresponded to 17%. The second more polar component was characterised as the hydroxy-aldehyde (170) in 65% yield (78% corrected yield on starting aldehyde returned). O-Demethylation of this material is confirmed from ^{1}H n.m.r. and u.v. spectroscopy studies based on a similar analysis as previously described, with further support from solution i.r. spectroscopy, which showed an extra strong band between 3500-3600 cm^{-1} attributable to the phenolic hydroxy OH stretch. Interestingly, in the spectrum recorded as a Nujol mull, this band is too weak to be observed, and only one sharp stretching band associated with the indolic NH at 3360 cm^{-1} is visible. A single proton resonance at δ 11.06 in the 270 MHz ^{1}H n.m.r. spectrum and a stretching band at 1635 cm^{-1} in the i.r. spectrum (Nujol mull) establishes the presence of an aldehyde group, but unfortunately no molecular ion corresponding to the hydroxy-aldehyde could be obtained from mass spectrometric studies. Furthermore, the compound proved relatively insoluble in most common solvents. This made its isolation difficult, and during the aqueous work-up and subsequent basification procedure large volumes of chloroform are required in order to extract it. Purification of the compound on a silica column by chromatography also required large volumes of a polar elutant and this became a tedious procedure. Basic and neutral alumina were investigated as alternative chromatographic supports, but both proved less satisfactory. Fortunately, all the starting aldehyde was consumed in subsequent reaction runs which were left for longer than 24 hours,

and as a result it was possible to collect the hydroxy-aldehyde by filtration of the basified reaction mixture. This material, after drying over phosphorus pentoxide *in vacuo*, appeared to be homogeneous by t.l.c. and gave spectral data identical to the chromatographed material. Using this procedure, a large scale O-demethylation of the aldehyde (159) (400 mg) was used to give the hydroxy-aldehyde (170) in 90% yield.

Initially, a small scale O-demethylation of the aldehyde (159) was achieved using hot pyridine hydrochloride in large excess. However, difficulties were encountered on purification of the hydroxy-aldehyde (170) and although the best yield recorded corresponded to 54%, spectral analysis suggested this product to be relatively impure.

In an alternative attempt, a reaction with lithium ethanethiolate in dimethylformamide was employed. This reagent has been used extensively to cleave aromatic ethers, and is normally very effective.[114] Addition of a dry dimethylformamide solution containing a large excess of lithium ethanethiolate to a solution of the aldehyde (159) in dry dimethylformamide at room temperature resulted instantaneously in the formation of a deep-red colour. Heating the resulting mixture under nitrogen at 80 °C for several hours showed starting aldehyde and base line material, with no evidence of the hydroxy-aldehyde (by t.l.c. analysis). Except for an increase in the intensity of the base line material, no change occurred on raising the reaction temperature to 140 °C for several hours. Work-up and column chromatography returned the starting aldehyde in 23% yield. It is conceivable on mixing the reagents, that lithium ethanethiolate behaves as a base and abstracts the indolic NH proton, so giving rise to the corresponding anion and hence the production of the deep-red colour observed. It is noteworthy that the lithium salt formed (171) may be stabilised by the adjacent

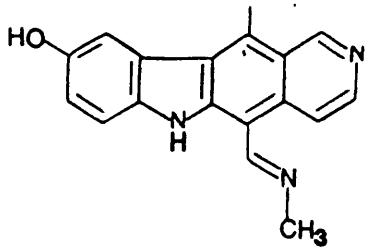
carbonyl group as shown. Since ethanethiolate anion O-demethylation



(171)

of aryl ethers is based on the nucleophilic attack at the alkyl carbon of the aryl ether, with subsequent displacement of the phenoxide anion,[114] formation of an anion *para* to the site of O-demethylation as in (171) would be expected to retard such a process. The low return of starting aldehyde is reflected by the t.l.c. evidence, which shows a strong base line spot. Unfortunately, the origin of this spot could not be ascertained, for after separation of the aldehyde, none of the additional fractions off the column gave rise to tangible products.

Treatment of a stirred suspension of the hydroxy-aldehyde (170) in dry methanol with gaseous methylamine led to formation of the corresponding imine (172) in 96% yield. The spectroscopic properties

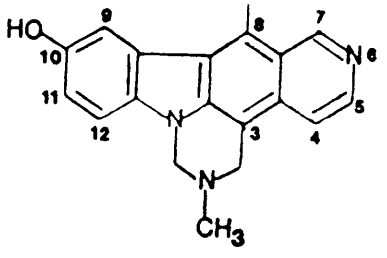


(172)

of this product are fully in accord with the structure (172), and in the ^1H n.m.r. spectrum the methine and N-methyl proton signals of the imine unit $CH{=}NCH_3$ are finely coupled with one another (J = 1 Hz). We assume that this function has the *E*-configuration.

For the preparation of this imine, we also tried to use methylamine in dry benzene as the reagent, but whereas this worked well for the methoxy- and desmethoxy-aldehydes, the hydroxylated aldehyde (170) proved to be insoluble in this mixture.

Addition of sodium borohydride (three equivalents) to a dry methanolic solution of the imine (172) at room temperature gave a complex mixture of products, together with much unreacted imine [as seen by a component corresponding in R_F to the hydroxy-aldehyde (170) on silica support]. This unusual observation contrasts sharply with the case of the other imines in this series, in which reduction is complete under these conditions. Subsequently, further portions of sodium borohydride were added (≈sixteen equivalents) and the mixture was refluxed for 1 hour. Re-analysis of the mixture after this time showed no evidence of the imine (172), but indicated a complex mixture containing a significant quantity of a more polar component. After an acid-base work-up, this component was purified by column chromatography and characterised as the pentacycle (173), which had formed in 17% yield. None of the desired amine was isolated. The structure of

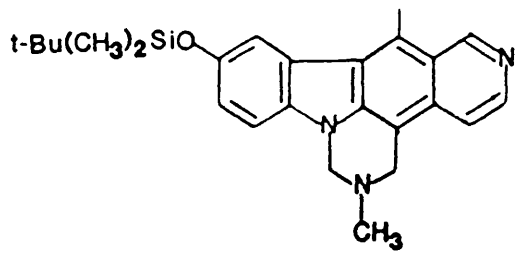


(173)

this pentacycle is consistent with its 270 MHz ^{1}H n.m.r. spectrum; in particular there is a singlet resonance for the methylene protons

between the two nitrogen atoms at δ 5.09. The benzylic methylene and N-methyl protons gave rise to singlet resonances at δ 4.40 and 2.51 respectively. Although no resonances for the indolic and secondary amino protons are observed, confirmation of the pentacyclic structure based on their absence is unambiguous, since similar signals were also absent from ^{1}H n.m.r. spectra of the amines (139) and (167). Unfortunately, no molecular ion corresponding to the pentacycle (173) was discernible in the mass spectrum (electron or chemical ionisation or fast atom bombardment), and little further evidence regarding the pentacyclic ring structure was afforded from u.v. or i.r. spectroscopy. Subsequently, in order to confirm the constitution of the pentacycle (173), we decided to protect the phenolic hydroxy group in the hope of obtaining a compound, the molecular ion of which might be more easily observed in the mass spectrometer.

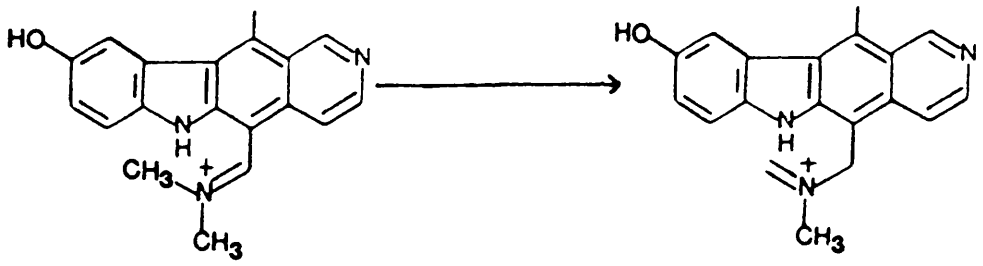
To this end, we initially treated the pentacycle (173) in dry dimethylformamide with chlorodimethyl-*t*-butylsilane in the presence of imidazole, literature conditions known to convert the phenolic hydroxy group to its dimethyl-*t*-butylsilyl protected analogue.[115] After 24 hours stirring under nitrogen, only starting material was evident by t.l.c. and u.v. studies. In an alternative approach, the phenoxide anion of the hydroxy-aldehyde (170) was generated *in situ* through the use of sodium hydride in dry dimethylformamide and quenched with a slight excess of chlorodimethyl-*t*-butylsilane. Using this procedure t.l.c. indicated absence of starting material and the formation of a less polar component. This new product was isolated, purified by column chromatography and fully characterised as the dimethyl-*t*-butylsilyl protected pentacycle (174) (39%). Thus, the electron impact



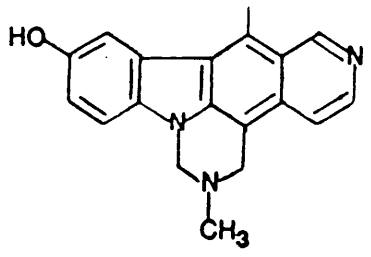
(174)

mass spectrum shows a molecular ion at *m/z* 417.2191 consistent with the protected pentacycle (174) (molecular mass required 417.2234), and this is further supported by major cleavage products of *m/z* 374 (M^+-CH_2=NCH_3) and 317 [*m/z* 374-$C(CH_3)_3$]. The 270 MHz ^1H n.m.r. spectrum contained the expected number of single proton resonances in the aromatic region and there are singlets at δ 4.96 (2H), 4.35 (2H) and 2.52 (3H) consistent with the resonances corresponding to the methylene group protons lying between the nitrogen atoms of the new ring, together with the benzylic methylene and N-methyl protons respectively of the same unit. The dimethyl-*t*-butylsilyl moiety not unexpectedly exhibits two singlet resonances at δ 1.06 (9H) and 0.27 (6H). In addition, the ^{13}C n.m.r. spectrum is fully in accord with presence of the pentacyclic ring system in the protected pentacycle (174). In particular, DEPT studies serve to distinguish between primary, secondary, tertiary and quaternary carbon resonances in questionable cases, and the presence of two secondary carbon resonances at δ 64.2 and 49.6, are assigned to the signals of the methylene carbon lying between the nitrogen atoms and benzylic methylene carbon in the pentacyclic ring respectively. The silyl-methyl carbon, quaternary *t*-butyl carbon and *t*-butyl methyl carbon of the dimethyl-*t*-butylsilyl moiety show characteristic resonances at δ -4.3, 18.3 and 25.8 res-

pectively. We envisage that the mechanism by which the pentacycle (173) is formed may have similarities to that of the methyl ether (142), thus the 'extra' carbon required to form the pentacyclic ring system is from a methoxy boron complex generated in solution from the methanolysis of sodium borohydride. The reaction of methanol with sodium borohydride is known to be a very facile process under these reaction conditions.[116] Once an intermediate of type (175) is formed, tautomerism to the iminium species (176) would facilitate ring closure to the pentacycle product (173). Although we are unsure what factors



(175) (176)



(173)

direct the observed ring closure in preference to imine reduction, it is possible that prior phenoxide formation reduces the rate of

reduction sufficiently to allow N-methylation to compete successfully. Lack of time prevented us from repeating this imine reduction in a solvent such as ethanol or 2-propanol where reaction of the solvent with sodium borohydride is stated to be considerably slower or occurs not at all.[116b]

In general, all the 9-hydroxyellipticine derivatives synthesised differ from the other ellipticines in: (i) much lower solubility in common solvents, and (ii) high polarity on silica supports. As a result, they proved difficult compounds to monitor in reactions, and subsequently to isolate and purify. Basic and neutral alumina supports were both less satisfactory than silica, and given these problems it may be altogether a far easier approach in future work directed towards the synthesis of the bis-9-hydroxy dimer (168) to prepare a large batch of the bis-9-methoxy dimer (148) and investigate its O-demethylation, possibly utilising the reagent boron tribromide.

Further future work may involve the use of other dicarboxylic acids than adipic acid in the acylation of the amines (139) and (167), so leading to analogues of the 6H-pyrido[4,3-*b*]carbazole dimers (140, n = 4) and (148) containing different lengths and functionality of spacer chain. In an alternative approach, it may be possible to synthesise totally new 6H-pyrido[4,3-*b*]carbazole dimers linked through C-5 from the chemistry of the aldehydes (29) and (159) as starting materials. In conclusion, as the search for even more potent anticancer ellipticine derived drugs continues, we have demonstrated a potential route to the efficient syntheses of new monomeric- and dimeric-ellipticine derivatives.

BIOLOGICAL RESULTS

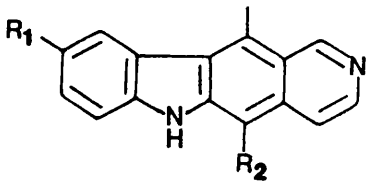
Several compounds were submitted for *in vitro* testing against leukaemia L1210 and Walker 180 tumour systems. The results of these tests are presented in Table 3. All the compounds analysed had ID_{50} values greater (less active) than that of 9-methoxyellipticine (2), but the aldehyde (159), amine (167), hydroxy-aldehyde (170) and the bis-9-methoxy dimer (148) exhibited much greater cytotoxicity than either the parent dimer (140, n = 4) or the 5-*n*-butylellipticine derivative (156). Although the most active compound synthesised is the bis-9-methoxy dimer (148), an investigation of the DNA binding parameters of this compound would have to be performed in order to establish whether it behaves as a bis-intercalator or, indeed, intercalates into DNA at all. The most striking contrast is the greater than one hundred fold increase in activity of this dimer compared to its desmethoxy analogue (140, n = 4). Although it is known that the presence of an alkoxy group at position 9 in the ellipticine ring usually leads to an enhancement in the antitumour activity,[6] the effect is not normally as pronounced as it is in this case. Thus 9-methoxyellipticine (2) is about one and a half times more active than ellipticine (1) against leukaemia L1210.[44] Such an observation may suggest that the bis-9-methoxy dimer does not behave as an intercalating agent, but has a cell killing action of a completely different nature. Clearly, further biological study of this dimer is warranted, as is the synthesis of analogues in which the length and nature of the linking chain is changed in order to gain a clear structure activity relationship.

Table 3 *In vitro* cytotoxicity of several ellipticine derivatives synthesised against L1210 and Walker 180 tumour systems[a]

Compound	Tumour system	
	Walker 180	L1210
		ID_{50} (μm)
(2)	-	0.6[b]
(156)	-	>400
(159)	-	6.5
(170)	-	>10
(167)	80% inhibition at 3.7 μm	
Dimers		
parent (140, n = 4)	-	>300
bis-9-methoxy (148)	-	2.6

[a]All compounds administered in dimethyl sulphoxide.

[b]Reference 44.



(2)	R_1	= OCH_3	R_2	=	CH_3
(156)	R_1	= OCH_3	R_2	=	n-C_4H_9
(159)	R_1	= OCH_3	R_2	=	CHO
(170)	R_1	= OH	R_2	=	CHO
(167)	R_1	= OCH_3	R_2	=	CH_2NHCH_3

EXPERIMENTAL

General

Melting points were recorded on an Electrothermal Mark II apparatus and are uncorrected. I.r. spectra were recorded on Perkin-Elmer 197 or 1310 grating spectrophotometers. U.v. spectra were recorded on Perkin-Elmer 402 and Lambda 3 instruments. ^{1}H N.m.r. spectra were run at 60 MHz on Perkin-Elmer R24B and Varian EM360 spectrometers; at 100 MHz on a JEOL PS 100 spectrometer; at 250 MHz on a Bruker instrument using the facility at Glaxo Group Research Ltd.; at 270 MHz on a JEOL JNM Fourier Transform spectrometer, and at 400 MHz using the SERC facility at Warwick University. ^{13}C N.m.r. were recorded at 67.8 MHz on a JEOL JNM Fourier Transform spectrometer and at 100 MHz using the SERC facility at Warwick University. Chemical shifts are expressed in parts per million downfield from tetramethylsilane (TMS) as internal standard. Mass spectra and high resolution accurate mass measurements were determined on a VG 7070E instrument with VG 2000 data system. T.l.c. analysis was performed on Merck DC-Alufolien plates coated with Kieselgel 60 F_{254}. Visualisation of reaction components was by u.v. light. Column chromatography was performed in short path columns packed with Merck 7736 Kieselgel and the solvent was eluted under pressure provided by hand bellows. Dry column chromatography was performed in cylindrical sinters packed with Merck 7736 Kieselgel and the solvent was eluted under water pump vacuum. The term "flash column chromatography" refers to the technique described by Still *et al.*,[118] and used Merck 9385 Kieselgel. Evaporations were carried out under water pump vacuum unless otherwise stated. Ethyl acetate, dichloromethane and light petroleum used for chromatography were distilled prior to use. The term "light petroleum" refers to the fraction boiling at 60-80 °C.

Reagents

Tetrahydrofuran was dried by distillation from sodium/benzophenone ketyl. Diethyl ether and benzene were dried by standing over sodium wire for at least 1 day. Dichloromethane was dried by distillation from phosphorus pentoxide. Dimethyl sulphoxide was dried by standing over activated 4 Å molecular sieves. Methanol and ethanol were dried by distillation from magnesium turnings. Triethylamine was dried by distillation from calcium hydride. Dimethylformamide was dried according to the procedure by Perrin *et al.*[119] N-Chlorosuccinimide was recrystallised from benzene prior to use. All molecular sieves were activated by heating to 150 °C overnight. Unless otherwise stated, all other solvents and reagents were used as supplied.

Ethyl 3-(3-pyridyl)but-2-enoate (116).-Triethyl phosphonoacetate (35 cm^3, 177 mmol) was added dropwise over a period of 1 hour to a cold (0 °C) suspension of sodium hydride (60% dispersion in oil) (7.10 g, 177 mmol) in dry tetrahydrofuran (75 cm^3) under an atmosphere of dry nitrogen. After all effervescence had ceased, 3-acetylpyridine (16.3 g, 135 mmol) was added dropwise over a period of 30 minutes. The resulting solution was stirred for a further 1 hour in the cold (0 °C), and then sealed under nitrogen and left at room temperature for 24 hours. The dark-red solution was poured into cold water (300 cm^3) and extracted with ethyl acetate (6 x 100 cm^3). The combined, dried (Na_2SO_4) extracts were evaporated to give a cloudy amber oil. On standing, two layers separated, the lower amber layer corresponding to the title ester (23.93 g, 93%), which was used without further purification, ν_{max} (thin film) 1715 (C=O), 1630 (C=C), 1585 and 1565; 60 MHz δ_H ($CDCl_3$) 8.8 (1H, d, J = 2 Hz, H-2'), 8.65 (1H, dd, J_1 = 5 Hz, J_2 = 2 Hz, H-6'), 7.85 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.35 (1H, dd, J_1 = 8 Hz, J_2 = 5 Hz, H-5'), 6.15 (0.8H, q, J = 1 Hz, C=C<u>H</u>, *E*-isomer), 6.05 (0.2H, q, J = 1 Hz, C=C<u>H</u>, *Z*-isomer), 4.25 (2H, m, $C\underline{H}_2CH_3$), 2.6 (2.4H, d, J = 1 Hz, $C\underline{H}_3C$=C, *E*-isomer), 2.25 (0.6H, d, J = 1 Hz $C\underline{H}_3C$=C, *Z*-isomer) and 1.3 (3H, t, J = 7 Hz $C\underline{H}_3CH_2$).

Ethyl 3-(3-pyridyl)butanoate (117).-A solution of ethyl 3-(3-pyridyl)but-2-enoate (10.01 g, 53 mmol) in 95% ethanol (100 cm^3) was hydrogenated at 125 psi and room temperature over 10% Pd/C (1.0 g) for 24 hours. The mixture was filtered through "Celite" and evaporated to afford a yellow oil. Distillation of the yellow oil under reduced pressure (80-83 °C/0.1 mmHg) gave *ethyl 3-(3-pyridyl)butanoate* as a clear colourless oil (8.92 g, 88%), ν_{max} (thin film) 1735 (C=O), 1590

and 1575 cm^{-1}; 100 MHz δ_H ($CDCl_3$) 8.7-8.4 (2H, m, H-2, H-6'), 7.65 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.3 (1H, dd, J_1 = 8 Hz, J_2 = 5 Hz, H-5'), 4.15 (2H, q, J = 7 Hz, $C\underline{H}_2CH_3$), 3.4 (1H, m, $CH_3C\underline{H}CH_2$), 2.65 (2H, d, J = 7 Hz, $CHC\underline{H}_2$), 1.35 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) and 1.20 (3H, t, J = 7 Hz, $C\underline{H}_3CH_2$).

3-*(3-Pyridyl)butan*-1-*ol* (118).-A solution of ethyl 3-(3-pyridyl)-butanoate (9.13 g, 47 mmol) in dry ether (30 cm^3) and dry tetrahydrofuran (30 cm^3) was added dropwise to a stirred suspension of lithium aluminium hydride (2.0 g, 52 mmol) in dry ether (100 cm^3) under dry nitrogen at room temperature. After all effervescence had ceased, the resulting mixture was stirred for 30 minutes and then cooled in ice. An aqueous solution of potassium sodium tartrate (33% w/w, 100 cm^3) was added cautiously with vigorous stirring. The two resulting phases were stirred at room temperature for a further 15 minutes, separated and the aqueous layer extracted with ethyl acetate (6 x 100 cm^3). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure to yield a slightly yellow oil. Bulb to bulb distillation of the yellow oil under reduced pressure (180 °C/0.1 mmHg) gave the *alcohol* (118) as a clear colourless oil (5.41 g, 76%), ν_{max} (thin film) 3275 (OH), 1590 and 1580 cm^{-1}; 100 MHz δ_H($CDCl_3$) 8.65-8.45 (2H, m, H-2', H-6'), 7.85 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.5 (1H, dd, J_1 = 8 Hz, J_2 = 5 Hz, H-5), 4.3 (1H, brs, exchanged with D_2O, OH), 3.65 (2H, td, J_1 = 6 Hz, J_2 = 2 Hz, $CH_2C\underline{H}_2OH$), 3.05 (1H, m, $CH_3C\underline{H}CH_2$) and 1.9 (2H, m, $C\underline{H}_2CH_2OH$).

3-*(3-Pyridyl)butanal* (119).-A stirred solution of freshly distilled oxalyl chloride (4.5 cm^3, 51.5 mmol) in dry dichloromethane (150 cm^3) was cooled to -60 °C (chloroform/liquid nitrogen) under a dry nitrogen atomosphere, and a solution of dry dimethyl sulphoxide (8.1 cm^3, 114 mmol) in dry dichloromethane (30 cm^3) was added dropwise, maintaining the

temperature of the mixture below -60 °C. After standing this mixture at -60 °C for 10 minutes, a solution of 3-(3-pyridyl)butan-1-ol (7.0 g, 47 mmol) in dry dichloromethane (25 cm^3) was added dropwise, once more maintaining the low temperature throughout and for an additional 30 minutes after the addition had been completed. Dry triethylamine was then added dropwise at -60 °C and the resulting brown suspension allowed to warm to room temperature over 2 hours. Water (100 cm^3) was added and the resulting layers thoroughly stirred, separated and the aqueous layer further extracted with dichloromethane (4 x 50 cm^3). The combined, dried ($MgSO_4$) organic layers were evaporated under reduced pressure and the residue was purified by dry column chromatography (eluted with ethyl acetate) to give the *aldehyde* (119) as a dark amber oil (6.45 g, 93%), ν_{max} (thin film) 2720 (CHO), 1725 (C=O), 1585 and 1570 cm^{-1}; 100 MHz δ_H ($CDCl_3$) 9.65 (1H, t, J = 1 Hz, $CH_2\underline{C}HO$), 8.55-8.35 (2H, m, H-2', H-6'), 7.55 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.2 (1H, dd, J_1 = 8 Hz, J_2 = 5 Hz, H-5'), 3.45 (1H, m, $CH_3\underline{C}HCH_2$), 2.8 (2H, dd, J_1 = 7 Hz, J_2 = 1 Hz, $CHC\underline{H}_2CHO$) and 1.35 (3H, d, J = 7 Hz, $C\underline{H}_3CH$).

3-*[1-(3-Pyridyl)ethyl]indole* (120).-A mixture of phenylhydrazine hydrochloride (11.85 g, 82 mmol) and 3-(3-pyridyl)butanal (11.85 g, 79 mmol) in absolute ethanol (100 cm^3) was stirred at room temperature under a dry nitrogen atmosphere for 1 hour. To the dark-red solution, dry ethanolic hydrogen chloride (200 cm^3) was added and the solution heated to reflux for 30 minutes. After allowing to cool, the solvent was removed under reduced pressure and the residue dissolved in water (250 cm^3) and made basic by the addition of 2 M ammonia solution. The mixture was extracted with dichloromethane (8 x 100 cm^3) and the combined, dried ($MgSO_4$) extracts were evaporated under reduced pressure to afford a partially solid red oil. Trituration of the oil with ethyl acetate

gave the *pyridylethylindole* (120) as an off-white solid. The solid was collected by filtration, washed with a little ethyl acetate and air dried (7.71 g). Column chromatography (eluted with ethyl acetate) of the filtrate afforded a red oil, which on re-columning (eluted with ethyl acetate/light petrolum 1:1 v/v) gave a partially solid red oil. Trituration with ethyl acetate as previously described, resulted in a further batch of the title compound (1.10 g) (total yield 8.81 g, 50%). The pyridylethylindole crystallised from 95% ethanol as colourless prisms, m.p. 175-176 °C (lit.,[117] 172 °C), ν_{max} (Nujol) 3140 (NH), 1590 and 1580 cm^{-1}; 100 MHz δ_H [$(CD_3)_2SO$] 10.9 (1H, brs, exchanged with D_2O, NH), 8.55 (1H, d, J = 2 Hz, H-2'), 8.30 (1H, dd, J_1 = 5 Hz, J_2 = 2 Hz, H-6'), 7.6 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.4-6.7 (6H, m, H-6', H-2, H-4, H-5, H-6, H-7), 4.35 (1H, q, J = 7 Hz, $CH_3\underline{CH}$) and 1.6 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit) (Found: C, 80.7; H, 6.3; N, 12.4. Calc. for $C_{15}H_{14}N_2$ C, 81.0; H, 6.4; N, 12.6%).

1-*Acetyl*-3-*[1-(3-pyridyl)ethyl]indole* (121).-A solution of 3-[1-(3-pyridyl)ethyl]indole (6.84 g, 31 mmol) in acetic anhydride (45 cm^3) and dry triethylamine (10 cm^3) was heated under reflux for 45 minutes. After cooling, the solvents were removed under reduced pressure (50 °C/ 0.1 mmHg) to afford a green gum, which was dissolved in dichloromethane (150 cm^3) and the solution washed with a saturated aqueous solution of sodium hydrogen carbonate (3 x 50 cm^3). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to give a partially solid yellow oil. Column chromatography (eluted with ethyl acetate/light petroleum 3:2 v/v) gave the product as an off-white solid (5.85 g, 72%), which crystallised from 95% ethanol, m.p. 122-124 °C (lit.,[117] 124 °C), ν_{max} (Nujol) 1695 (C=O), 1600, 1590 and 1575 cm^{-1}; 100 MHz δ_H ($CDCl_3$)

8.6 (1H, d, J = 2 Hz, H-2'), 8.45-8.25 (2H, m, H-6', H-7), 7.5 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.35-6.9 (5H, m, H-5', H-2, H-4, H-5, H-6), 4.25 (1H, q, J = 7 Hz, $CH_3C\underline{H}$), 2.6 (3H, s, $COC\underline{H}_3$) and 1.7 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit) (Found: C, 77.6, H, 6.1; N, 10.7. Calc. for $C_{17}H_{16}N_2O$ C, 77.3; H, 6.1; N, 10.6%).

1-*Amino*-3-*[1-(1-acetylindol-3-yl)ethyl]pyridinium mesitylenesulphonate* (122).-A solution of 1-acetyl-3-[1-(3-pyridyl)ethyl]indole (2.56 g, 9.7 mmol) in dry dichloromethane (20 cm^3) was cooled to 0 °C. An ice cold solution of O-mesityl sulphonylhydroxylamine (MSH)[84] (123) (2.13 g, 9.9 mmol) in dry dichloromethane (15 cm^3) was added dropwise, maintaining the temperature below 5 °C. The resulting orange solution was allowed to warm to room temperature and stirred for 20 minutes before addition to ice cold dry ether (500 cm^3). The resulting cream precipitate was stirred vigorously in the cold for a further 25 minutes under anhydrous conditions, and then collected by filtration, washed with a little dry ether and dried *in vacuo* to afford the *N-amino* salt (122) (4.39 g, 95%), m.p.130-135 °C, ν_{max} (Nujol) 3275 (NH_2) 3230 (NH_2), 1710 (C=O), 1600 and 1565 cm^{-1}; 250 MHz δ_H [$(CD_3)_2SO$] 8.85 (1H, s, H-2'), 8.67 (1H, d, J = 6 Hz, H-6'), 8.45 (2H, brs, NH_2), 8.36 (1H, d, J = 7 Hz, H-7), 8.29 (1H, d, J = 8 Hz, H-4'), 7.96 (2H, dd, J_1 = 8 Hz, J_2 = 6 Hz, H-5' + s, H-2), 7.45 (1H, d, J = 7 Hz, H-4), 7.35 (1H, td, J_1 = 7 Hz, J_2 = 1 Hz, H-6), 7.23 (1H, td, J_1 = 7 Hz, J_2 = 1 Hz, H-5), 6.77 (2H, s, aromatic protons mesitylene group), 4.66 (1H, q, J = 7 Hz, $CH_3C\underline{H}$), 2.72 (3H, s, $COC\underline{H}_3$), 2.52 (6H, s, *ortho*-$C\underline{H}_3$ mesitylene group), 2.19 (3H, s, *para*-$C\underline{H}_3$ mesitylene group) and 1.74 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit).

Decouplings

Resonance irradiated (δ)	Decoupling effect
8.67, H-6'	7.96, dd, H-5' → d (J = 8 Hz)
8.36, H-7	7.35, td, H-6 → d (J = 7 Hz)
8.29, H-4'	7.96, dd, H-5' → d (J = 6 Hz)
7.96, H-5'	8.67, d, H-6' → s
	8.29, d, H-4' → s
7.45, H-4	7.23, td, H-5 → d (J = 7 Hz)

3-*[1-(1-Acetylindol-3-yl)ethyl]-1-methylacetamidopyridinium iodide* (124).-Acetic anhydride (75 cm^3) was added to a stirred suspension of the N-amino salt (122) (4.32 g, 9.0 mmol) in ice cold water (60 cm^3), maintaining the temperature below 0 °C. The mixture was allowed to warm to room temperature and then stirred for 40 minutes. To the resulting ice cold pale-yellow solution an aqueous solution of potassium carbonate (30% w/w) was added dropwise maintaining the temperature below 15 °C. When the pH had reached 8, potassium carbonate addition was ceased and the mixture was stirred in the cold for 30 minutes to destroy excess acetic anhydride. The mixture was extracted with cold dichloromethane (6 x 100 cm^3) and the combined, dried ($MgSO_4$) extracts evaporated under reduced pressure at room temperature to afford a viscous yellow oil. The oil was dissolved in a mixture of acetone (70 cm^3) and iodomethane (50 cm^3) and heated under reflux for 45 minutes. The resulting yellow suspension was evaporated under reduced pressure to afford an orange gum. Trituration of the gum with acetone gave the *N-methylacetamido* salt (124) as a bright yellow solid, which was collected by filtration, washed with a little acetone and dried *in vacuo* (2.92 g, 70%), m.p. 205-207 °C, ν_{max} (Nujol) 1695 (C=O), 1620, 1600, 1590 and 1580 cm^{-1}; high temperature (+72 °C), 250 MHz δ_H [$(CD_3)_2SO$] 9.40 (1H, s, H-2'), 9.18 (1H, d, J = 6 Hz, H-6'),

8.78 (1H, d, J = 8 Hz, H-4'), 8.34 (1H, d, J = 7 Hz, H-7), 8.27 (1H, dd, J_1 = 8 Hz, J_2 = 6 Hz, H-5'), 7.85 (1H, s, H-2), 7.47 (1H, d, J = 7 Hz, H-4), 7.35 (1H, t, J = 7 Hz, H-6), 7.22 (1H, t, J = 7 Hz, H-5), 4.74 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.72 (3H, s, $NC\underline{H}_3$), 2.70 (3H, s, $NCOC\underline{H}_3$) 2.21 (3H, brs, $NNCOC\underline{H}_3$) and 1.82 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit) (Found: C, 51.9; H, 4.8; N, 9.1. Calc. for $C_{20}H_{22}N_3O_2I$ C, 51.7; H, 4.8; N, 8.8%).

1-Acetyl-3-{1-[3-(4-cyanopyridyl)]ethyl}indole (125).-A stirred suspension of the N-methylacetamido salt (124) (2.65 g, 5.7 mmol) and ammonium chloride (0.63 g, 11.6 mmol) in water (50 cm^3) was treated dropwise with a solution of potassium cyanide (0.80 g, 12.1 mmol) in water (20 cm^3). The resulting pink suspension was stirred at room temperature for 90 minutes and then extracted with dichloromethane (4 x 100 cm^3). The combined extracts were washed with water (2 x 150 cm^3), dried (Na_2SO_4) and solvent removed under reduced pressure to afford a pink foam. The foam was dissolved in absolute ethanol (100 cm^3) and irradiated with u.v. light (125 W) for 45 minutes under nitrogen. Evaporation of the solvent under reduced pressure gave a viscous oil, which was purified by column chromatography (eluted with ethyl acetate/light petroleum 3:1 v/v) to give the product as a brown coloured gum (0.75 g, 46%). The gum slowly solidified on standing to an off-white solid, which crystallised from 95% ethanol, m.p. 135-137 °C (lit.,[117] 111-112 °C), ν_{max} (Nujol) 2240 (C≡N), 1700 (C=O), 1600, 1580 and 1560 cm^{-1}; 400 MHz δ_H ($CDCl_3$) 8.66 (1H, s, H-2'), 8.64 (1H, d, J = 5 Hz, H-6'), 8.42 (1H, brd, J = 7 Hz, H-7), 7.52 (1H, dd, J_1 = 5 Hz, J_2 = 0.5 Hz, H-4'), 7.45 (1H, brs, H-2), 7.34 (1H, m, H-6), 7.18 (2H, d, J = 4 Hz, H-5, H-4), 4.70 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 2.69 (3H, s, $COC\underline{H}_3$) and 1.84 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit).

Decouplings

Resonance irradiated (δ)	Decoupling effect
7.18, H-5, H-4	7.34, m, H-6 → d (J = 7 Hz)
8.42, H-7	7.34, m, H-6 → t (J = 4 Hz)

3-{1-*[3-(4-Cyanopyridyl)]ethyl}indole* (114).-To a stirred suspension of the N-acetylcarbonitrile (125) (3.99 g, 13.8 mmol) in 95% ethanol (100 cm^3) a solution of potassium hydroxide (0.84 g, 15 mmol) in 95% ethanol (40 cm^3) was added, and the mixture stirred for 40 minutes at room temperature. The resulting light-brown solution was evaporated under reduced pressure, and the residue partitioned between chloroform (100 cm^3) and water (100 cm^3). After thorough mixing, the layers were separated and the aqueous layer further extracted with chloroform (100 cm^3). The combined, dried (Na_2SO_4) extracts were evaporated under reduced pressure to give a viscous oil, which was purified by dry column chromatography (eluted with ethyl acetate) to afford the *carbonitrile* (114) as a light-brown gum (3.41 g, 100%), ν_{max} (Nujol) 3140 (NH), 2235 (C≡N), 1615, 1590, 1575 and 1545 cm^{-1}; 400 MHz δ_H ($CDCl_3$)[a] 8.66 (1H, s, H-2'), 8.57 (1H, d, J = 5 Hz, H-6'), 8.23 (1H, brs, exchanged with D_2O, NH), 7.47 (1H, dd, J_1 = 5 Hz, J_2 = 1 Hz, H-5'), 7.36 (1H, dt, J_1 = 8 Hz, J_2 = 1Hz, H-7), 7.27 (1H, dd, J_1 = 8 Hz, J_2 = 1 Hz, H-4), 7.21 (1H, dd, J_1 = 2.5 Hz, J_2 = 1 Hz, H-2), 7.17 (1H, ddd, J_1 = 8 Hz, J_2 = 7.5 Hz, J_3 = 1 Hz, H-6), 7.02 (1H, ddd, J_1 = 8 Hz, J_2 = 7.5 Hz, J_3 = 1 Hz, H-5), 4.77 (1H, q, J = 7 Hz, $CH_3\underline{CH}$) and 1.82 (3H, d, J = 7 Hz, $\underline{CH_3}CH$) (primed numbers refer to the pyridyl unit; [a]assignments from COSY).

5-*(1,3-Dithian-2-yl)*-11-*methyl*-6*H*-*pyrido[4,3—b]carbazole* (30).-A dry tetrahydrofuran solution (18 cm^3), containing freshly sublimed 1,3-dithiane (1.64 g, 13.7 mmol), was left to stand for 30 minutes in the

presence of activated 4 Å molecular sieves under nitrogen. The solution was cooled to -20 °C (carbon tetrachloride/dry ice) and treated dropwise with 1.56 M *n*-butyllithium (8.75 cm^3, 13.6 mmol). The resulting mixture was stirred for 3 hours maintaining the temperature between -20 °C to -15 °C (anion formation was judged >95% efficient after 150 minutes by addition of a small sample of the mixture to D_2O and measurement of the deuterium incorporation at C-2 of the 1,3-dithiane ring by ^1H n.m.r. spectroscopy). To a separate flask a solution of the carbonitrile (114) (506 mg, 2.1 mmol) in dry tetrahydrofuran (6 cm^3) was left to stand for 30 minutes in the presence of activated 4 Å molecular sieves under nitrogen. The pink coloured solution was cooled to -78 °C (acetone/dry ice) and treated dropwise with the 2-lithio-1,3-dithiane solution (seven-fold excess). The resulting mixture, after stirring for a further 30 minutes at this temperature, was sealed under nitrogen and kept at -20 °C for 15 hours. After allowing to warm to room temperature over a 30 minute period, a saturated aqueous solution of sodium chloride (15 cm^3) was added, the resulting layers thoroughly stirred and filtered to remove the 4 Å molecular sieves. The two layers were separated and the aqueous layer further extracted with chloroform (2 x 25 cm^3). The combined extracts were evaporated under reduced pressure to afford a yellow brown oil, which was taken up in aqueous acetic acid (30% v/v, 90 cm^3) and heated on a steam bath for 2 hours. On cooling, the mixture was basified with a saturated aqueous solution of sodium hydrogen carbonate and extracted with chloroform (3 x 100 cm^3). Evaporation under reduced pressure of the dry combined extracts (Na_2SO_4) gave a dark-yellow oil, which was purified by column chromatography (eluted initially with ethyl acetate/light petroleum 2:1 v/v followed by ethyl acetate) to afford the *thioacetal* (30)

as a yellow solid (670 mg, 93%), which crystallised from methanol/chloroform, m.p. 294-296 °C (lit.,[23] 236-240 °C), t.l.c. R_F 0.23 (ethyl acetate/light petroleum/triethylamine 10:5:1 v/v); ν_{max} (Nujol) 3380 (NH), 1605 and 1580 cm^{-1}; λ_{max} (95% EtOH) 203 (ε 26930), 223 (19460), 238sh (14830), 279sh (36600), 288 (55800) and 322 nm (3380); 400 MHz δ_H ($CDCl_3$) 9.71 (1H, s, H-1), 9.54 (1H, brs, NH), 8.55 (1H, d, J = 6 Hz, H-3), 8.34 (1H, d, J = 8 Hz, H-10), 7.98 (1H, dbr, H-4), 7.58-7.51 (2H, m, H-8, H-7), 7.31 (1H, t, J = 7.5 Hz, H-9), 6.38 (1H, s, SC<u>H</u>S), 3.30 (3H, s, C<u>H</u>$_3$), 3.26 (2H, dd, J_1 = 14 Hz, J_2 = 12 Hz, H-4'ax, H-6'ax), 3.04 (2H, brd, J = 14 Hz, H-4'eq, H-6'eq), 2.31 (1H, brd, J = 14.5 Hz, H-5'eq) and 2.14 (1H, m, H-5'ax) (primed numbers refer to the 1,3-dithiane protons); 100 MHz δ_C ($CDCl_3$) 150.0, 141.8, 141.4, 140.8, 132.3, 131.2, 127.4, 125.0, 123.8, 123.1, 122.5, 120.0, 114.7, 110.6, 107.9, 44.5 (S<u>C</u>S), 32.2 (S<u>C</u>C), 25.1 (SC<u>C</u>) and 14.9 (<u>C</u>H$_3$); *m/z* (low eV electron impact) 352 (M^+ +2, 15%), 351 (M^+ +1, 27), 350 (M^+, 100), 276 (27), 260 (24) and 232 (31) (Found: C, 65.4; H, 5.5; N, 7.6. Calc. for $C_{20}H_{18}N_2S_2.H_2O$ C, 65.2; H, 5.4; N, 7.5%).

A large scale reaction of the carbonitrile (114) (3.41 g, 13.8 mmol) with 2-lithio-1,3-dithiane under analogous conditions afforded a 64% yield of the *thioacetal* (30) (3.08 g). Prior to acid hydrolysis, a small amount of the intermediate was purified by column chromatography (eluted with ethyl acetate). The resulting yellow foam corresponded to the *aminoketene thioacetal* (132), which by n.m.r. existed as a mixture of diastereomers, t.l.c. R_F 0.27 (ethyl acetate): 270 MHz δ_H ($CDCl_3$) 8.69-8.42 (2H, m), 8.30-8.16 (1H, brd, exchanged with D_2O, indolic NH), 7.54-6.96 (6H, m), 4.71-4.67 (1H, m, $CH_3C\underline{H}$), 4.26 (6/13 x 2H, brs, exchanged with D_2O, NH_2), 4.10 (7/13 x 2H, brs, exchanged with D_2O, NH_2), 2.90-2.05 (6H, m) and 1.76 (3H, d, J = 7 Hz, C<u>H</u>$_3$CH); 67.8 MHz, δ_C ($CDCl_3$)

150.0, 149.5, 147.3, 147.2, 146.4, 146.3, 144.4, 144.2, 139.4, 139.0, 136.6, 136.5, 126.6, 126.3, 123.8, 123.5, 122.2, 122.0, 121.8, 121.5, 120.9, 119.9, 119.4, 119.3, 119.1, 111.3, 110.9, 92.5, 92.3, 32.6, 32.3, 31.8, 31.6, 31.1, 26.6, 26.4, 22.9 and 22.1; *m/z* (low eV electron impact) 369 (M^+ +2, 15%), 368 (M^+ +1, 25), 367 (M^+, 100), 261 (11), 248 (17), 247 (22) and 222 (47).

5-(1,3-Dithian-2-yl)-11-methyl-6H-pyrido[4,3-b]carbazole 2-oxide (136).-To an acetone solution (18 cm^3) of the thioacetal (30) (55 mg, 0.16 mmol) a solution of silver nitrate (54 mg, 0.32 mmol) in water (1 cm^3) was added dropwise over a 25 minute period. The resulting cloudy mixture was stirred for 48 hours at room temperature under nitrogen. The mixture was filtered and to the filtrate a saturated aqueous solution of sodium chloride was added (15 cm^3). The solid collected was washed with chloroform (4 x 25 cm^3), which was subsequently used to extract the aqueous filtrate. The dry combined extracts (Na_2SO_4) were evaporated under reduced pressure to afford a yellow solid, which was purified by column chromatography (eluted with ethyl acetate) to give the title compound as a yellow solid (25 mg, 44%), m.p. 254-260 °C, ν_{max} ($CHCl_3$) 3400 (NH) and 1600 cm^{-1}; λ_{max} (95% EtOH) 289 nm; 400 MHz δ_H ($CDCl_3$) 9.41 (1H, s, NH), 8.64 (1H, vbrs, H-1), 8.35 (1H, d, J = 8 Hz, H-10), 8.00 (1H, vbrd, H-3), 7.65-7.45 (3H, m, H-4, H-7, H-8), 7.32 (1H, ddd, J_1 = 8 Hz, J_2 = 6.5 Hz, J_3 = 1.5 Hz, H-9), 6.37 (1H, s, SC<u>H</u>S), 3.31 (3H, s, C<u>H</u>$_3$), 3.28 (2H, ddd, J_1 = 14 Hz, J_2 = 13 Hz, J_3 = 2.5 Hz, H-4'ax, H-6'ax), 3.06 (2H, ddd, J_1 = 14 Hz, J_2 = 4.5 Hz, J_3 = 3 Hz, H-4'eq, H-6'eq), 2.32 (1H, dtt, J_1 = 14 Hz, J_2 = 4.5 Hz, J_3 = 2.5 Hz, H-5'$_{eq}$) and 2.16 (1H, dtt, J_1 = 14 Hz, J_2 = 13 Hz, J_3 = 3 Hz, H-5'ax) (primed numbers refer to the 1,3-dithiane protons); *m/z* (low eV electron impact) 366 (M^+, 36%), 350 (100), 276 (35) and 232 (24).

5-Formyl-11-methyl-6H-pyrido[4,3-b]carbazole (*17-oxoellipticine*) (29).-Nitrogen was continuously bubbled through a solution of the thioacetal (30) (457 mg, 1.3 mmol) in tetrahydrofuran (175 cm^3) and 2 M nitric acid (150 cm^3). After a period of 30 minutes, a solution of silver nitrate (466 mg, 2.7 mmol) in 2 M nitric acid (25 cm^3) was added and the resulting mixture was heated to between 40-50 °C. After 20 hours the mixture was allowed to cool, added to a saturated aqueous solution of sodium chloride (100 cm^3) and basified with solid sodium hydrogen carbonate. Chloroform (200 cm^3) was added to the mixture, the layers thoroughly stirred and filtered. The two layers were separated and the solid collected, washed with chloroform (3 x 100 cm^3), which was further used to extract the aqueous phase. The dry combined extracts (Na_2SO_4) were evaporated under reduced pressure to afford a partially solid yellow oil, which was purified by column chromatography (eluted with ethyl acetate/light petroleum/triethylamine 20:15:1 v/v) to give the *aldehyde* (29) as a bright yellow solid (214 mg, 63%), m.p. 256-258 °C (lit.,[23] 266-268 °C), t.l.c. R_F 0.40 (ethyl acetate/light petroleum/triethylamine 10:5:1 v/v); ν_{max} ($CHCl_3$) 3400 (NH), 1645 (C=O), 1600, 1590 and 1570 cm^{-1}; ν_{max} (Nujol) 3340 (NH) and 1645 (C=O) cm^{-1}; λ_{max} (95% EtOH) 231 (ε 22050), 238 (19830), 290 (56990), 357 (7340) and 408 nm (6450); 400 MHz δ_H ($CDCl_3$) 11.23 (1H, brs, NH), 11.03 (1H, s, C<u>H</u>O), 9.75 (1H, s, H-1), 8.66[a] (1H, d, J = 6 Hz, H-3), 8.43[a] (1H, d, J = 6 Hz, H-4), 8.34 (1H, d, J = 8 Hz, H-10), 7.61-7.55 (2H, m, H-7, H-8), 7.41 (1H, ddd, J_1 = 8 Hz, J_2 = 6 Hz, J_3 = 2 Hz, H-9) and 3.35 (3H, s, C<u>H</u>$_3$) ([a]assignment of signals to H-3 and H-4 may be reversed); *m/z* (low eV electron impact) 261 (M^+ +1, 17%), 260 (M^+, 100) and 231 (7) (Found: M^+, 260.0913. Calc. for $C_{17}H_{12}N_2O$ M^+, 260.0948).

5-(Methyliminomethyl)-11-methyl-6H-pyrido[4,3-b]carbazole (141).- Gaseous methylamine was bubbled through a cold (0 °C) stirred orange suspension of the aldehyde (29) (550 mg, 2.1 mmol) in dry benzene (150 cm^3) containing activated 3 Å molecular sieves. After 90 minutes, the flow of gaseous methylamine was ceased (no carbonyl stretch in the solution i.r. spectrum) and the dark-red solution was stirred for a further 1 hour in the cold (0 °C). The solution was filtered, the 3 Å molecular sieves collected washed with dry benzene (75 cm^3), and the benzene filtrate evaporated under reduced pressure to afford the *imine* (141) (535 mg, 93%) as a red gum, which was used without further purification, ν_{max} ($CHCl_3$) 1630 (C=N), 1600 and 1575 cm^{-1}; 250 MHz δ_H ($CDCl_3$) 11.87 (1H, brs, NH), 9.71 (1H, s, H-1), 9.36 (1H, q, J = 1.5 Hz, $CH_3N=C\underline{H}$), 8.56 (1H, d, J = 6 Hz, H-3), 8.36 (1H, d, J = 8 Hz, H-10), 8.16 (1H, d, J = 6 Hz, H-4), 7.56 (2H, d, J = 4 Hz, H-7, H-8), 7.36 (1H, m, H-9), 3.78 (3H, d, J = 1.5 Hz, $C\underline{H}_3N=CH$) and 3.31 (3H, s, $C\underline{H}_3$).

Decouplings

Resonance irradiated (δ)	Decoupling effect
9.36, $CH_3N=C\underline{H}$	3.78, d → s
8.36, H-10	7.36, m → t, J = 4 Hz
7.56, H-7, H-8	7.36, m → d, J = 8 Hz

5-(Methylaminomethyl)-11-methyl-6H-pyrido[4,3-b]carbazole (139).- Sodium borohydride (114 mg, 3.0 mmol) was added portionwise to a cold (0 °C) stirred red solution of the imine (141) (535 mg, 2.0 mmol) in dry methanol (75 cm) under an atmosphere of dry nitrogen. The resulting mixture was further stirred in the cold for 3 hours and then the solvent was removed under reduced pressure. The resulting residue was partitioned between water (60 cm^3) and chloroform (60 cm^3), the layers thoroughly

stirred and separated. The aqueous phase was further extracted with chloroform (6 x 60 cm^3), and the dry combined extracts ($MgSO_4$) evaporated under reduced pressure to give an orange gum, which was purified by column chromatography (eluted first dichloromethane/methanol/ammonia (880) 100:5:1 v/v and then dichloromethane/methanol/ammonia (880) 100:10:1 v/v) to afford the *amine* (139) as an orange solid (322 mg, 60%), m.p. 190-191 °C, t.l.c. R_F 0.11 (dichloromethane/methanol/ammonia (880) 125:8:1 v/v); ν_{max} (Nujol) 1600 cm^{-1}; λ_{max} (95% EtOH) 225 (ε 15330), 238 (16020), 286 (47100), 293 (39600), 331 (4180), 382 (3380) and 395 nm (3260); 250 MHz δ_H ($CDCl_3$) 9.70 (1H, s, H-1), 8.46 (1H, d, J = 6 Hz, H-3), 8.36 (1H, d, J = 8 Hz, H-10), 7.88 (1H, d, J = 6 Hz, H-4), 7.51-7.49 (2H, m, H-7, H-8), 7.31-7.27 (1H, m, H-9), 4.54 (2H, s, $CH_3NC\underline{H}_2$), 3.28 (3H, s, $C\underline{H}_3$) and 2.58 (3H, s, $C\underline{H}_3NCH_2$) (indolic and secondary amino protons not observed); *m/z* (low eV electron impact) 275 (M^+, 49%) and 244 (45).

N,N'-Dimethyl-N,N'-bis(11-methyl-6H-pyrido[4,3-b]carbazol-5-ylmethyl)-hexanediamide [(140), n = 4].-Freshly distilled diphenylphosphoryl azide (0.49 cm^3, 625 mg, 2.27 mmol) was added to a stirred orange solution of the amine (139) (312 mg, 1.1 mmol) and adipic acid (83 mg, 0.57 mmol) in dry dimethylformamide (25 cm^3) under a nitrogen atmosphere. The resulting mixture was cooled to 0 °C, dry triethylamine added dropwise (0.63 cm^3, 459 mg, 4.5 mmol) and then sealed under nitrogen and kept at -20 °C. After 1 hour, a dark-red solution had formed, in which, on leaving a further 22 hours, contained a yellow precipitate. The mixture was allowed to warm to room temperature and stirred for 2 hours, before collecting the yellow solid by filtration, which was washed with a little ethyl acetate and dried *in vacuo*. Flash chromatography of the solid (eluted with ethyl acetate/methanol/ammonia (880) 100:8:3 v/v) afforded the title

compound as a bright yellow solid (109 mg). The remaining filtrate was added to a mixture of dichloromethane (80 cm^3) and 2 M hydrochloric acid (60 cm^3), the layers thoroughly stirred and separated. The organic phase was further extracted with 2 M hydrochloric acid (4 x 80 cm^3). The combined 2 M hydrochloric acid extracts were basified with solid sodium hydrogen carbonate and extracted with dichloromethane (2 x 100 cm^3). The combined organic extracts were dried ($MgSO_4$) and solvent removed under reduced pressure (55 °C/0.1 mmHg). Purification of the resulting residue by flash chromatography (eluted initially with ethyl acetate/methanol/ammonia (880) 100:8:1 v/v and then dichloromethane/methanol/ammonia (880) 100:8:1 v/v) gave further amounts of the title compound (31 mg) (total yield 140 mg, 37%), m.p. 305-308 °C, t.l.c. R_F 0.4 (dichloromethane/methanol/triethylamine 100:2:1 v/v); ν_{max} (Nujol) 1620sh (C=O) and 1600 cm^{-1}; λ_{max} (95% EtOH) 225 (ε 34240), 238 (33630), 285 (106270), 292 (84890), 317 (5920), 330 (7840), 380 (6500) and 396 nm (6360); 270 MHz δ_H ($CDCl_3$) 10.63 (1H, s, NH), 9.74 (1H, s, H-1), 8.53 (1H, d, J = 6 Hz, H-3), 8.35 (1H, d, J = 8 Hz, H-10), 7.98 (1H, d, J = 6 Hz, H-4), 7.55-7.46 (2H, m, H-7, H-8), 7.29 (1H, ddd, J_1 = 8 Hz, J_2 = 6 Hz, J_3 = 1.5 Hz, H-9), 5.26 (2H, s, $CH_3NC\underline{H}_2$), 3.33 (3H, s, $C\underline{H}_3$), 2.89 (3H, s, $C\underline{H}_3NCH_2$), 2.41 (2H, m, $CH_3NCOC\underline{H}_2CH_2$) and 1.75 (2H, m, $CH_3NCOCH_2C\underline{H}_2$); *m/z* (fast atom bombardment, glycerol/thioglycerol/0.1 M hydrochloric acid matrix) 661 (M^++1, 9%) (Found: C, 74.3; H, 6.0; N, 12.3. $C_{42}H_{40}N_6O_2.H_2O$ requires C, 74.3; H, 6.3; N, 12.4%).

Reductive amination of 17-*oxoellipticine* (29) → 5-*(Methoxymethyl)*-11-*methyl*-6*H*-*pyrido*[4,3-*b*]*carbazole* (142).-Ethanolic methylamine (33% w/w, 0.7 cm^3, 5.8 mmol) was added to a stirred suspension of the aldehyde (29) (152 mg, 0.58 mmol) in dry methanol (25 cm^3) containing activated 3 Å molecular sieves. The mixture was adjusted to pH 6 with dry methanolic hydrogen chloride and sodium cyanoborohydride (55 mg, 0.87 mmol) added.

The resulting orange solution was left to stir at room temperature. After 23 hours, further quantities of ethanolic methylamine (33% w/w, 0.7 cm^3, 5.8 mmol) and sodium cyanoborohydride (75 mg, 1.2 mmol) were added, the pH re-adjusted to 6 with methanolic hydrogen chloride and the resulting solution left to stir for a further 25 hours. After this period of time, the solution was filtered, and the 3 Å molecular sieves collected, washed with little dry methanol. The pH of the filtrate was increased to 2 with concentrated hydrochloric acid and the methanol removed by evaporation under reduced pressure. The resulting residue was basified with a saturated aqueous solution of sodium hydrogen carbonate, extracted with chloroform (6 x 25 cm^3) and the combined extracts dried (Na_2SO_4). Removal of solvent under reduced pressure gave an off-yellow solid, which was purified by flash chromatography (eluted initially with ethyl acetate/hexane/triethylamine 30:10:2 v/v and then ethyl acetate/methanol 50:1 v/v) to afford 5-*(methoxymethyl)*-11-*methyl*-6*H*-*pyrido*[4,3-*b*]-*carbazole* (142) as a bright yellow solid (72 mg, 45%), m.p. 247-248 °C, t.l.c. R_F 0.16 (ethyl acetate/hexane/triethylamine 10:5:1 v/v); ν_{max} (Nujol) 1600 cm^{-1}; λ_{max} (95% EtOH) 224 (ε 14200), 238 (13300), 285 (51400), 292 (44100), 317 (2450), 330 (3300), 382 (2600) and 398 nm (2500); 250 MHz δ_H [$(CD_3)_2SO$]; 11.17 (1H, s, H-N), 9.70 (1H, H-1), 8.44 (1H, d, J = 6 Hz, H-3), 8.39 (1H, d, J = 8 Hz, H-10), 8.01 (1H, d, J = 6 Hz, H-4), 7.62-7.53 (2H, m, H-7, H-8), 7.29 (1H, t, J = 8 Hz, H-9), 5.20 (2H, s, $CH_3O\underline{CH}_2$) 3.43 (3H, s, $\underline{CH}_3O$) and 3.31 (3H, s, $\underline{CH}_3$); *m/z* (70 eV electron impact) 277 (M^++1, 15%), 276 (M^+, 71), 245 (70) and 244 (100).

Sodium cyanoborohydride reduction of 17-*oxoellipticine* (29) *at pH* 3.- Sodium cyanoborohydride (83 mg, 1.3 mmol) was added to a stirred suspension of the aldehyde (29) (170 mg, 0.65 mmol) in dry methanol (40 cm^3) containing

activated 3 Å molecular sieves. The pH of the mixture was adjusted to 3 with dry methanolic hydrogen chloride and the resulting solution was stirred at room temperature. After a period of 5 days the solution was filtered and the 3 Å molecular sieves collected washed with a little dry methanol. The methanol was evaporated under reduced pressure and the resulting residue basified with a saturated aqueous solution of sodium hydrogen carbonate. The mixture was extracted with dichloromethane (5 x 30 cm^3) and the dry combined extracts (Na_2SO_4) evaporated under reduced pressure to give an off-yellow solid. Column chromatography gave two components. The first (eluted with ethyl acetate/light petroleum/triethylamine 15:5:1 v/v) was collected as a yellow solid and corresponded to 5-*(methoxymethyl)*-11-*methyl*-6*H*-*pyrido*[4,3-*b*]-*carbazole* (142) (41 mg, 23%) (^{1}H n.m.r., t.l.c. same as authentic sample). The second component (eluted with ethyl acetate/methanol/triethylamine 50:1:1 v/v) was isolated as a yellow solid and corresponded to 5-*(hydroxymethyl)*-11-*methyl*-6*H*-*pyrido*[4,3-*b*]*carbazole* (72) (44 mg, 26%), m.p. 259-263 °C (lit.,[36] 257-258 °C), t.l.c. R_F 0.13 (dichloromethane/methanol/ammonia (880) 125:8:1 v/v); ν_{max} (Nujol) 3150 (NH, OH) and 1600 cm^{-1}; λ_{max} (95% EtOH) 226 (ε 19840), 239 (15740), 276 (36230), 286 (54760), 293 (48035), 331 (3770) and 342 nm (2300); 270 MHz δ_H [$(CD_3)_2SO$] 11.50 (1H, s, exchanged with D_2O, NH), 9.72 (1H, s, H-1), 8.44 (1H, d, J = 6 Hz), 8.39 (1H, d, J = 8 Hz, H-10), 8.09 (1H, d, J = 6 Hz), 7.63-7.49 (2H, m, H-7, H-8), 7.27 (1H, t, J = 8 Hz, H-9), 5.25 (3H, brs, $HOC\underline{H}_2$, OH on addition of D_2O the integral decreased to that required for 2H, OH exchanged) and 3.28 (3H, s, $C\underline{H}_3$); *m/z* (low eV electron impact) 262 (M^+, 24%) and 246 (15).

3-*[1-(3-Pyridyl)ethyl]*-5-*methoxyindole* (150).-A mixture of *para*-methoxyphenylhydrazine hydrochloride (7.35 g, 4.2 mmol) and 3-(3-pyridyl)-butanal (6.29 g, 4.2 mmol) in absolute ethanol (60 cm^3) was stirred at

room temperature under a dry nitrogen atmosphere for 1 hour. To the dark-red solution, dry ethanolic hydrogen chloride (100 cm^3) was added and the resulting solution heated to reflux for 4 hours. After allowing to cool, the solvent was removed under reduced pressure, the residue dissolved in water (150 cm) and made basic by the addition of 2 M ammonia solution. The mixture was extracted with chloroform (6 x 100 cm^3), the combined extracts dried ($MgSO_4$) and the solvent removed under reduced pressure. Dry column chromatography of the residue (eluted with chloroform) gave a partially solid red oil. Trituration of the oil with ethyl acetate gave the 5-*methoxypyridylethylindole* (150) as an off-white solid, which was collected by filtration, washed with a little ethyl acetate and air dried (3.80 g). Dry column chromatography of the remaining filtrate (eluted with ethyl acetate/light petroleum 2:1 v/v) gave a further partially solid red oil, which on trituration with ethyl acetate as previously described, afforded a further batch of the title compound (410 mg) (total yield 4.21 g, 40%). The 5-methoxypyridylethylindole crystallised from ethyl acetate as colourless prisms, m.p. 149-151 °C, ν_{max} (Nujol) 3160 (NH), 1590 and 1580 cm^{-1}; 60 MHz δ_H [$(CD_3)_2SO$] 10.85 (1H, brs, NH), 8.6 (1H, d, J = 2 Hz, H-2'), 8.35 (1H, dd, J_1 = 5 Hz, J_2 = 2 Hz, H-6'), 7.65 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.4-7.1 (3H, m, H-5', H-2, H-7), 6.85-6.5 (2H, m, H-4, H-6), 4.35 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.65 (3H, s, $\underline{CH_3}O$) and 1.65 (3H, d, J = 7 Hz, $\underline{CH_3}CH$) (primed numbers refer to the pyridyl unit) (Found C, 76.0; H, 6.3; N, 10.7. Calc. for $C_{16}H_{16}N_2O$ C, 76.2; H, 6.4; N, 11.1%).

1-*Acetyl*-3-*[1-(3-pyridyl)ethyl]*-5-*methoxyindole* (151).-A solution of 3-[1-(3-pyridyl)ethyl]-5-methoxyindole (4.11 g, 16.3 mmol) in acetic anhydride (25 cm^3) and dry triethylamine (6 cm^3) was heated under reflux for 3 hours. After allowing to cool, the solvents were removed under reduced

pressure (50 °C/0.1 mmHg) to give a green gum, which was dissolved in chloroform (60 cm^3) and the solution washed with a saturated aqueous solution of sodium hydrogen carbonate (100 cm^3). The two layers were separated, the aqueous phase further extracted with chloroform (100 cm^3) and the combined extracts dried ($MgSO_4$). Removal of the solvent under reduced pressure gave a partially solid yellow oil, which, on trituration with ethyl acetate, afforded the title compound as an off-white solid. The solid was collected by filtration, washed with a little ethyl acetate and air dried (4.12 g). Dry column chromatography of the remaining filtrate (eluted with ethyl acetate/light petroleum 1:1 v/v) gave a further batch of the title compound as an off-white solid (290 mg) (total yield 4.41 g, 92%). Crystallisation from ethyl acetate gave white microcrystals, m.p. 135-136 °C, ν_{max} (Nujol) 1690 (C=O), 1590 and 1570 cm^{-1}; 60 MHz δ_H ($CDCl_3$) 8.75 (1H, d, J = 2 Hz, H-2'), 8.6 (1H, dd, J_1 = 5 Hz, J_2 = 2 Hz, H-6'), 8.4 (1H, d, J = 9 Hz, H-7), 7.65 (1H, dt, J_1 = 8 Hz, J_2 = 2 Hz, H-4'), 7.4-7.2 (2H, m, H-5', H-2), 7.0 (1H, dd, J_1 = 9 Hz, J_2 = 2 Hz, H-6), 6.75 (1H, d, J = 2 Hz, H-4), 4.35 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.8 (3H, s, $\underline{CH_3}O$), 2.65 (3H, s, $COC\underline{H_3}$) and 1.75 (1H, d, J = 7 Hz, $C\underline{H_3}CH$) (primed numbers refer to the pyridyl unit) (Found: C, 73.1; H, 6.2; N, 9.5. Calc. for $C_{18}H_{18}N_2O_2$ C, 73.4; H, 6.2; N, 9.5%).

1-*Amino*-3-*[1-(1-acetyl*-5-*methoxyindol*-3-*yl)ethyl]pyridinium mesitylenesulphonate* (152).-A solution of 1-acetyl-3-[1-(3-pyridyl)ethyl]-5-methoxyindole (8.26 g, 28.1 mmol) in dry dichloromethane (30 cm^3) was cooled to 0 °C. An ice cold solution of O-mesityl sulphonylhydroxylamine (MSH)[84] (123) (7.55 g, 35.1 mmol) in dry dichloromethane (30 cm^3) was added dropwise, maintaining the temperature below 5 °C. The resulting orange solution was allowed to warm to room temperature and

stirred for 30 minutes before addition to ice cold dry ether (400 cm^3). The resulting cream precipitate was stirred vigorously in the cold for a further 20 minutes under anhydrous conditions, and then collected by filtration, washed with a little dry ether and dried *in vacuo* to afford the *N-amino* salt (152) (13.6 g, 95%), m.p. 98-100 °C. Prolonged exposure of the solid to air gave the N-amino salt as a gum, ν_{max} (thin film) 3280 (NH_2), 3240 (NH_2), 1690 (C=O) and 1590 cm^{-1}; 270 MHz δ_H ($CDCl_3$) 9.19 (1H, s, H-2'), 8.86 (2H, brs, NH_2), 8.73 (1H, d, J = 6.5 Hz, H-6'), 8.29 (1H, d, J = 9 Hz, H-7), 7.73 (1H, d, J = 8 Hz, H-4'), 7.52 (1H, s, H-2), 7.47 (1H, dd, J_1 = 8 Hz, J_2 = 6.5 Hz, H-5'), 6.89 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-6), 6.79 (2H, s, aromatic protons mesitylene group), 6.68 (1H, d, J = 2.5 Hz, H-4), 4.32 (1H, q, J = 7 Hz, $CH_3C\underline{H}$), 3.73 (3H, s, $C\underline{H}_3O$), 2.58 (6H, s, *ortho*-$C\underline{H}_3$ mesitylene group), 2.50 (3H, s, $COC\underline{H}_3$), 2.21 (3H, s, *para*-$C\underline{H}_3$mesitylene group) and 1.70 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit).

3-*[1-(1-Acetyl-5-methoxyindol-3-yl)ethyl]-1-methylacetamidopyridinium iodide* (153).-Ice cold acetic anhydride (60 cm^3) was added to the N-amino salt (152) (13.6 g, 27.6 mmol), maintaining the temperature below 0 °C. The mixture was allowed to warm to room temperature and then stirred for 1 hour. To the resulting ice cold pale-yellow solution, an aqueous solution of potassium carbonate (30% w/w) was added dropwise, maintaining the temperature below 15 °C. When the pH had reached 8, potassium carbonate addition was ceased and the mixture stirred in the cold for 30 minutes to destroy excess acetic anhydride. The mixture was then extracted with cold dichloromethane (3 x 200 cm^3) and the combined, dried ($MgSO_4$) extracts evaporated under reduced pressure at room temperature to give a white foam. The foam was dissolved in a mixture of acetone (250 cm^3) and iodomethane (75 cm^3) and heated under

reflux for 90 minutes. The resulting yellow suspension was evaporated under reduced pressure and the residue triturated with acetone to afford the 5-*methoxy-N-methylacetamido* salt (153) as a bright-yellow solid, which was collected by filtration, washed with a little acetone and dried *in vacuo* (9.6 g, 73%), m.p. 222-224 °C, ν_{max} (Nujol) 1700 (C=O), 1615 and 1595 cm^{-1}; 270 MHz δ_H [$(CD_3)_2SO$] 9.52 (1H, brs, H-2'), 9.17 (1H, brd, H-6'), 8.80 (1H, d, J = 8 Hz, H-4'), 8.30 (1H, dd, J_1 = 8 Hz, J_2 = 6.5 Hz, H-5'), 8.21 (1H, d, J = 9 Hz, H-7), 7.91 (1H, s, H-2), 6.98-6.90 (2H, m, H-4, H-6), 4.70 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.74 (6H, s, $\underline{CH_3}O$, $NC\underline{H_3}$), 2.67 (3H, s, $NCOC\underline{H_3}$), 2.32 (3H, brs, $NNCOC\underline{H_3}$) and 1.79 (3H, d, J = 7 Hz, $C\underline{H_3}CH$) (primed numbers refer to the pyridyl unit) (Found: C, 51.5; H, 5.0; N, 8.3. Calc. for $C_{21}H_{24}N_3O_3I$ C, 51.1; H, 4.9; N, 8.5%).

1-*Acetyl*-3-{1-*[3-(4-cyanopyridyl)]ethyl*}-5-*methoxyindole* (154).-A stirred suspension of the 5-methoxy-N-methylacetamido salt (153) (7.50 g, 15.2 mmol) and ammonium chloride (3.33 g, 62.2 mmol) in a mixture of water (100 cm^3) and 95% ethanol (30 cm) was treated dropwise with a solution of potassium cyanide (1.29 g, 19.8 mmol) in water (30 cm^3). The resulting pink suspension was stirred at room temperature for 2 hours and then extracted with dichloromethane (2 x 100 cm^3). The combined extracts were washed with water (2 x 100 cm^3), dried ($MgSO_4$) and solvent removed under reduced pressure. Column chromatography of the residue (eluted with ethyl acetate/light petroleum 3:1 v/v) gave the *N-acetyl-5-methoxycarbonitrile* (154) as a brown coloured gum (2.89 g, 60%), which on standing, slowly solidified to an off-white solid, m.p. 117-119 °C (lit.,[83a] 95-96 °C), ν_{max} (Nujol) 2230 (C≡N), 1695 (C=O), 1600 and 1590 cm^{-1}; 270 MHz δ_H ($CDCl_3$) 8.67 (1H, s, H-2'), 8.64 (1H, d, J = 5 Hz, H-6'), 8.32 (1H, d, J = 9 Hz, H-7), 7.52 (1H, d, J = 5 Hz, H-5'), 7.41

(1H, brs, H-2), 6.92 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-6), 6.64 (1H, d, J = 2.5 Hz, H-4), 4.65 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.75 (3H, s, $\underline{CH_3}O$), 2.66 (3H, s, $COCH_3$) and 1.83 (3H, d, J = 7 Hz, $\underline{CH_3}CH$) (primed numbers refer to the pyridyl unit).

3-{1-*[3-(4-Cyanopyridyl)]ethyl*}-5-*methoxyindole* (149).-To a stirred suspension of the N-acetyl-5-methoxycarbonitrile (154) (5.9 g, 18.5 mmol) in 95% ethanol (100 cm^3) a solution of potassium hydroxide (1.4 g, 25 mmol) in 95% ethanol (50 cm^3) was added and the mixture stirred for 90 minutes at room temperature. The resulting light-brown solution was then evaporated under reduced pressure and the residue partitioned between chloroform (75 cm^3) and an aqueous solution of potassium carbonate (30% w/w, 75 cm^3). After thorough mixing, the layers were separated and the aqueous phase further extracted with chloroform (2 x 100 cm^3). The combined, dried ($MgSO_4$) extracts were evaporated under reduced pressure to give an oil, which was purified by flash chromatography (eluted with ethyl acetate/light petroleum 3:1 v/v) to afford the 5-*methoxycarbonitrile* (149) as a light-brown gum (4.4 g, 86%), ν_{max} (Nujol) 3140 (NH), 2235 (C≡N), 1615 and 1580 cm^{-1}; 270 MHz δ_H ($CDCl_3$) 8.67 (1H, s, H-2'), 8.57 (1H, d, J = 5 Hz, H-6'), 8.28 (1H, brs, NH), 7.47 (1H, d, J = 5 Hz, H-5'), 7.25 (1H, d, J = 9 Hz, H-7), 7.20 (1H, d, J = 2.5 Hz, H-2), 6.83 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-6), 6.73 (1H, d, J = 2.5 Hz, H-4), 4.72 (1H, q, J = 7 Hz, $CH_3\underline{CH}$), 3.74 (3H, s, $\underline{CH_3}O$) and 1.82 (3H, d, J = 7 Hz, $\underline{CH_3}CH$) (primed numbers refer to the pyridyl unit).

5-*(1,3-Dithian-2-yl)*-9-*methoxy*-11-*methyl*-6*H*-*pyrido[4,3-b]carbazole* (155).-A dry tetrahydrofuran solution (15 cm^3) containing freshly sublimed 1,3-dithiane (1.53 g, 12.8 mmol) was left to stand for 30 minutes in the presence of activated 4 Å molecular sieves under nitrogen. The

solution was then cooled to -20 °C (carbon tetrachloride/dry ice) and treated dropwise with 1.62 M *n*-butyllithium (8.0 cm^3, 13.0 mmol). The resulting mixture was stirred for 3 hours maintaining the temperature between -20 °C to -15 °C (anion formation was judged ≈100% efficient after 165 minutes by addition of a small sample of the mixture to D_2O and measurement of the deuterium incorporation at C-2 of the 1,3-dithiane ring by ^1H n.m.r. spectroscopy). To a separate flask, a solution of the 5-methoxycarbonitrile (149) (845 mg, 3.1 mmol) in dry tetrahydrofuran (15 cm^3) was left to stand for 3 hours in the presence of activated 4 Å molecular sieves under nitrogen. The pink-coloured solution was then cooled to -78 °C (acetone/dry ice) and treated dropwise with the 2-lithio-1,3-dithiane solution (four-fold excess). The resulting mixture, after stirring for a further 1 hour at this temperature, was sealed under nitrogen and kept at -20 °C for 15 hours. After allowing to warm to room temperature over a 1 hour period, a saturated aqueous solution of sodium chloride (15 cm^3) was added, the two layers thoroughly stirred and filtered to remove the 4 Å molecular sieves. The layers were separated and the aqueous phase further extracted with chloroform (4 x 25 cm^3). The combined extracts were evaporated under reduced pressure to give a yellow-orange oil, which was taken up in aqueous acetic acid (30% v/v, 100 cm^3) and heated on a steam bath for 4 hours. On cooling, the mixture was basified with a saturated aqueous solution of sodium hydrogen carbonate, extracted with chloroform (3 x 200 cm^3) and the combined, dried extracts ($MgSO_4$) evaporated under reduced pressure. Column chromatography (eluted initially with ethyl acetate/light petroleum 2:1 v/v, followed by ethyl acetate) of the residue gave two components. The first was collected as a yellow solid (52 mg, 5%) and corresponded to 5-*n-butyl*-9-*methoxy*-11-*methyl*-6*H*-*pyrido*[4,3-*b*]*carbazole* (156), m.p. 268-269 °C, t.l.c. R_F 0.47 (ethyl acetate/light petrolum/triethylamine 10:5:1 v/v); ν_{max} (Nujol) 3320 (NH) and 1590 cm^{-1}; λ_{max} (95% EtOH) 240,

276, 293, 336 and 354 nm; 270 MHz δ_H [$(CD_3)_2SO$] 11.19 (1H, s, NH), 9.69 (1H, s, H-1), 8.40 (1H, d, J = 6 Hz, H-3), 7.91-7.87 (2H, m, H-4, H-10), 7.50 (1H, d, J = 9 Hz, H-7), 7.20 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 3.91 (3H, s, $C\underline{H}_3O$), 3.30-3.26 [5H, m, $C\underline{H}_3$, $CH_3(CH_2)_2C\underline{H}_2$], 1.65 (2H, m, $CH_3CH_2C\underline{H}_2CH_2$), 1.49 [2H, m, $CH_3C\underline{H}_2(CH_2)_2$] and 0.94 [3H, t, J = 7 Hz, $C\underline{H}_3(CH_2)_3$]; *m/z* (low eV electron impact) 318 (M^+, 100%) (Found: M^+, 318.1725. $C_{21}H_{22}N_2O$ requires M^+, 318.1730).

The second component was isolated as a yellow solid (946 mg, 82%), which crystallised from ethyl acetate to afford the *thioacetal* (155), m.p. 266-267 °C, t.l.c. R_F 0.30 (ethyl acetate/light petroleum/triethylamine 10:5:1 v/v); ν_{max} (Nujol) 3390 (NH), 1615sh, 1605 and 1585 cm^{-1}; λ_{max} (95% EtOH) 244 (ε 25650), 264sh (33510), 275sh (40380), 294 (64450) and 337 nm (7240); 270 MHz δ_H ($CDCl_3$) 9.71 (1H, s, H-1), 9.40 (1H, brs, exchanged with D_2O, NH), 8.54 (1H, d, J = 6 Hz, H-3), 7.97 (1H, brd, J = 6 Hz, H-4), 7.87 (1H, d, J = 2.5 Hz, H-10), 7.49 (1H, d, J = 9 Hz, H-7), 7.19 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 6.38 (1H, s, $SC\underline{H}S$), 3.29 (3H, s, $C\underline{H}_3$), 3.28 (2H, ddd, J_1 = 14 Hz, J_2 = 12.5 Hz, J_3 = 2 Hz, H-4'ax, H-6'ax), 3.05 (2H, ddd, J_1 = 14 Hz, J_2 = 4 Hz, J_3 = 3 Hz, H-4'eq, H-6'eq), 2.33 (1H, dtt, J_1 = 14.5 Hz, J_2 = 4 Hz, J_3 = 2 Hz, H-5'eq) and 2.14 (1H, dtt, J_1 = 14.5 Hz, J_2 = 12.5 Hz, J_3 = 3 Hz, H-5'ax) (primed numbers refer to the 1,3-dithiane ring); 67.8 MHz δ_C (CF_3COOD) 156.1, 144.4, 139.6, 139.4, 136.0, 133.3, 130.6, 129.4, 124.8, 121.6, 120.3, 116.0, 114.7, 112.9, 112.7, 59.4 ($\underline{C}H_3O$), 46.1 ($S\underline{C}S$), 33.7 ($S\underline{C}C$), 26.5 ($SC\underline{C}$) and 15.9 ($\underline{C}H_3$); *m/z* (low eV electron impact) 382 (M^++2, 12%), 381 (M^++1, 28), 380 (M^+, 100), 306 (39) and 290 (6) (Found: C, 66.1; H, 5.5; N, 7.2. $C_{21}H_{20}N_2OS_2$ requires C, 66.3; H, 5.3; N, 7.4%).

By using a slight excess of 1,3-dithiane over *n*-butyllithium in generation of 2-lithio-1,3-dithiane, a large scale reaction of the 5-methoxycarbonitrile (149) (4.4 g, 15.8 mmol) under analogous conditions afforded a 62% yield of the thioacetal (155) (3.75 g) only. Prior to

acid hydrolysis a small amount of the intermediate was purified by column chromatography (eluted with ethyl acetate). The resulting yellow foam corresponded to the *aminoketene thioacetal* (158), which by ^{1}H n.m.r. (at room temperature) existed as a mixture of diastereomers, t.l.c. R_F 0.15 (ethyl acetate/light petroleum 1:1 v/v); room temperature (+25 °C) 270 MHz δ_H ($CDCl_3$) 8.7-8.40 (3H, m, on addition of D_2O the integral decreased to that required for 2H, indolic NH exchanged), 7.21-6.76 (5H, m), 4.65-4.59 (1H, m, $CH_3C\underline{H}$), 4.32 (13/30 x 2H, brs, exchanged with D_2O, NH_2), 4.13 (17/30 x 2H, brs, exchanged with D_2O, NH_2), 3.80 (22/40 x 3H, s, $C\underline{H}_3O$), 3.67 (18/40 x 3H, s, $C\underline{H}_3O$), 2.90-2.02 (6H, m), 1.74 (22/40 x 3H, d, J = 7Hz, $C\underline{H}_3$) and 1.73 (18/40 x 3H, d, J = 7 Hz, $C\underline{H}_3$); high temperature +127 °C), 250 MHz δ_H [$(CD_3)_2SO$] 10.38 (1H, brs, indolic NH), 8.44 (1H, brs, H-2'), 8.35 (1H, d, J = 5 Hz, H-6'), 7.22 (1H, d, J = 9 Hz, H-7), 7.14 (1H, s, H-2), 7.08 (1H, d, J = 5 Hz, H-5'), 6.94 (1H, d, J = 2 Hz, H-4), 6.68 (1H, dd, J_1 = 9 Hz, J_2 = 2 Hz, H-6), 5.04 (2H, brs, NH_2), 4.54 (1H, q, J = 7 Hz, $CH_3C\underline{H}$), 3.67 (3H, s, $C\underline{H}_3O$), 3.08-1.92 (6H, m, $SC\underline{H}_2C\underline{H}_2C\underline{H}_2S$) and 1.67 (3H, d, J = 7 Hz, $C\underline{H}_3CH$) (primed numbers refer to the pyridyl unit), 67.8 MHz δ_C ($CDCl_3$) 153.7, 149.8, 149.4, 147.1, 146.4, 144.7, 144.3, 139.3, 139.2, 131.6, 126.9, 126.6, 123.7, 123.4, 122.9, 122.3, 120.4, 119.0, 111.9, 111.8, 111.7, 101.9, 101.2, 92.3, 56.0, 55.9, 32.5, 32.3, 31.8, 31.6, 30.9, 26.5, 26.4, 22.9 and 22.0); *m/z* (low eV electron impact) 401 (M^++2, 13%), 400 (M^++1, 27), 397 (M^+, 100), 291 (18), 278 (20), 277 (42) and 252 (35).

Bis[5-(1,3-dithian-2-yl)-9-methoxy-11-methyl-6H-pyrido[4,3-b]carbazol-7-yl] (160).-Nitrogen was continuously bubbled through a solution of the thioacetal (155) (28 mg, 0.07 mmol) in tetrahydrofuran (15 cm^3) and 2 M nitric acid (10 cm^3). After a period of 45 minutes a solution of silver nitrate (26 mg, 0.15 mmol) in 2 M nitric acid (5 cm^3) was

added and the resulting mixture heated between 40-50 °C. After 16 hours the mixture was allowed to cool, added to a saturated aqueous solution of sodium chloride (10 cm^3) and basified with solid sodium hydrogen carbonate. The mixture was extracted with chloroform (5 x 40 cm^3), the combined organic extracts dried ($MgSO_4$) and solvent removed under reduced pressure. The residue was purified by preparative t.l.c. (eluted with ethyl acetate/light petroleum/triethylamine 20:10:1 v/v) to give the title compound as a rusty red solid (19 mg, 68%), m.p. 279-283 °C, t.l.c. R_F 0.21 (ethyl acetate/light petroleum/triethylamine 15:5:1 v/v); ν_{max} (Nujol) 3380 (NH), 1610sh, 1600sh and 1575 cm^{-1}; λ_{max} (95% EtOH) 274 nm; 270 MHz δ_H ($CDCl_3$) 11.28 (2H, brs, 2 x NH), 9.76 (2H, s, H-1,1'), 8.63 (2H, d, J = 6 Hz, H-3,3'), 8.23 (2H, d, J = 2.5 Hz, H-10,10'), 8.04 (2H, d, J=6 Hz, H-4,4'), 7.94 (2H, d, J = 2.5 Hz, H-8,8'), 6.38 (2H, s, 2 x SC<u>H</u>S), 4.02 (6H, s, 2 x C<u>H</u>$_3$O), 3.29 (6H, s, 2 x C<u>H</u>$_3$), 3.28 (4H, ddd, J_1 = 14.5 Hz, J_2 = 12 Hz, J_3 = 2 Hz, 2 x H-4ax, 2 x H-6ax), 3.08 (4H, ddd, J_1 = 14.5 Hz, J_2 = 4.5 Hz, J_3 = 3 Hz, 2 x H-4eq, 2 x H-6eq), 2.37 (2H, dtt, J_1 = 14.5 Hz, J_2 = 4.5 Hz, J_3 = 2 Hz, 2 x H-5eq) and 2.23 (2H, dtt, J = 14.5 Hz, J_2 = 12 Hz, J_3 = 3 Hz, 2 x H-5ax) (Found: C, 66.5, H, 5.2; N, 7.8. $C_{42}H_{38}N_4O_2S_4$ requires C, 66.5; H, 5.0; N, 7.4%).

Bis[5,11-dimethyl-9-methoxy-6H-pyrido[4,3-b]carbazol-7-yl] (161).- Nitrogen was continuously bubbled through a solution of 9-methoxy-ellipticine (2) (81 mg, 0.29 mmol) in tetrahydrofuran (30 cm^3) and 2 M nitric acid (20 cm^3). After a period of 30 minutes a solution of silver nitrate (102 mg, 0.6 mmol) in 2 M nitric acid (10 cm^3) was added and the resulting mixture heated between 40-50 °C. After 17 hours the mixture was allowed to cool, basified with a saturated aqueous solution of sodium hydrogen carbonate and chloroform added (50 cm^3). The two resulting layers were thoroughly stirred, filtered and separated. The solid collected was washed with chloroform (2 x 50 cm^3), which was further used to extract the aqueous phase. The combined, dried

extracts (Na_2SO_4) were evaporated under reduced pressure to afford a red gum, which was purified by flash chromatography (eluted with ethyl acetate/light petroleum/triethylamine 20:10:1 v/v) to give the title compound as a red solid (4 mg, 5%), m.p. >350 °C, t.l.c. R_F 0.27 (ethyl acetate/light petroleum/triethylamine 10:10:1 v/v); ν_{max} (Nujol) 3370 (NH), 1615, 1600 and 1580 cm^{-1}; λ_{max} (95% EtOH) 271 nm; 270 MHz δ_H ($CDCl_3$) 9.88 (2H, brs, 2 x NH), 9.76 (2H, s, H-1,1'), 8.58 (2H, d, J = 6 Hz, H-3,3'), 8.25 (2H, d, J = 2 Hz, H-10,10'), 7.91-7.90 (4H, m, H-4,4', H-8,8'), 4.01 (6H, s, 2 x $C\underline{H}_3O$), 3.29 (6H, s, 2 x 11-$C\underline{H}_3$) and 2.84 (6H, s, 2 x 5-$C\underline{H}_3$).

5-Formyl-9-methoxy-11-methyl-6H-pyrido[4,3-b]carbazole (159).-A suspension of the thioacetal (155) (283 mg, 0.74 mmol) in acetonitrile (150 cm^3) was warmed until all had dissolved, placed under nitrogen in an ultrasonic bath and allowed to cool to room temperature. Nitrogen was continuously bubbled through an ice cold aqueous acetonitrile solution (60% v/v, 100 cm^3) containing N-chlorosuccinimide (403 mg, 3.0 mmol) and silver nitrate (603 mg, 3.5 mmol). After a period of 1 hour the thioacetal solution was added to the stirred aqueous acetonitrile solution under nitrogen (*via* cannula). A white precipitate separated as the liquid phase became yellow. Once addition was complete, the resulting mixture was stirred at 0 °C for 30 minutes, then allowed to warm to room temperature and stirred for a further 30 minutes, before being treated successively at 1 minute intervals with saturated aqueous solutions of sodium sulphite, sodium hydrogen carbonate and sodium chloride (20 cm^3 each). After thorough stirring, the two resulting layers were filtered and separated. The solid collected was washed with chloroform (4 x 100 cm^3), which was further used to extract the aqueous phase. The combined, dried extracts ($MgSO_4$) were evaporated under reduced pressure and column chromatography of the residue gave two components. The first (eluted initially with ethyl

acetate/light petroleum 2:1 v/v, followed by ethyl acetate) was obtained as a yellow solid (153 mg, 71%), which crystallised from ethyl acetate to give the *aldehyde* (159) as yellow microcrystals, m.p. 248-250 °C, t.l.c. R_F 0.34 (ethyl acetate/light petroleum/triethylamine 10:5:1 v/v); ν_{max} (Nujol) 3350 (NH), 1640 (C=O), 1595 and 1570 cm^{-1}; λ_{max} (95% EtOH) 234 (ε 23040), 261 (18330), 297 (49540) and 361 nm (4900); 270 MHz δ_H [$(CD_3)_2SO$] 12.18 (1H, s, NH), 11.03 (1H, s, C<u>H</u>O), 9.74 (1H, s, H-1), 8.70[a] (1H, d, J = 6 Hz, H-3), 8.58[a] (1H, d, J = 6 Hz, H-4), 7.83 (1H, d, J = 2.5 Hz, H-10), 7.76 (1H, d, J = 9 Hz, H-7), 7.21 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 3.91 (3H, s, C<u>H</u>$_3$O) and 3.30 (3H, s, C<u>H</u>$_3$)([a]assignment of signals to H-3 and H-4 may be reversed); *m/z* (low eV electron impact) 291 (M^++1, 23%), 290 (M^+, 100) (Found: C, 74.3; H, 4.7; N, 9.4. $C_{18}H_{14}N_2O_2$ requires C, 74.5; H, 4.9; N, 9.7%).

The second component (eluted with ethyl acetate/methanol 100:3 v/v) was isolated as a yellow solid (32 mg, 10%), which crystallised from dichloromethane/light petroleum to afford 5-*(1,1-dioxy-1,3-dithian-2-yl)-9-methoxy-11-methyl-6H-pyrido[4,3-b]carbazole* (163c), m.p.304-305 °C, t.l.c. R_F 0.12 (ethyl acetate/methanol 25:1 v/v); ν_{max} (Nujol) 3410 (NH), 1610sh, 1600, 1590, 1290 (SO_2), 1135 (SO_2) and 1110 cm^{-1} (SO_2); λ_{max} (95% EtOH) 242, 275, 294 and 335 nm; 270 MHz δ_H ($CDCl_3$) 9.71 (2H, s, NH, H-1), 8.56 (1H, d, J = 6 Hz, H-3), 7.90 (1H, d, J = 6 Hz, H-4), 7.86 (1H, d, J = 2.5 Hz, H-10), 7.48 (1H, d, J = 9 Hz, H-7), 7.20 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 6.29 (1H, s, SC<u>H</u>S), 3.96 (3H, s, C<u>H</u>$_3$O), 3.52-3.41 (2H, m), 3.31 (3H, s, C<u>H</u>$_3$), 3.28-3.19 (1H, m), 3.10-2.91 (2H, m) and 2.81-2.68 (1H, m); 67.8 MHz δ_C (CF_3COOD) 156.4, 144.4, 139.6, 139.2, 135.6, 133.2, 131.2, 130.6, 124.5, 121.0, 120.5, 117.8, 115.0, 113.7, 113.0, 64.8 (SO_2<u>C</u>S), 59.3 (C<u>H</u>$_3$O), 56.0 (SO_2<u>C</u>C), 31.7[a] (SO_2C<u>C</u>), 31.2[a] (S<u>C</u>C) and 16.2 (<u>C</u>H$_3$) ([a]assignment of signals may be reversed); *m/z* (low eV electron impact) 412 (M^+, 31%), 349 (31), 348 (100) and 290 (49) (Found: C, 58.5; H, 4.8; N, 6.6 $C_{21}H_{20}N_2O_3S_2.H_2O$ requires C, 58.6, H, 5.2; N, 6.5%).

A large scale hydrolysis of the 1,3-dithiane ring in the thioacetal (155) (1.01 g, 2.7 mmol) under analogous conditions afforded a 64% yield of the corresponding aldehyde (159), together with a 8% yield of the sulphone by-product (163c).

9-Methoxy-5-(methyliminomethyl)-11-methyl-6H-pyrido[4,3-b]carbazole (166).-Gaseous methylamine was bubbled through a cold (0 °C) stirred orange suspension of the aldehyde (159) (334 mg, 1.2 mmol) in dry benzene (150 cm^3) containing activated 3 Å molecular sieves. After 135 minutes the flow of gaseous methylamine was ceased (no carbonyl stretch in the Nujol mull i.r. spectrum) and the dark-orange solution sitrred for a further 1 hour in the cold (0 °C). The solution was filtered, the 3 Å molecular sieves collected, washed with dry benzene (100 cm^3), and the benzene filtrate evaporated under reduced pressure to afford the *imine* (166) (346 mg, 99%) as an orange solid, which was used without further purification, m.p. 161-163 °C, ν_{max} (Nujol) 1625 (C=N) and 1600 cm^{-1}; 270 MHz δ_H ($CDCl_3$) 11.65 (1H, brs, NH), 9.56 (1H, s, H-1), 9.20 (1H, q, J = 1 Hz, $CH_3N{=}C\underline{H}$), 8.43 (1H, d, J = 6 Hz, H-3), 8.00 (1H, d, J = 6 Hz, H-4), 7.71 (1H, d, J = 2.5 Hz, H-10), 7.34 (1H, d, J = 9 Hz, H-7), 7.08 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 3.88 (3H, s, $C\underline{H}_3O$), 3.64 (3H, d, J = 1 Hz, $C\underline{H}_3N{=}CH$) and 3.13 (3H, s, $C\underline{H}_3$).

Decouplings

Resonance (δ)	Decoupling effect
9.20, $CH_3N{=}C\underline{H}$	3.64, d → s

9-Methoxy-5-(methylaminomethyl)-11-methyl-6H-pyrido[4,3-b]carbazole (167).-Sodium borohydride (124 mg, 3.3 mmol) was added portionwise to a stirred orange solution of the imine (166) (380 mg, 1.3 mmol) in dry ethanol (75 cm^3) at room temperature under an atmosphere of dry nitrogen.

The resulting mixture was further stirred for 150 minutes, before removing the solvent under reduced pressure. Water (60 cm^3) was added to the resulting residue, which was then made acidic with concentrated hydrochloric acid. The resulting yellow solution was stirred for 5-10 minutes and basified with a saturated aqueous solution of sodium hydrogen carbonate. The mixture was extracted with chloroform (7 x 100 cm^3), the combined extracts dried ($MgSO_4$) and the solvent removed under reduced pressure. Column chromatography of the residue (eluted initially with dichloromethane/methanol/triethylamine 100:5:1 v/v, followed by dichloromethane/methanol/triethylamine 200:15:2 v/v) gave the *amine* (167) as a yellow solid (189 mg, 49%), m.p. 178-180 °C, t.l.c. R_F 0.16 (dichloromethane/methanol/ammonia (880) 125:8:1 v/v); ν_{max} (Nujol) 1600 cm^{-1}; λ_{max} (95% EtOH) 244 (ε 23450), 279 (36320), 294 (48760) and 335 nm (6000); 270 MHz δ_H ($CDCl_3$) 9.68 (1H, s, H-1), 8.44 (1H, d, J = 6 Hz, H-3), 7.88 (1H, d, J = 2.5 Hz, H-10), 7.86 (1H, d, J = 6 Hz, H-4), 7.41 (1H, d, J = 9 Hz, H-7), 7.16 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 4.53 (2H, s, $CH_3NC\underline{H}_2$), 3.97 (3H, s, $C\underline{H}_3O$), 3.28 (3H, s, $C\underline{H}_3$) and 2.58 (3H, s, $C\underline{H}_3NCH_2$) (indolic and secondary amino protons not observed): *m/z* (low eV electron impact) 305 (M^+, 11%) and 274 (16) (Found M^+, 305.1528. $C_{19}H_{19}N_3O$ requires M^+, 305.1528).

N,N'-Dimethyl-N,N'-bis(9-methoxy-11-methyl-6H-pyrido[4,3-b]carbazol-5-ylmethyl)hexanediamide (148).-Freshly distilled diphenylphosphoryl azide (0.20 cm^3, 256 mg, 0.93 mmol) was added to a stirred orange solution of the amine (167) (142 mg, 0.47 mmol), adipic acid (34 mg, 0.23 mmol), 4-dimethylaminopyridine (114 mg, 0.93 mmol) and 1-hydroxybenzotriazole (126 mg, 0.93 mmol) in dry dimethylformamide (20 cm^3) under a nitrogen atmosphere. The resulting mixture was cooled to 0 °C, dry triethylamine added dropwise (0.26 cm^3, 188 mg, 1.9 mmol), and then sealed under nitrogen and kept at -20 °C for 41 hours. After this

period of time the solution was allowed to warm to room temperature and stirred for 5 hours, before addition to a mixture of dichloromethane (80 cm^3) and 2 M hydrochloric acid (60 cm^3). After thorough mixing, the layers were separated and the organic phase further extracted with 2 M hydrochloric acid (5 x 80 cm^3). The combined 2 M hydrochloric acid extracts were basified with solid sodium hydrogen carbonate and extracted with chloroform (3 x 100 cm^3). The combined organic extracts were dried (Na_2SO_4) and solvent removed under reduced pressure (50 °C/0.05 mmHg). Purification of the resulting residue by flash chromatography (eluted initially with ethyl acetate/methanol/triethylamine 100:8:1 v/v, followed by dichloromethane/methanol/triethylamine 100:8:1 v/v) gave the title compound as a yellow solid (76 mg, 45%), which crystallised from ethyl acetate/dichloromethane as yellow microcrystals, m.p. 207-209 °C, t.l.c. R_F 0.4 (dichloromethane/methanol/triethylamine 100:2:1 v/v); ν_{max} (Nujol) 3180 (NH), 1615sh (C=O) and 1605 cm^{-1}; λ_{max} (95% EtOH) 243, 271, 292 and 334 nm; 270 MHz δ_H ($CDCl_3$) 10.40 (1H, brs, NH), 9.66 (1H, s, H-1), 8.44 (1H, brd, J = 6 Hz, H-3), 7.89 (1H, d, J = 6 Hz, H-4), 7.81 (1H, d, J = 2.5 Hz, H-10), 7.37 (1H, d, J = 9 Hz, H-7), 7.07 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8), 5.17 (2H, s, $CH_3NC\underline{H}_2$), 3.87 (3H, s, $C\underline{H}_3O$), 3.24 (3H, s, $C\underline{H}_3$), 2.82 (3H, s, $C\underline{H}_3NCH_2$), 2.33 (2H, m, $CH_3NCO-C\underline{H}_2CH_2$) and 1.67 (2H, m, $CH_3NCOCH_2C\underline{H}_2$); *m/z* (fast atom bombardment, ethylene glycol/0.1 M hydrochloric acid matrix) 721 (M^++1, 0.7%) (Found: C, 73.0; H, 6.1; N, 11.4. $C_{44}H_{44}N_6O_4$ requires C, 73.3; H, 6.2; N, 11.7%).

5-Formyl-9-hydroxy-11-methyl-6H-pyrido[4,3-b]carbazole (170).-To a stirred yellow solution of the aldehyde (159) (400 mg, 1.4 mmol) in dry dichloromethane (250 cm^3) under a dry nitrogen atmosphere was added 1.0 M boron tribromide (in dichloromethane 8.4 cm^3, 8.4 mmol). The resulting deep-red solution was sealed under nitrogen, and further stirred at

room temperature for 71 hours before removal of the solvent under reduced pressure. The residue was taken up in 2 M hydrochloric acid (100 cm^3), heated to gentle reflux for 30 minutes and the hot mixture then basified by addition to a saturated aqueous solution of sodium hydrogen carbonate. The resulting precipitate was collected by filtration, washed with a little water (2 x 20 cm^3) and dried over phosphorus pentoxide *in vacuo* to afford the *hydroxy-aldehyde* (170) as a yellow solid (342 mg, 90%), which was used without further purification, m.p. >350 °C, t.l.c. R_F 0.08 (dichloromethane/methanol 25:1 v/v); ν_{max} (Nujol) 3360 (NH), 1635 (C=O), 1610, 1600 and 1580 cm^{-1}; ν_{max} (CH_3CN) 3600 (OH), 3525 (NH) and 1620 vbr cm^{-1} (C=O, aromatic C...C); λ_{max} (95% EtOH) 236, 243, 260, 299 and 364 nm; λ_{max} (95% EtOH/NaOH) 247 and 321 nm; 270 MHz δ_H [$(CD_3)_2SO$] 12.15 (1H, brs, exchanged with D_2O, NH), 11.06 (1H, s, CHO), 9.75 (1H, s, H-1), 9.42 (1H, brs, exchanged with D_2O, OH), 8.73[a] (1H, d, J = 6 Hz, H-3), 8.58[a] (1H, d, J = 6 Hz, H-4), 7.79 (1H, d, J = 2.5 Hz, H-10), 7.68 (1H, d, J = 9 Hz, H-7), 7.07 (1H, dd, J_1 = 9 Hz, J_2 = 2.5 Hz, H-8) and 3.30 (3H, s, CH_3) ([a]assignment of signals to H-3 and H-4 may be reversed) (Found: C, 71.3; H, 4.4; N, 9.4. $C_{17}H_{12}N_2O_2.\frac{1}{2}H_2O$ requires C, 71.6; H, 4.6; N, 9.8%).

9-Hydroxy-5-(methyliminomethyl)-11-methyl-6H-pyrido[4,3-b]carbazole (172).-Gaseous methylamine was bubbled through a stirred orange suspension of the crude hydroxy-aldehyde (170) (1.20 g, 4.3 mmol) in dry methanol (250 cm^3) containing activated 3 Å molecular sieves. After 90 minutes the flow of gaseous methylamine was ceased (no carbonyl stretch in the Nujol mull i.r. spectrum) and the dark-red solution was stirred for a further 17 hours at room temperature. The solution was filtered, the 3 Å molecular sieves collected washed with dry methanol (160 cm^3), and the methanol filtrate evaporated under reduced

pressure to afford the *imine* (172) (1.21 g, 96%) as a red solid which was used without further purification, m.p. > 350 °C; ν_{max} (Nujol) 1625 (C=N), 1595 and 1580 cm^{-1}; 270 MHz δ_H [$(CD_3)_2SO$] 11.88 (1H, brs, exchanged with D_2O, NH), 9.71 (1H, s, H-1), 9.53 (1H, q, J = 1 Hz, $CH_3N{=}C\underline{H}$), 8.49-8.45 (3H, m, H-3, H-4, OH on addition of D_2O the integral decreased to that required for 2H, OH exchanged), 7.81 (1H, d, J = 2 Hz, H-10), 7.65 (1H, d, J = 8.5 Hz, H-7), 7.05 (1H, dd, J_1 = 8.5 Hz, J_2 = 2 Hz, H-8), 3.72 (3H, d, J = 1 Hz, $C\underline{H}_3N{=}CH$) and 3.27 (3H, s, $C\underline{H}_3$).

Sodium borohydride reduction of imine (172) → 2-Methylpyrido[3,4-h]-pyrimido[5,6,1-jk]carbazol-10-ol (173).-Sodium borohydride (0.51 g, 13.4 mmol) was added portionwise to a stirred red solution of the crude imine (172) (1.21 g, 4.2 mmol) in dry methanol at room temperature under an atmosphere of dry nitrogen. The resulting mixture was further stirred for 1 hour, after which t.l.c. analysis still revealed the presence of starting material (t.l.c. run initially in dichloromethane/methanol 50:3 v/v and then dichloromethane/methanol/triethylamine 100:4:1 v/v). The solution was then heated to gentle reflux, further portions of sodium borohydride added (2 g, 53 mmol) and refluxing continued for 1 hour (no starting material by t.l.c.). After allowing the solution to cool, the solvent was removed under reduced pressure and the residue made acidic with concentrated hydrochloric acid. The mixture was then basified with a saturated aqueous solution of sodium hydrogen carbonate and exhaustively extracted with chloroform (7 x 350 cm^3). The combined, dried extracts (Na_2SO_4) were evaporated under reduced pressure and the residue purified by column chromatography (eluted initially with dichloromethane/methanol 150:9 v/v, followed by dichloromethane/methanol/triethylamine 300:18:3 v/v) to give the *pentacycle* (173) as a light-yellow gum (216 mg, 17%), t.l.c. R_F

0.11 (dichloromethane/methanol 50:3 v/v); ν_{max} (Nujol) 1600 cm^{-1}; λ_{max} (95% EtOH) 252, 278, 297, 338 and 354 nm; λ_{max} (95% EtOH/NaOH) 259 and 309 nm; 270 MHz δ_H [$(CD_3)_2SO$] 9.68 (1H, s, H-7), 9.26 (1H, s, exchanged with D_2O, OH), 8.39 (1H, d, J = 6 Hz, H-5), 7.81 (1H, d, J = 2 Hz, H-9), 7.78 (1H, d, J = 6 Hz, H-4), 7.44 (1H, d, J = 8.5 Hz, H-12), 7.08 (1H, dd, J_1 = 8.5 Hz, J_2 = 2 Hz, H-11), 5.09 (2H, s, $NC\underline{H}_2N$), 4.40 (2H, s, $CH_3NC\underline{H}_2$), 3.22 (3H, s, $C\underline{H}_3$) and 2.51 (3H, s, $C\underline{H}_3NCH_2$).

10-*(Dimethyl*-t-*butylsilyloxy)*-2-*methylpyrido[*3,4-*h]pyrimido[*5,6,1-*jk]carbazole* (174).-To a stirred solution of the pentacycle (173) (82 mg, 0.27 mmol) in dry dimethylformamide (5 cm^3) under a dry nitrogen atmosphere was added sodium hydride (60% dispersion in oil) (86 mg, 3.6 mmol). The mixture was stirred at room temperature for 30 minutes and then chlorodimethyl-*t*-butylsilane (606 mg, 4.0 mmol) added. The resulting mixture was further stirred at room temperature for 5 hours, before addition to water (30 cm^3) and extraction with chloroform (3 x 50 cm^3). The combined, dried extracts (Na_2SO_4) were evaporated under reduced pressure (50 °C/0.1 mmHg) to give a red gum, which was purified by column chromatography (eluted with dichloromethane/methanol 25:1 v/v) to afford the title compound as a yellow gum (44 mg, 39%), t.l.c. R_F 0.32 (dichloromethane/methanol 50:3 v/v); ν_{max} ($CHCl_3$) 1605 cm^{-1}; λ_{max} (95% EtOH) 277, 296, 336 and 352 nm; 270 MHz δ_H ($CDCl_3$) 9.65 (1H, s, H-7), 8.42 (1H, d, J = 6 Hz, H-5), 7.80 (1H, d, J = 2 Hz, H-9), 7.58 (1H, d, J = 6 Hz, H-4), 7.18 (1H, d, J = 8.5 Hz, H-12), 7.09 (1H, dd, J_1 = 8.5 Hz, J_2 = 2 Hz, H-11), 4.96 (2H, s, $NC\underline{H}_2N$), 4.35 (2H, s, $CH_3NC\underline{H}_2$), 3.19 (3H, s, $C\underline{H}_3$), 2.52 (3H, s, $C\underline{H}_3NCH_2$), 1.06 [9H, s, $(CH_3)_3$] and 0.27 (6H, s, 2 x $SiC\underline{H}_3$); partial 67.8 MHz δ_C ($CDCl_3$) 64.2 ($N\underline{C}N$), 49.6 ($N\underline{C}H_2$), 42.0 ($\underline{C}H_3NC$), 25.8 [$C(\underline{C}H_3)_3$], 18.3 [$\underline{C}(CH_3)_3$], 14.6 ($\underline{C}H_3$) and -4.3 ($Si\underline{C}H_3$); *m/z* (70 eV electron impact) 418 (M^++1, 9%), 417 (M^+, 27), 375 (17), 374 (53), 318 (16) and 317 (43) (Found:

M^+, 417.2191. $C_{25}H_{31}N_3OSi$ requires M^+, 417.2234).

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