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# APPROACHES TO THE SYNTHESIS OF CAMPTOTHECIN

by

Woldeab Isaac

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Medicinal Chemistry)
in The University of Michigan
1972

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To my parents
to whom I owe a debt of gratitude
I can never adequately repay.

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# I. INTRODUCTION

The two major criteria that limit therapeutic effectiveness of anticancer agents are a lack of tissue selectivity and the development of cell lines that are resistant to inhibitory effects. Most of the agents used today can be thought of as inhibiting preferentially cell divisions of rapidly dividing clones of cells. However, tumor cells are not always rapidly proliferating and, besides, the presence of rapidly proliferating normal cells (intestinal mucosa, bone marrow, etc.) limit distinction between cancerous and normal cells. The ultimate achievement of highly selective drugs awaits an insight leading to a qualitative difference in the biochemistry between the two.

In the absence of such fundamental insight, compounds from natural sources have not only provided useful drugs but are also serving as probes into understanding the metabolism and biochemistry of the disease, opening up the way for the design of better and more effective drugs. The use in medicine of drugs derived from plant origin goes back to antiquity. When one considers natural products such as morphine, digitalis, ergot, cocaine, quinine, reserpine, vincaleukoblaskne - just to cite a few, it is more than evident how great is the debt of medicine to plant derived drugs even today.

Camptothecin, the unusual quinoline alkaloid, was isolated from a plant native to China, Camptothececa accuminata, by Wall and coworkers in 1966. Its remarkable activity in L1210 leukemia and its very promising results in preliminary clinical studies, coupled with the great

difficulty in obtaining it, generated a great deal of interest in developing a laboratory synthesis of the alkaloid.

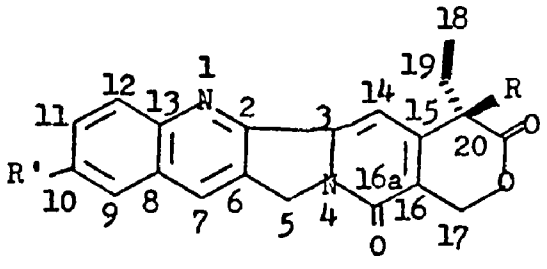
This thesis is primarily concerned with the development of various approaches to its synthesis, and these will be discussed in detail. The chemistry, pharmacology and biochemistry of the alkaloid will also be reviewed in order to provide background information and emphasize its medicinal potential.

## II. BACKGROUND

### A. CHEMISTRY

#### 1. Isolation and Structure Determination

Camptothecin (1) a novel pentacyclic quinoline alkaloid was first isolated by Wall and coworkers.[1] The dried wood was continuously extracted with hot hexane-heptane solvent followed by a similar extraction with 95% ethanol. The ethanol extract was partitioned between chloroform and water. Silica gel chromatography of the methanol-insoluble material from the chloroform extract followed by crystallization from methanol-acetonitrile gave camptothecin as pale yellow needles: $M^+$ at m/e 348.17 calcd for $C_{20}H_{16}N_2O_4$, 348.1111; mp 264-267° dec.; $[\alpha]_D$ +31.3° (chloroform-methanol 8:2); $\lambda_{max}$ 220 mμ (τ 37,320), 254 (29,230), 290 (4980) and 370 (19,900); $\nu_{max}$ 3440 (hydroxy), 1760-1745 (lactone), 1660 (lactam), and 1610, 1585 $cm^{-1}$ (aromatic c=c); nmr (60 MHZ $CD_3SOCD_3$): 0.91 δ (3H, triplet, $-CH_2\underline{CH_3}$), 1.90 (2H, multiplet, $\rangle COH\underline{CH_2}CH_3$), 5.45 (2H, singlet, Ar-$\underline{CH_2}$-O), and 5.28 (2H, S Ar-$\underline{CH_2}$-O), and 5.28 (2H, S Ar-$\underline{CH_2}$-N), aromatic proton signals were poorly resolved.




1, R = OH,

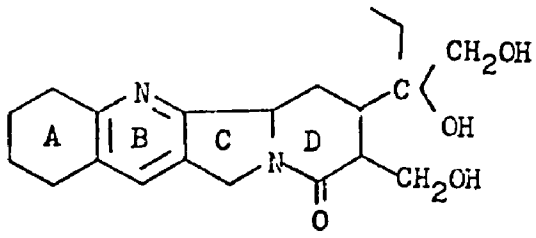
2, R = OAc

3, R = $-O-\overset{O}{\overset{\|}{C}}-CH_2Cl$

4, R = $O-\overset{O}{\overset{\|}{C}}-CH_2I$

5, R = Cl

The alkaloid does not form stable salts, with acids; however its lactone portion is fairly reactive and several derivatives (2 → 5) have been prepared and characterized. Prolonged treatment of 1 with acetic anhydride-pyridine yielded 2 and treatment of 1 with chloroacetic anhydride yielded 3. The iodo acetate 4 was prepared from 3 and sodium iodide in acetone, and 5 was obtained from the reaction of 1 with thionyl chloride and pyridine. Exhaustive hydrogenation of 1 in acetic acid with Adams catalyst gave deodecahydro derivative 6. Most of these derivatives have also been tested for biological



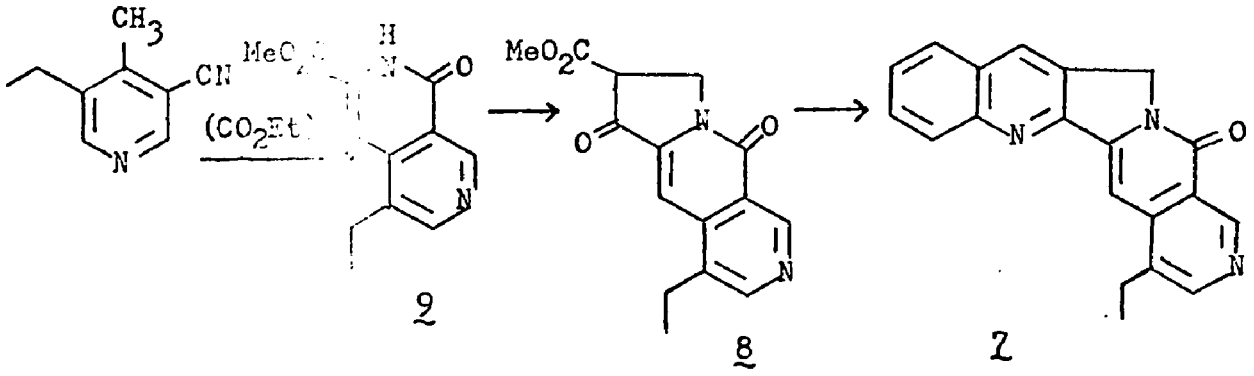

6

activity.[31] Deductions from the detailed X-ray analysis of the iodo acetate derivative 5 established the structure and absolute stereochemistry of camptothecin as 1.[2]

## 2. Synthesis

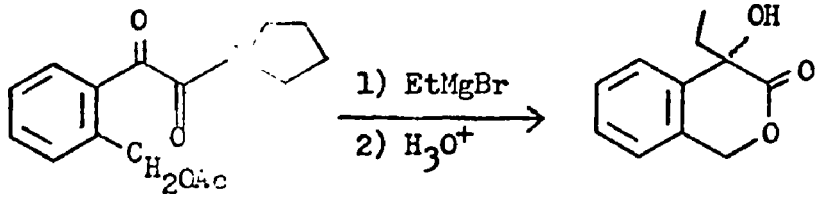
Camptothecin's remarkable biological activity against L 1210 leukemia and very promising preliminary clinical results, coupled with the great difficulty in obtaining the alkaloid from natural sources, generated considerable enthusiasm for assembling the compound in various laboratories. Very recently, various synthetic approaches and several total syntheses of the alkaloid have been reported in the literature.

Hoping that 7 would be a potential precursor to camptothecin, Wenkert[3] reported the synthesis of the heteropentacycle 7. Potassium t-butoxide induced condensation of 4-methyl-5-ethylnicotinonitrile with dimethyloxalate to 9. Condensation thereof with methylacrylate produced 8 which after decarboxylation and Friedlander condensation with o-nitrobenzaldehyde gave 7.



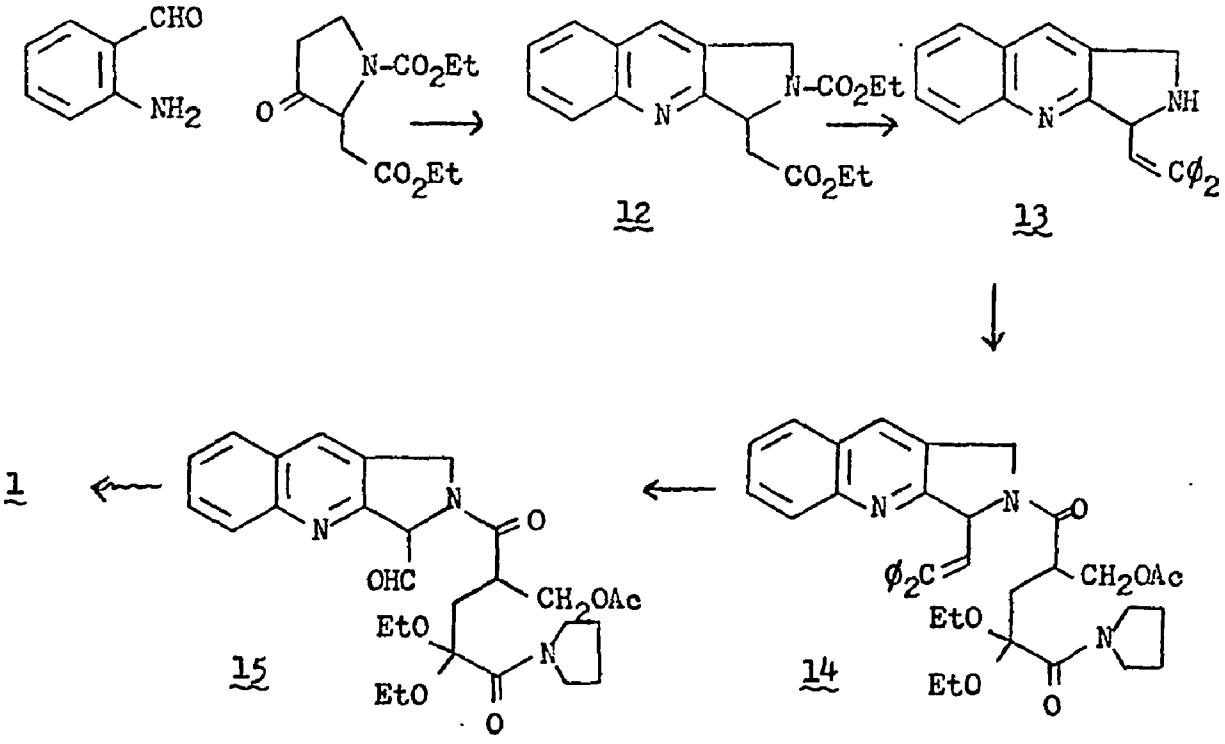

9 8 7

Wall[4] and coworkers felt that a successful synthesis of the alkaloid would have to make provision for the synthesis of the labile lactone at the final step. Following a model reaction that they developed in the conversion of 10 to 11, they pursued an approach outlined below. They were able to carry out successfully the reaction sequence

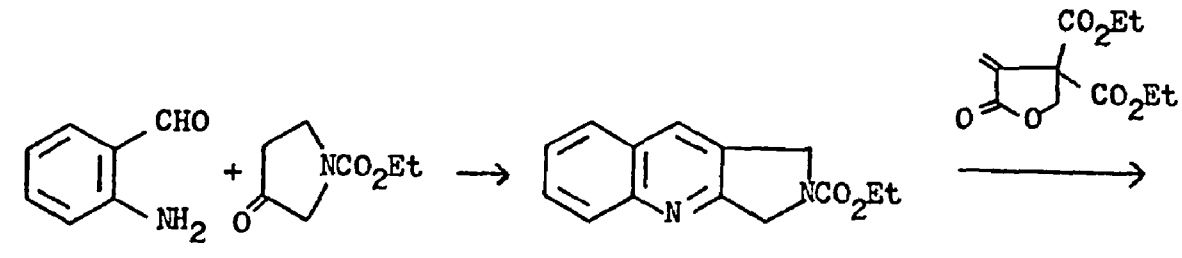



10 11

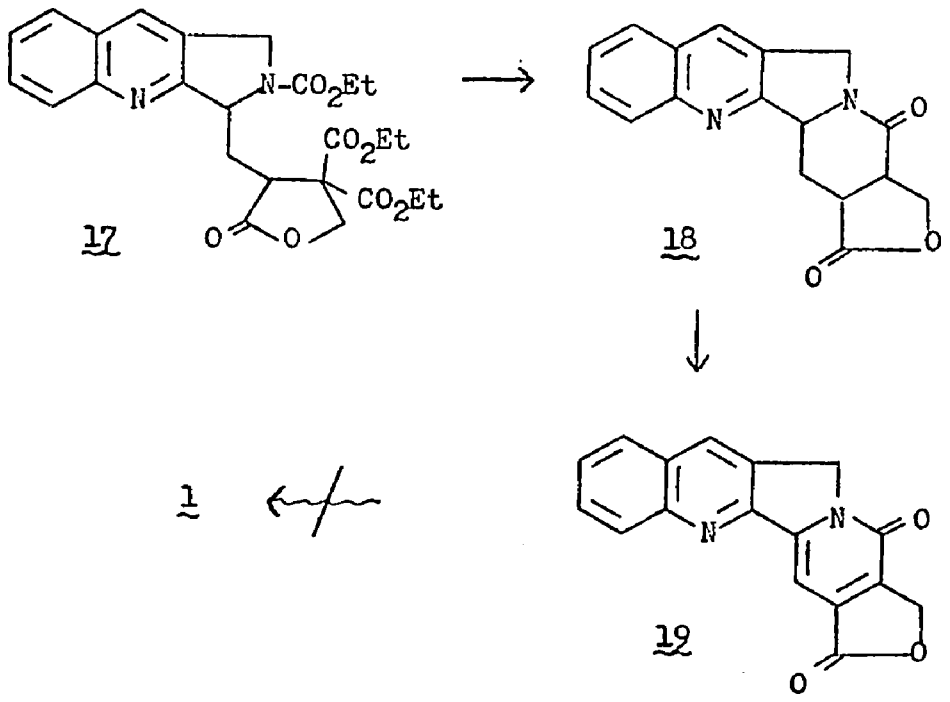
up to 14; however various conditions and oxidation procedures failed to convert 14 to the multifunctional compound 15. In 1970, they




reported[5] a different approach. This involved the Michael condensation of a pyrroloquiniline derivative 16b with an α-methylene-β,β-diethoxycarbonyl-γ-butyrolactone as the key reaction. The reaction sequence up to 19 was successful, but any success in converting 19 to 1 has not been reported.




16b




17 18 19 1

As a further modification of the reactions already investigated, Wall[6] altered the Michael adduct to methyl 2-carbomethoxy-3-methylene-4-ketohexanoate so as to include all the functionalities necessary for the E ring. Condensation of 16 with methyl 2-carbomethoxy-3-methylene-4-ketohexanoate gave 20 and treatment of 20 with sodium cyanide and hydrochloric acid gave 21, this in turn was treated with strong base (NaH) and methyl chloromethyl ether to give 22. Finally 22 was successfully converted to camptothecin (Figure 1).[7] No yields were given thus the efficiency of the synthesis probably cannot be compared with other syntheses of camptothecin.

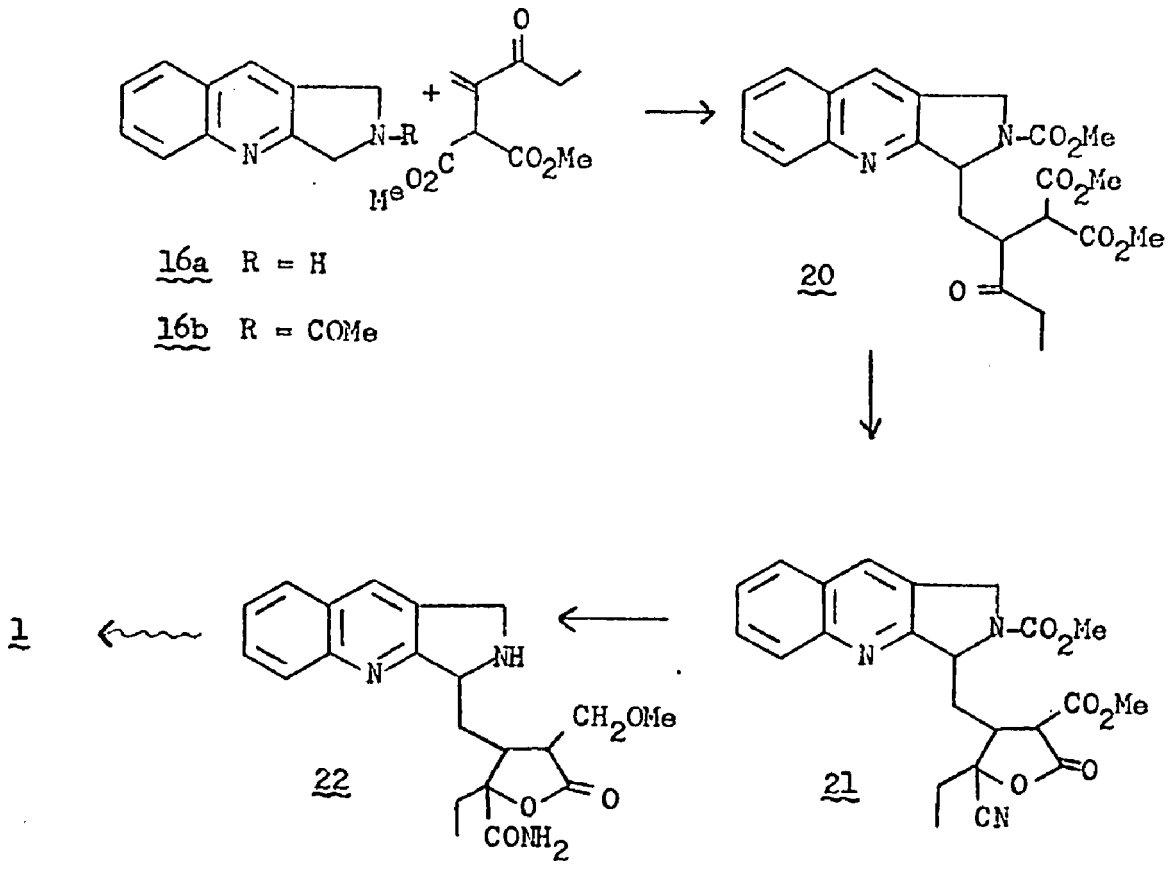
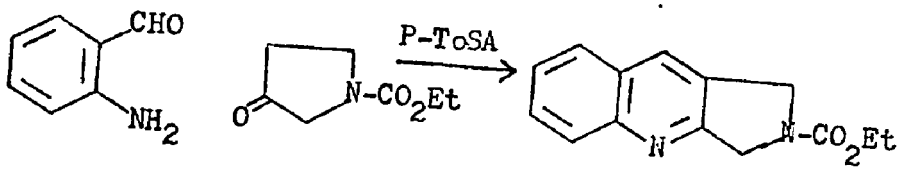
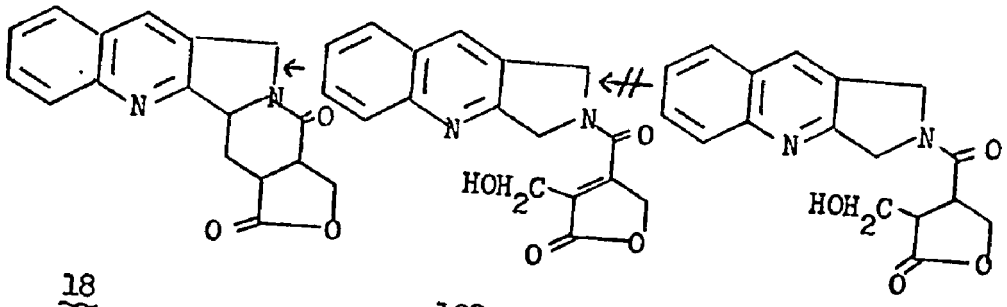



Figure 1. Wall's synthesis of camptothecin

An efficient synthesis of N-carbethoxy-1,3-dihydro-2H-pyrrolo-(3,4-b)quinoline 16b through the condensation of N-carbethoxy-3-pyrrolidone and o-aminobenzaldehyde in the presence of p-toluenesulfonic acid was reported by Zalkow.[8,9] Attempts were made to utilize 16b in the synthesis of camptothecin through the conversion of 16a to 197. However, all attempts to transform 197 either to 198 or to 18 were unsuccessful.

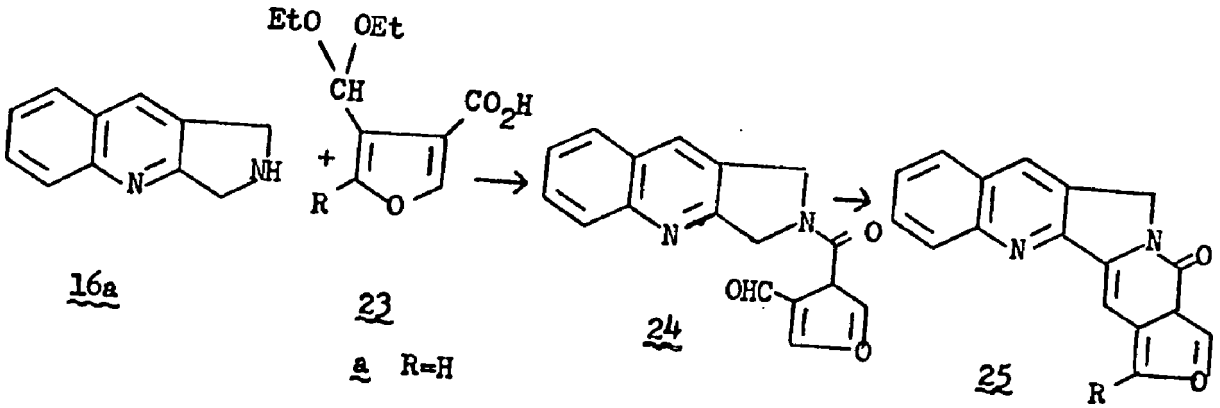



16b




18 19a 19

Kende[10] attempted the synthesis of the alkaloid through the pentacyclic intermediate 25 where the E ring of camptothecin is replaced by a furan ring. However, all efforts to open the furan ring and modify it to the desired lactone ring were fruitless. Compound 25 was obtained from the reaction of 16a with 23 by means of dicyclohexylcarbodiimide to form the amide 24 followed by potassium acetate catalysed cyclization of the aldehyde.



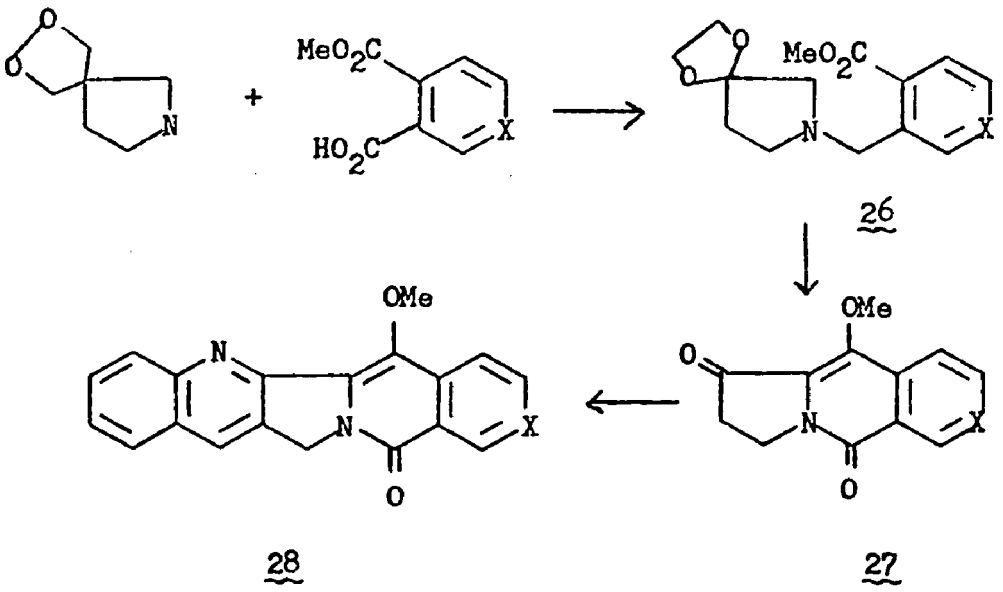

16a 23 24 25

a R=H

b R=Et

1

Shamma and coworkers[11,12] successfully incorporated the ABCD ring system of camptothecin; however, ring E was an aromatic or hetero aromatic group, instead of a lactone. Several E ring aromatic analogues were prepared but no provision nor effort was reported to incorporate the lactone portion of the molecule. 3-Pyrrolidinone ethylene ketal was converted to the ketal amido ester 26. Mild hydrolysis followed by cyclization in polyphosphoric acid gave the hydroxypyridone 27. The pentacyclic analogue of camptothecin was then derived by condensation of 27 with o-aminobenzaldehyde.



26

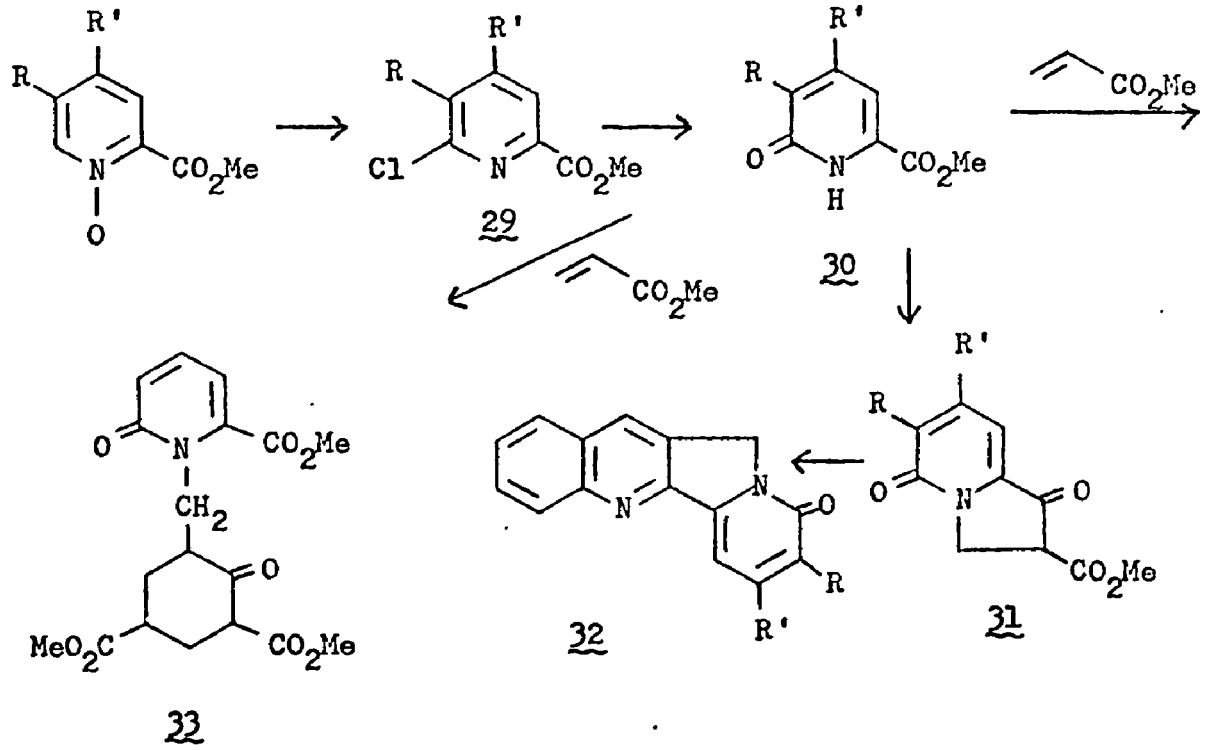
28 27

X = N

= C

Kametani's[13-15] approach utilized a simple picolinic acid for the construction of the ABCD ring system of the molecule. Treatment of methyl picolinate N-oxide with phosphorus oxychloride gave 29a which after hydrolysis and esterification led to 30a. The bicyclic compound 31a was obtained from the reaction of 30a with methyl acrylate;

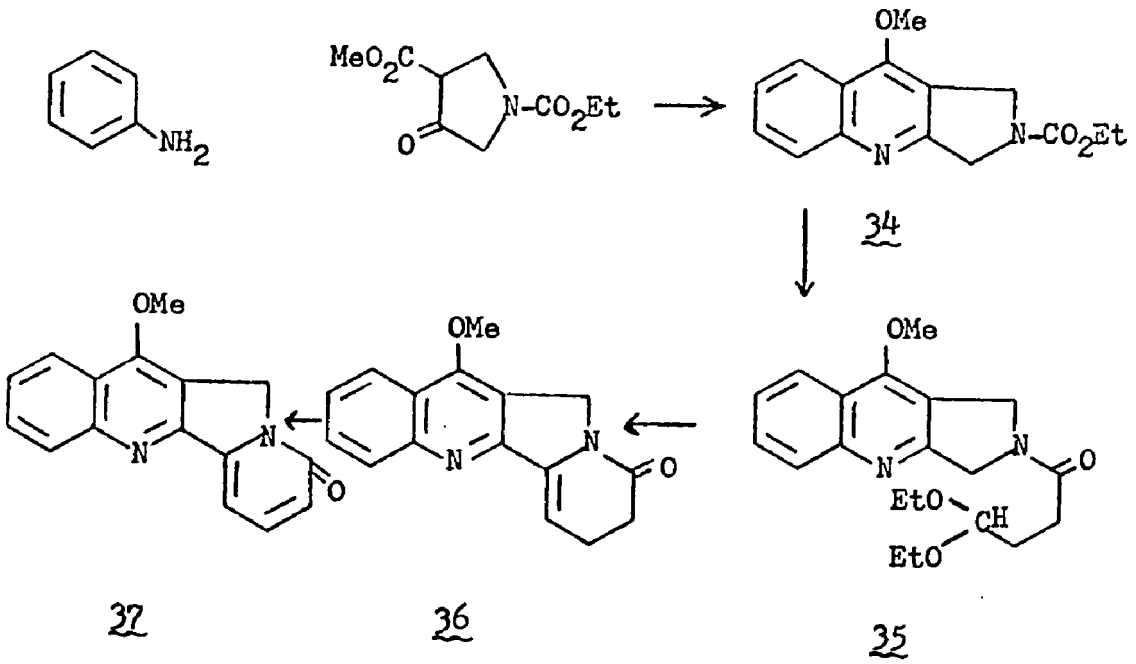
decarboxylation followed by the Friedlander condensation of 31a with o-aminobenzaldehyde gave the tetracyclic derivative 32a. When the substituted pyridone 30b was used in the same reaction sequence, none of the desired bicyclic compound 31b was obtained; instead, 33 was the product isolated.



a, R = H  R' = H  b, R = $CO_2Et$  R' = CN

Another method of constructing the ABCD ring system of camptothecin involves the condensation of aniline with 3-oxopyrrolidine derivative to give the tricyclic compound 34.[16] Condensing the free base of 34 with β-formylpropionic acid diethyl ketal gave amide 35 which was cyclized to 36 by means of acetic anhydride. The final product 37 was obtained by treating 36 with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. This approach provides however, no added advantage

over Kametani's. Furthermore, it seems it would be more difficult to incorporate the E ring.




A biogenetically patterned route to the camptothecin chromophore involving an efficient conversion of an indole system to quinolones has been reported.[17-19] The key reaction in this approach, i.e. the conversion of the indole to quinolone, is achieved in one step through a base catalysed autoxidation ($KOtBu/O_2$) in good yield. Winterfeldt autoxidized 41 and converted it to 42 which was chlorinated and reduced to give 43. Selective reduction of 43 using diisobutylaluminum hydride afforded 44, which after alkylation and cupric chloride catalysed oxidation was successfully converted to D,L-camptothecin (Figure 2).[105]

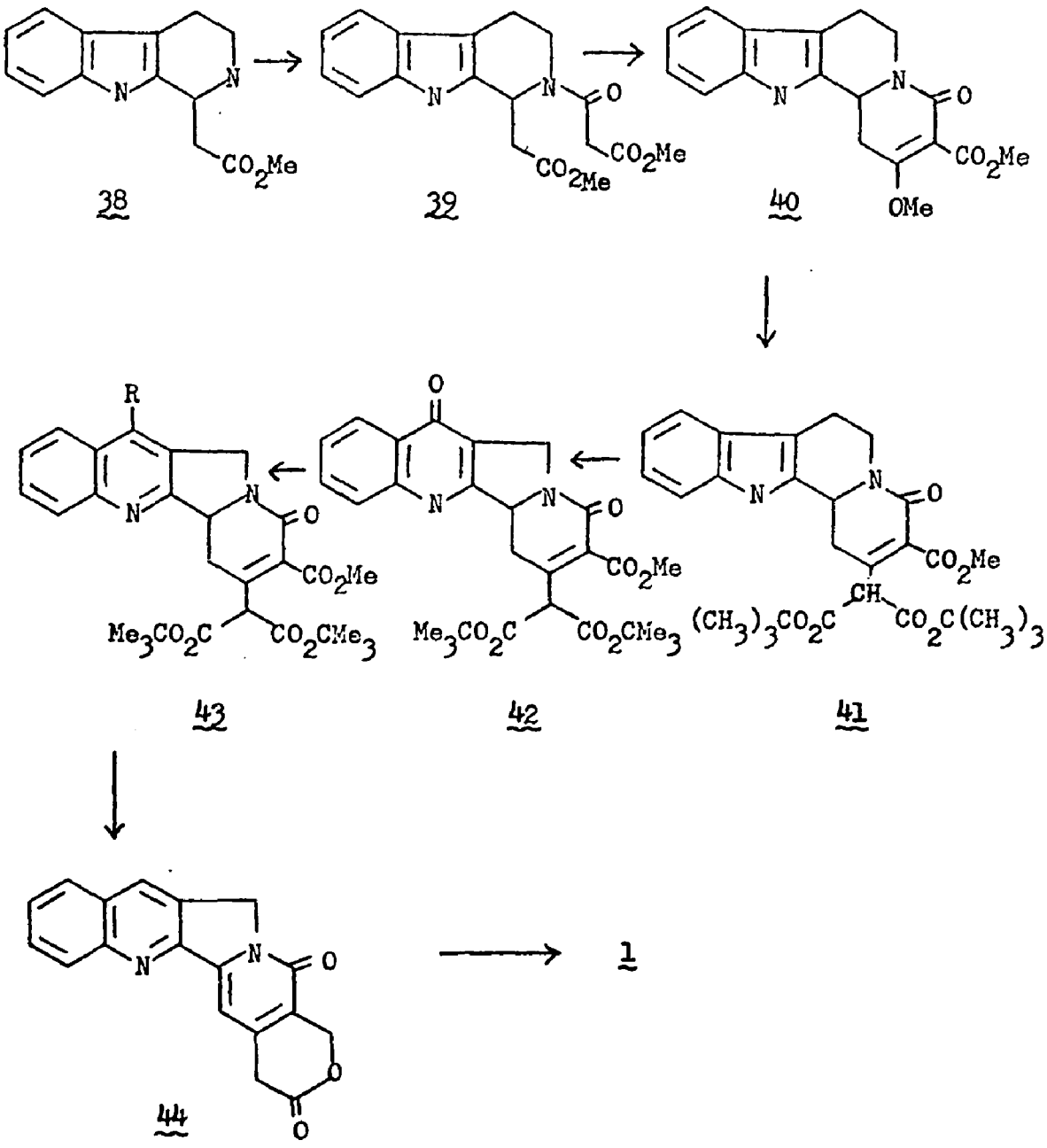
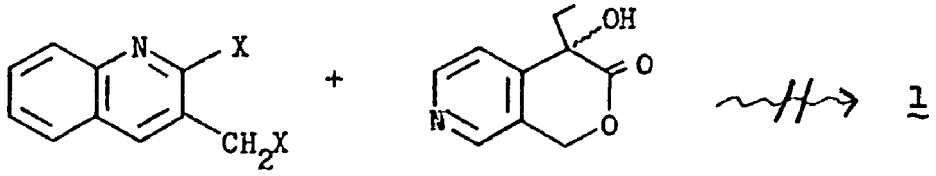


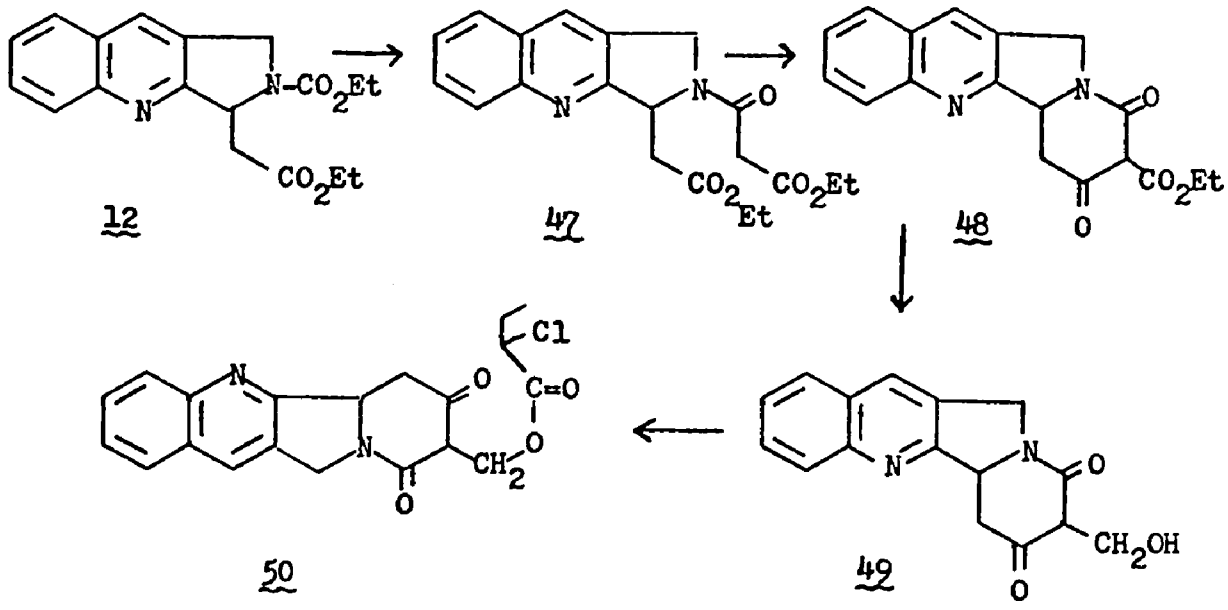

**Figure 2.** Winterfeldt's synthesis of camptothecin

In a short communication, Lyle[20] reported an approach that would involve a photochemical coupling of 45 and 46; however, no success has been reported. Cheng[21] constructed the ABCD ring system by means of procedures similar to those already described; however, attempts



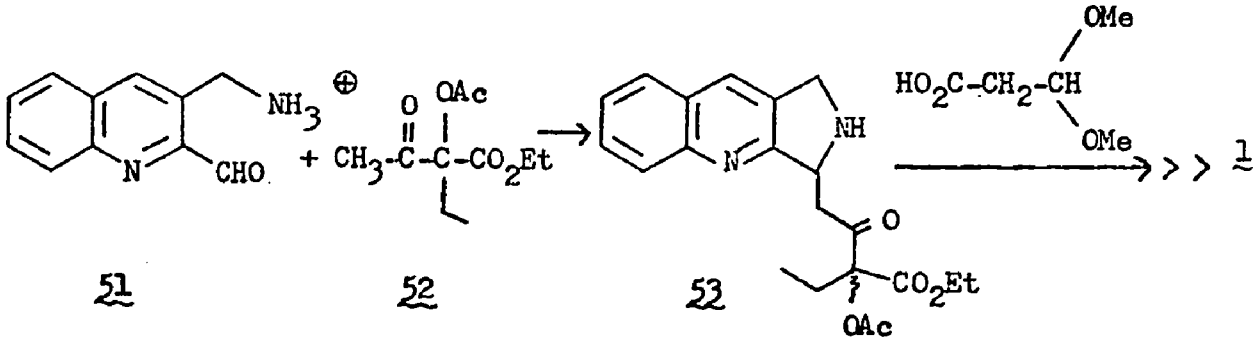
46 45

to construct ring E were unsuccessful. Hydrolysis of 12 followed by condensation of the half ester of malonic ester gave 47, Dieckmann cyclization of which led to 48. Reduction of the ester of 48 followed by esterification with the appropriate acid chloride led to 50. All the reactions are claimed to proceed in good yield, and 49 was also converted to various other esters that might possibly simulate the biological action of camptothecin.

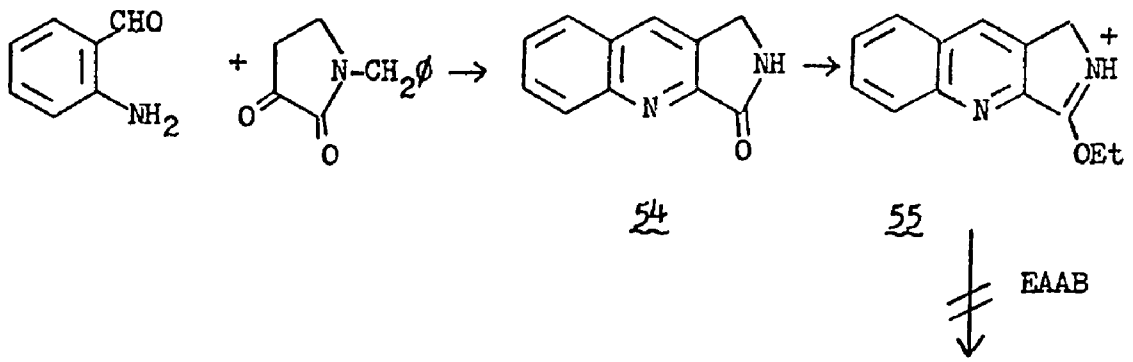


12 47 48 50 49

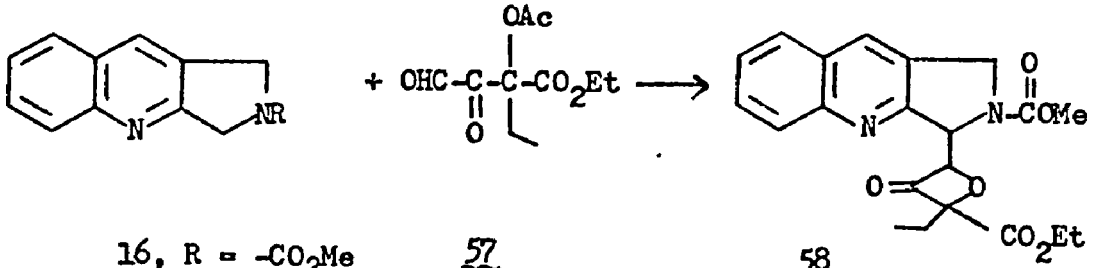
Burckhalter and coworkers investigated various approaches to the synthesis of camptothecin which center around the key intermediate 52, ethyl α-acetoxy butyrate (EAAB) which incorporates all the necessary functionalities for both rings D and E. The first approach[101] involved an intramolecular Mannich type reaction of EAAB and the aldehyde 51 so as to obtain 53. The reaction of which with dimethoxy acetal of 3-al-propionic acid followed by aldol cyclization, reduction and lactonization would have led to 1. However, numerous attempts to prepare the aldehyde 51 were unsuccessful.




As a modification of the foregoing scheme,[102] attempts were made to utilize the synthetically more accessible lactam 54. The desired lactam was prepared through the acid catalysed condensation of o-aminobenzaldehyde and N-benzyl-2,3-dioxopyrrolidine. The lactam 54 was converted to the enol ether 55 by treatment with triethyloxonium fluoroborate (Meerwein reagent); however, further attempts to condense 55 with EAAB proved fruitless.



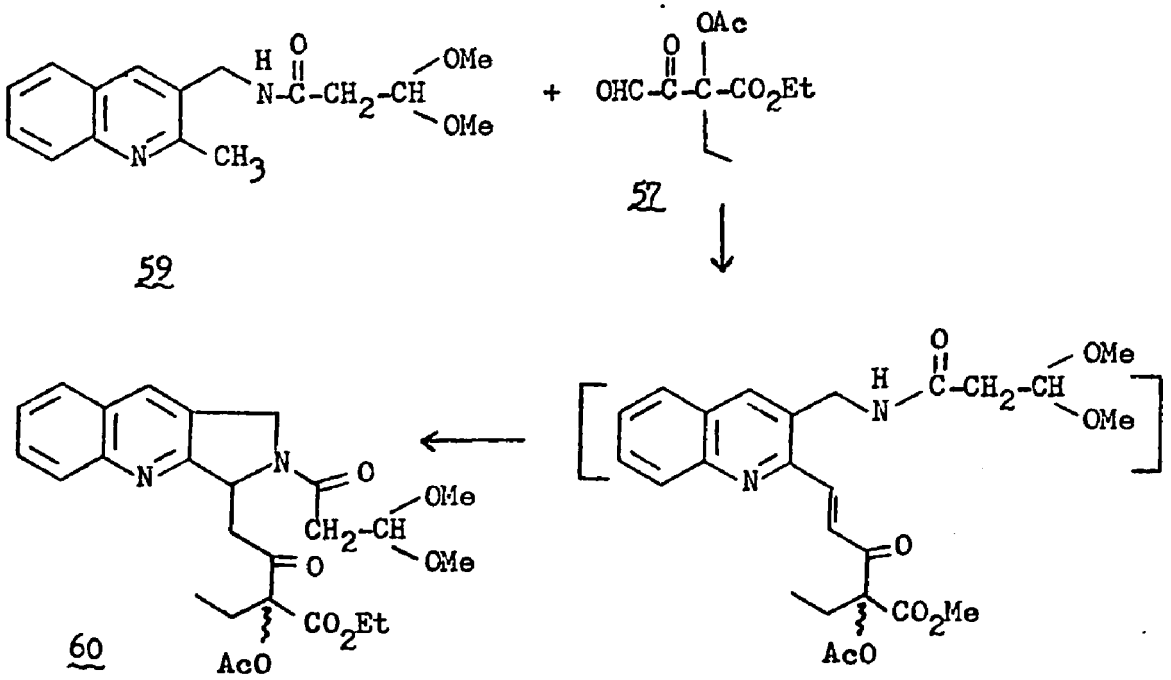

In a further synthetic effort, Burckhalter[103] et al. converted EAAB into ethyl α-glyoxyl-α-acetoxybutyrate 57 through an oxidation sequence involving p-nitrosodimethylaniline. Acetic anhydride catalyzed condensation of 16 with 57 gave 5% yield of 58; however, all efforts to condense the properly functionalized compound 56 with 57 failed to give any condensed material. Attempts[103] were also made to condense 59 or




16, R = $-CO_2Me$ 57 58

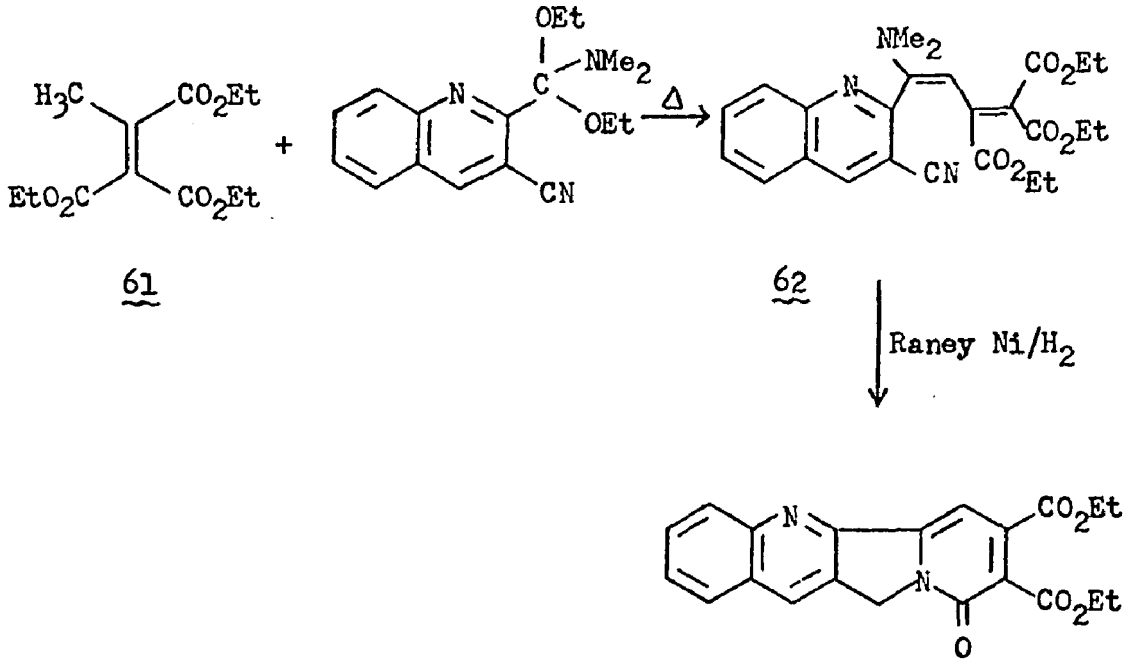
56, R = $-\overset{O}{\overset{\|}{C}}-CH_2CH_2OBz$

its N-oxide with 57 under various basic catalysts but no traces of 60 nor of the styryl intermediate could be obtained.



Many other approaches to the synthesis of camptothecin,[22,23] to naphthalene A/B camptothecin[24] and to the D and E ring system of the alkaloid[25,26] have also been reported. Borch[104] recently reported the acylation of 3-carbalkoxycitraconic ester 61 with amide acetals to obtain in good yield dimethylaminoalkylidene malonate 62, and, upon treatment with the primary amine derived from 62, cyclized to the properly substituted pyridone 63. This procedure has successfully been utilized to construct the ABCD ring system. However, no success in the construction of the final E ring has been reported. Conversions on the average were poor, with 62 → 63 proceeding in only 11% yield.

Very recently, the total synthesis of D,L-camptothecin has been achieved independently in the laboratories of Stork and Daniskefsky. Stork[27] utilized a new and ingenious annelation of an α,β-unsaturated lactam. He effected the fusion of a lactone ring 66 by addition-cycliza-



61 62 63

tion of the enolate of a carbonate of an α-hydroxy ester to an α,β-unsaturated lactam 65. Such an annelation allowed the introduction of all the necessary atoms of the camptothecin E ring (Figure 3).

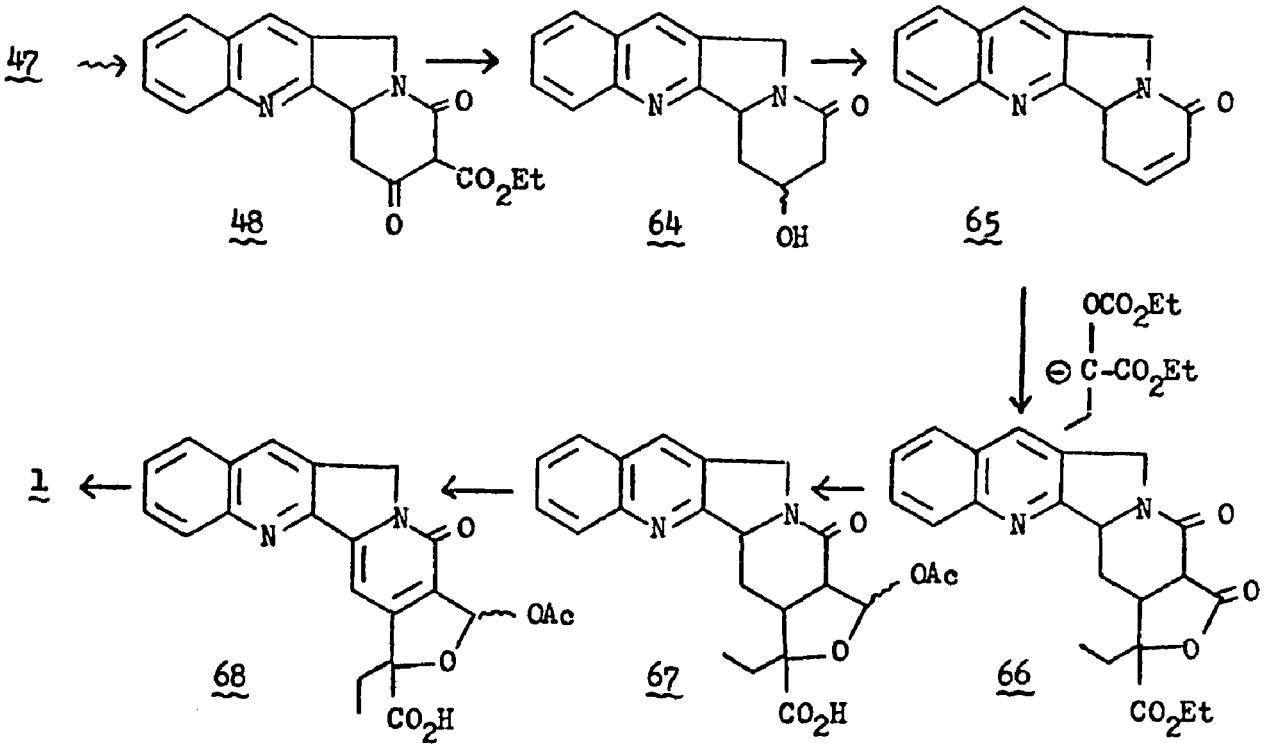


Figure 3. Stork's synthesis of camptothecin

Hydrolysis followed by reduction of 66 gave 67, which after aromatization with palladium on carbon led to 68. Sodium borohydride reduction of 68 followed by lactonization led to D,L-camptothecin.

Danishefsky's group[28-30] approached the problem by developing a new α-pyridone synthesis through the condensation of 1,3-dicarboalkoxyallene 69 with equimolar amounts of ethyl trans-β-aminoacronate 70. This synthesis has been successfully applied in the total synthesis of D,L-camptothecin as in Figure 4. It should be mentioned that this scheme not only involves numerous steps but also low yield reactions.

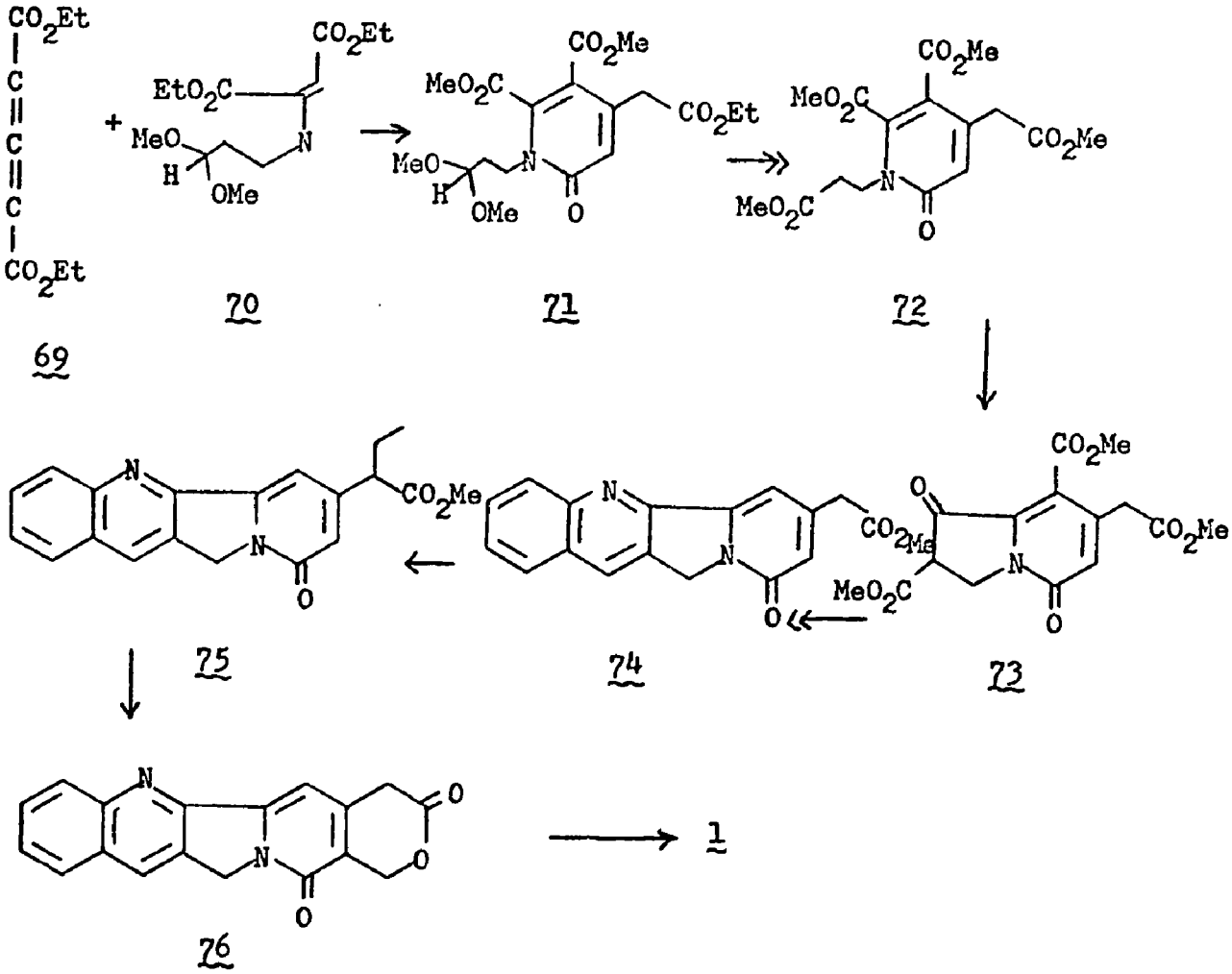



Figure 4. Danishefsky's synthesis of camptothecin

## B. BIOSYNTHESIS

The alkaloids quinine and cinchonine were the first quinoline alkaloids to be isolated from nature (in 1820). Since then numerous substances with a quinoline ring system have been isolated from higher plants, animals, fungi and bacteria. The fundamental role of the quinoline alkaloids - for that matter, of alkaloids as a whole - is not known[32] but attempts have been made to relate alkaloid formation with protein synthesis,[34,35] carbohydrate metabolism,[36,33] and the age of the plant. Other propositions claim protective means of plants against insects. Any progress in this area will most likely influence the current status of drug design.

A significant portion of the quinoline alkaloids are derived from anthranilic acid and acetate[37,38] to give the quinoline moiety, and further condensation with an isoprenoid[39-41] unit or with m-tyrosine[42-44] or phenylalanine yield various types of quinoline alkaloids. Camptothecin differs from these alkaloids in that, like quinine and cinchonine, it is believed to be biogenetically very closely related to the indole alkaloids. Speculation[45,19,3,2] involves the origin of its unique ring system from tryptophan and a $C_{10}$ terpenoid fragment namely loganin. These two form the building blocks of all indole alkaloids and the mechanistic details in which they give rise to the various classes of indole alkaloids has been clarified mainly through the work of Battersby, Angoni and Scott.[46,47] The quinoline portion of camptothecin is considered to be derived from an oxidative cleavage of the 2,3 carbon bond of the indole molecule followed by transannular cyclization to give the quinoline moiety. Such a transformation is both biologically precedented[48] and

chemically feasible.[49,50] The mechanistic details and sequences of the transformations involved in the biosynthesis of camptothecin are yet to be worked out; however, the gross biosynthetic path can be summarized as in Figure 5 with the sequences of the transformations not necessarily in the correct order.

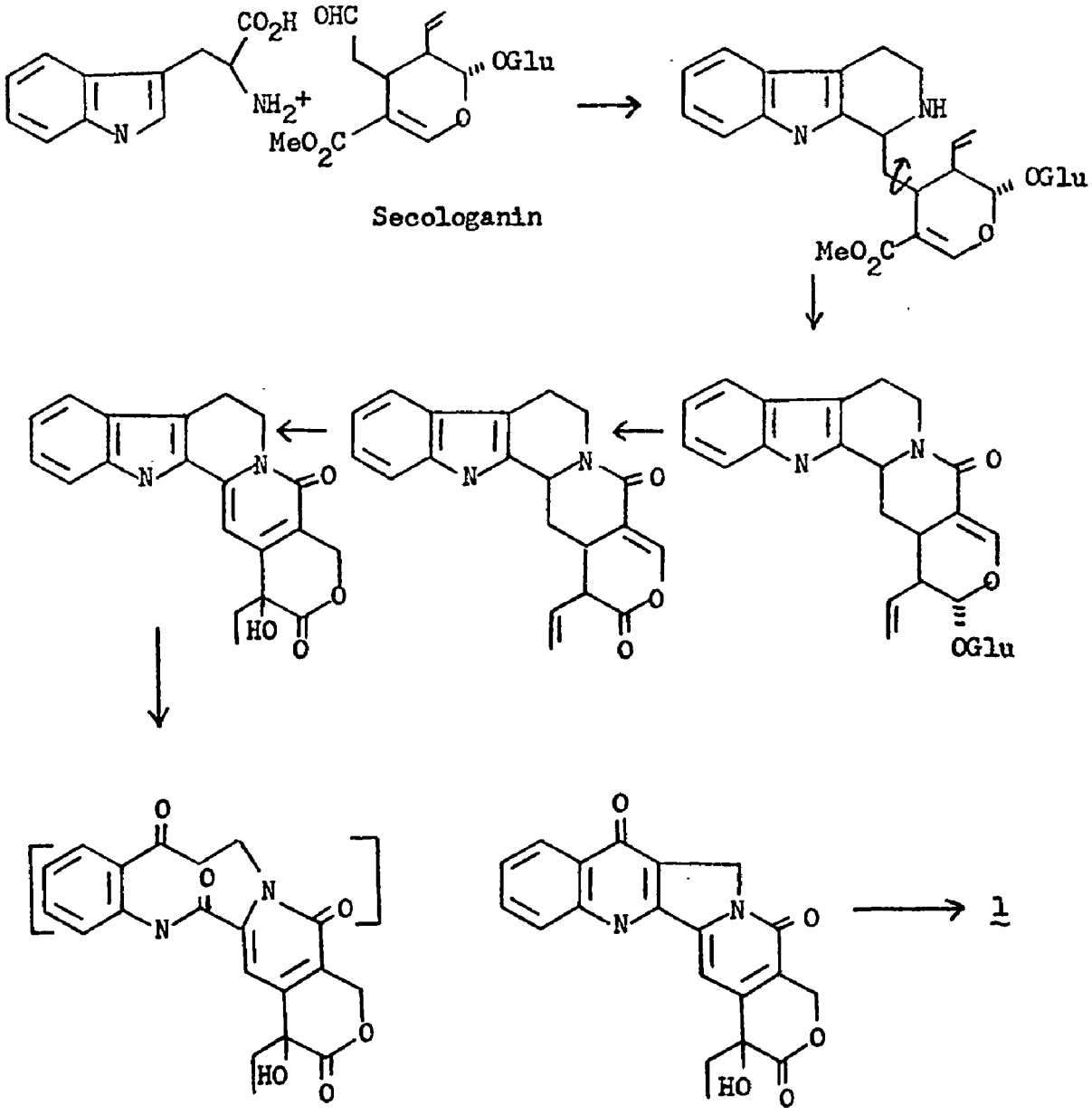



Figure 5. Biosynthesis of camptothecin

## C. MECHANISM OF ACTION

### 1. Relation to Other Anticancer Agents

The E ring of camptothecin is made up of an α-hydroxy-$\delta$-lactone which if dehydrated would yield and α,β-unsaturated lactone. Though a detailed structure-activity relationship of camptothecin is not yet available; preliminary results from the ABCD ring analogs of the alkaloid[51] and also molecular orbital calculations[52] tend to indicate the lactone portion of the molecule to be essential for biological activity. Furthermore, it is now well known that compounds containing the unsaturated lactone function exhibit a broad range of biological and pharmacological properties,[53] e.g. selective inhibition of growth and germination, antibiotic, cardiac, insecticidal, carcinogenic[54] and carcinostatic[1,55-58] activities.

Study of lactones as carcinogens started with the important observation that repeated subcutaneous injection of β-propiolactone produced sarcoma at the site of injection.[59] The first systematic and careful survey of a variety of saturated and unsaturated lactones, many of which were derived from natural sources, was performed for carcinogenic activity by Dickens.[54,60-64] Their structure-activity studies revealed that α,β unsaturation is the key structural feature for activity (Table I), with some resemblance to the lactones with antitumor activity (Table II).

The bacteriostatic and fungistatic activity of α,β unsaturated lactones are largely abolished by incubation with thiol groups. Thus, interaction with essential sulfahydryl groups in bacterial metabolism

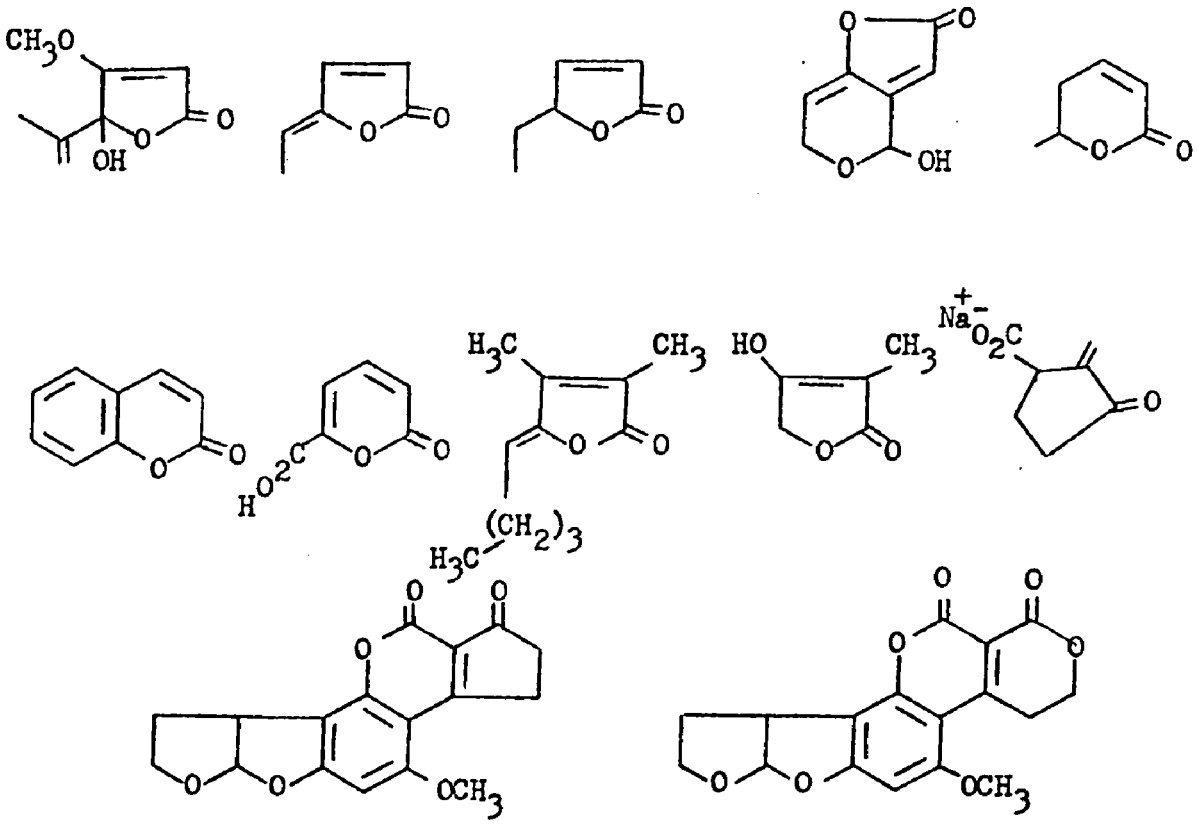
has been proposed as their possible mode of action.[65] Studies[66,67] of the reaction of these lactones with cysteine showed that the thiol in preference to the amine added across the double bond in a Michael fashion. Attempts to correlate the rate of reaction with the potency of the lactone, however, proved unsuccessful.[63]

The biological function of proteins as measured by the inhibition of enzymatic action, for example, does not appear to be very sensitive to alkylation,[68] whereas the inactivation of bacteriophage $T_2$ and $T_4$, which is due to alkylation of double stranded DNA,[69] requires only a low concentration of alkylating agent. Thus, it is not unreasonable to ascribe the cytotoxic effects of alkylating agents to their effects on DNA rather than on protein. The greater cytotoxic action of bifunctional agents is attributed to their ability to cross-link the twin molecular chains of double-stranded DNA, thus inhibiting their separation which is necessary for cell division.[70-72]

Jones and Young,[73,74] therefore, argued that the active lactones are alkylating agents and their biological effects probably result from an in vivo modification of the nucleophilic groups which are either DNA or RNA bases. They thus chose a primary amine instead of a thiol as a model for a reactive biological nucleophile, and they studied a few of both the active and inactive lactones. They found that the carcinolytically inactive lactones acylated the amine while the active ones alkylated it. They reasoned that the reaction of a non-carcinogenic lactone with a biological nucleophile produces an acyl derivative. However, there are several enzymatically catalysed processes capable of reversing such reactions, making these compounds ineffective. On the other hand, when a carcinogenic lactone alkylates a biological nucleo-

TABLE I

Representative structures of carcinogenic lactones

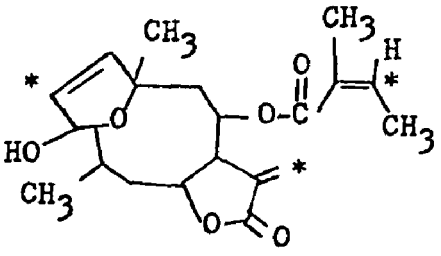


phile via Michael addition, the reverse reaction may occur thermodynamically. However, no enzyme systems are as yet known which can reverse such reactions. Furthermore, the products of such initial alkylation reactions are capable of undergoing further nucleophilic attack at the carbonyl group introducing the possibility for cross-linking the twin molecular chains of DNA.

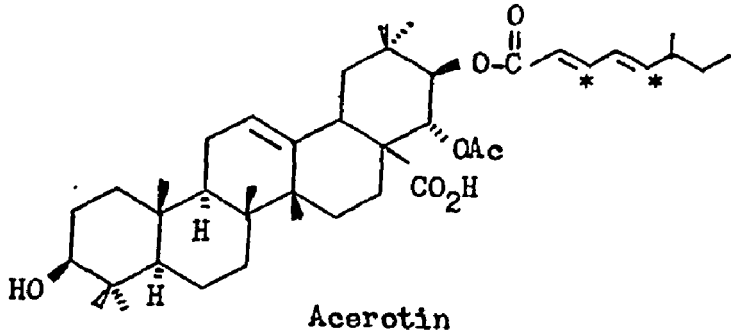
Recently, a large number of compounds from plant sources have shown anticancer activity. The structural features of these compounds are

TABLE II

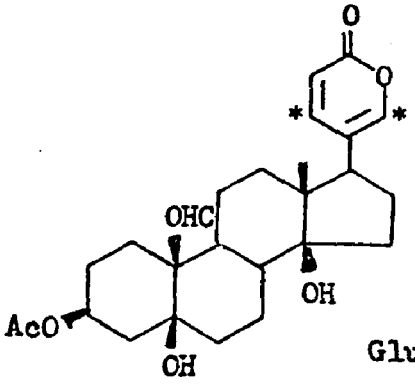
Representative structures of naturally occurring antitumor compounds



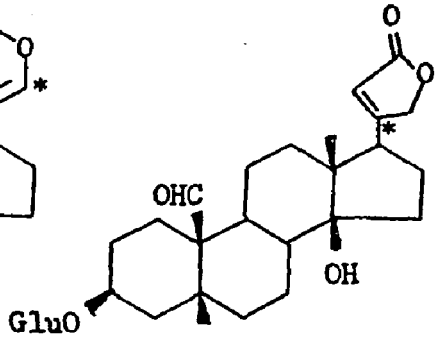
Liatrine



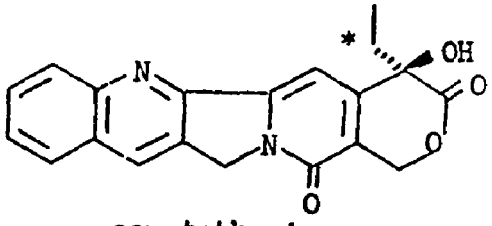
Acerotin



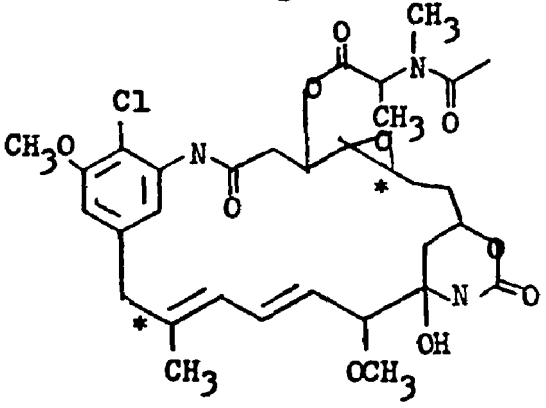
hellebrigenin



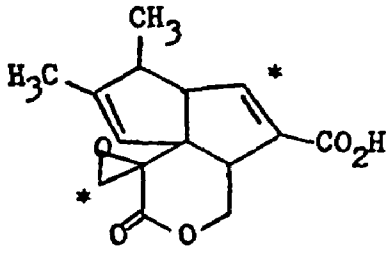
Appocanoside



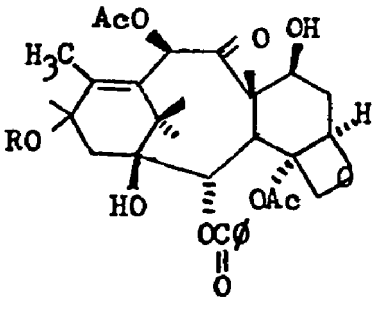
camptothecin



maytansine



Pentalenolactone



Taxol

vastly varied including such classes as sesquiterpenes,[75,76,57] triterpenes,[77] cardenolides,[78] bufadienolides,[79] quinoline alkaloids,[1] ansa compounds[80] and taxanes.[58] The great structural diversity (Table II) precludes the determination of free energy parameters required for a rigorous Hansch analysis.[81] However, it can easily be seen that all the compounds have an alkylation site marked by an asterisk (Table II). A structure-activity relationship among the sesquiterpene lactones[31,56] showed that $\alpha,\beta$ unsaturation was essential for antitumor activity, and the presence of more than one $\alpha,\beta$ unsaturation enhanced activity. With the less complicated sesquiterpenes, it was also shown that increase in cytotoxicity was accompanied by increase in lipophilicity. The selectivity and some specificity shown by antitumor agents is believed to be related[84] to the forces that control movements or transport of molecules within cells and these in turn are related to molecular structures.[8]

The alkaloid camptothecin needs only an in vivo dehydration of the $\alpha$-hydroxy $\delta$-lactone to fit into this group of alkylating agents. However, unlike the rest of the lactones it has a quinoline moiety as its AB ring system and this could provide an additional site of interaction with DNA. The antiparasitic and antimicrobial activity of acridines, phenanthridines and quinolines was found to depend upon their existence as cations, and those activities could also be correlated with the area of flatness of the molecule.[85,86] As early as 1945, it was proposed that planar heterocyclic molecules, such as acridines and quinolines, could intercalate between adjacent base pairs in the DNA double helix.[87] Lerman[88] suggested that the interaction between the drug and DNA would result in a local unwinding of the helix creating enough space for the

drug to slip in between the two strands. Intercalated drug molecules are thought to be held firmly in position by strong electronic interactions (type of charge transfer complex), with base pairs above and below the drug.[89]

Although the general concept of intercalation now seems well supported by experimental findings, Lerman's proposed model is by no means the only one. Pritchard[90] described a model where the drug reacts with two adjacent bases in the same polynucleotide rather than with H-bonded base pairs. The details of intercalation mechanisms and the nature of the forces that control them are not fully clarified yet; however, the combination of many quinoline-containing drugs with double stranded DNA have resulted in a stabilization of the helix as measured by the temperature requirement to denature DNA.[91-94] The increase in temperature (Tm) is proportional to the log of drug concentration. Presumably, Tm in drug treated DNA corresponds to extra energy required to separate the polynucleotide strands of DNA. DNA, however, needs to separate and replicate in order to have mitosis, and such stabilization could retard such a replicatory mechanism. Although the camptothecin molecule is very flat, its quinoline nitrogen is not sufficiently basic to be protonated at physiological pH. Thus, its intercalating mechanism may not be significant. In the light of these facts, therefore, it seems plausible that the camptothecin molecule may exert its cytotoxic effect primarily by alkylation through its lactone moiety, and this action may be enhanced by extra energy gained if the flat ring system intercalates between DNA strands.

2. Structure-Activity Relationship

The preparation of a few derivatives of camptothecin (see p. 2) followed by the isolation of hydroxy and methoxy camptothecin from nature makes it possible to draw some broad preliminary conclusions with regard to structure-activity relationship.[31] Nuclear substitution in the benzene ring has little influence on antitumor activity with hydroxy camptothecin appearing to be slightly more potent than the parent compound. Oxidation of the quinoline nitrogen to the N-oxide reduces but does not destroy activity. Replacement of the $\alpha$-hydroxy group with a chlorine destroys activity; however, replacing it with a hydrogen as in desoxy camptothecin reduces the potency but does not affect activity. But since the C-20 position of camptothecin is very susceptible to oxidation an *in vivo* conversion of the desoxy camptothecin to camptothecin can not be ruled out. The open E ring compound and its sodium salt are as active as the parent compound, and this is believed to result from subsequent cyclization to the lactone following administration to the animal.

3. Pharmacological Actions

In Hela cells and in L5187Y cells, camptothecin was found reversibly to inhibit DNA and RNA synthesis,[93,94] but no inhibition of mitochondrial DNA or the DNA-dependent RNA polymerase of *E. coli* could be detected even at high concentrations. Protein synthesis was slightly inhibited in mammalian cells, but glycoprotein synthesis was unaffected. Preliminary reports[95,96] also indicate that the alkaloid does not bind to calf thymus DNA as determined by thermal denaturation or changes in

absorption spectrum. This seems to indicate that the intercalatory contribution of the alkaloid to the inhibition of DNA synthesis is not very significant, but more work is needed to confirm such a conclusion. Several derivatives, including the sodium salt, methyl amide, 10-methoxy, 10-hydroxy and 20-desoxy analogs of camptothecin, show DNA-inhibiting activity comparable to the parent compound. The inhibition of DNA and RNA synthesis but not of protein synthesis by camptothecin was also confirmed in the L1210 leukemia system.[95] Recently, Gallo and coworkers[96,97] have demonstrated that camptothecin does inhibit DNA synthesis at dose levels that do not affect RNA or protein synthesis. They also showed that it is active against various mouse lymphocytic leukemias, including L1210, L5187Y and P388. In addition, it was active against variants of mouse leukemias resistant to dichloromethotrexate, BCNU, cytosine arabinoside, 6-mercaptopurine, L-asparaginase and vincristine (Table III), showing that the alkaloid is not cross-resistant to these conventional agents. It had no cytocidal effect on resting lymphocytes i.e. cells at $G^{o}$ stage. When camptothecin and $^{3}$H-TDR were added 42 hours after phytohemaglutinin (PHA) stimulation and the cells harvested after 48 hours, the percentage of cells transformed was not affected and the number of transformed cells labelled with $^{3}$H-TDR were only slightly reduced. However, mitosis was still markedly inhibited; none of the labelled transformed cells completed their cycle in the presence of camptothecin. Thus, camptothecin not only inhibits DNA synthesis but also causes a $G_2$ "lesion" which prevents subsequent mitosis. This is probably not caused by a primary defect in $G_2$ macromolecular synthesis; the major action seems to affect cells in S phase and the capacity of these cells to go through $G_2$.[97]

TABLE[97] III

Activity of camptothecin against variants of mouses leukemia resistant to conventional agents

| Tumor* | Optional dose mg/kg and schedule + | Increase in median survival over control | Long term survivors % ° |
|---|---|---|---|
| L1210/DCM | 40 q.4.d | 280 | 10 |
| L1210/BCNU | 40 " " " | 360 | 0 |
| L1210/CA | 40 " " " | 310 | 0 |
| L1210/6MP | " " " " | >1000 | 50 |
| L5187Y/L-Ase. | " " " " | >1000 | 50 |
| P388/VCR | " " " " | 260 | 0 |

* Variants of leukemia resistant to dichloromethotrexate (DCM), 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU); cytosine arabinoside (CA), 6-mercaptopurine (6MP), L-asparaginase (L-Ase), and vincristine (VCR).

+ Mice were treated IP at 40 mg/kg every 4 days as long as 6 months.

° Survivors 6 months or longer after death of all control.

4. Toxicity

Toxicological studies of camptothecin sodium salt in dogs, mice, rats, and monkeys were conducted.[98,99] In rodents, intravenous administration (i.v.) was better tolerated than oral ( os ); $LD_{50}$ single dose rat: i.v. 234.1 mg/kg per os 153.2 mg/kg; mouse: i.v. 57.3 mg/kg per os 26.9 mg/kg. Dogs treated with high doses died from severe, often hemmorhagic, enterocolitis. Death occurred after treatment with a single dose of 80 mg/kg in two out of two animals (2/2) and after 14 repeated doses of 2.5 in 2/2, 1.25 mg/kg in 1/2 and 0.625 mg/kg in 1/2 animals.

Several of the dogs treated demonstrated neutropenia, lymphopenia and thrombopenia with survivors exhibiting histopathological evidence of moderate liver toxicity. The toxicological results obtained from studies with monkeys was very similar to that observed with dogs. Toxicologically, the alkaloid does not seem to offer substantial advantage over conventional agents; in fact this might be its greatest limitation.

5. Clinical Studies

Clinical studies[100] were conducted on 18 adults, 17 with progressive tumors and one with advanced acute myclogenous leukemia. Patients received 0.5 to 10.0 mg/kg of camptothecin intravenously. The compound was highly bound to plasma protein (97.4-98.9%) and except for unchanged camptothecin no metabolite could be isolated in the urine by photometric determination. Distribution studies among body fluids show that it was only found in ascitic fluid and gastric aspirate.

Five patients whose disease was rapidly progressing with conventional chemotherapy had partial remission with greater than 50% measured reduction in tumor size. Six others demonstrated objective response; two of these had remarkable improvement in liver function. However, 78% of the patients developed leukopenia, 50% had thrombocytopenia and all those who received 7.5 mg/kg required platelet transfusions. Eight out of 18 patients developed alopecia and 7/18 had mild gastrointestinal toxicity. The drug thus does not seem to have advantage over conventional agents with regards to toxicity; in fact, the serious toxicological effects of the drug coupled with solubility problems of the compound has caused a

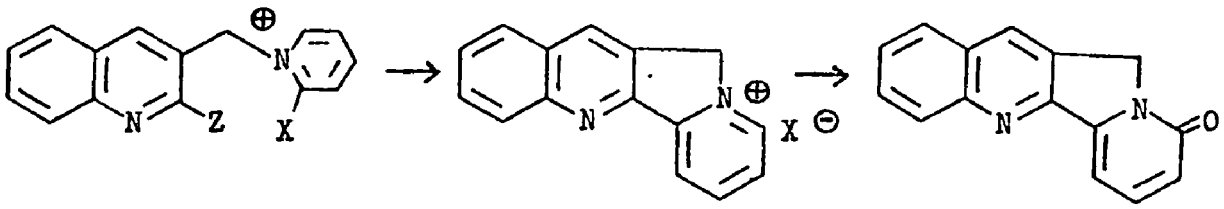
decline in the initial excitement and enthusiasm concerning the potential of this interesting alkaloid. But the fact that it is not cross resistant to conventional agents and that some tumors refractory to other agents may respond to it leaves room for structural-activity studies and molecular modification in the hope of finding a useful agent for cancer.

## III. RESULTS AND DISCUSSION

Four basic approaches to the ABCD ring system of camptothecin and a combination of various approaches to its E ring will be described.

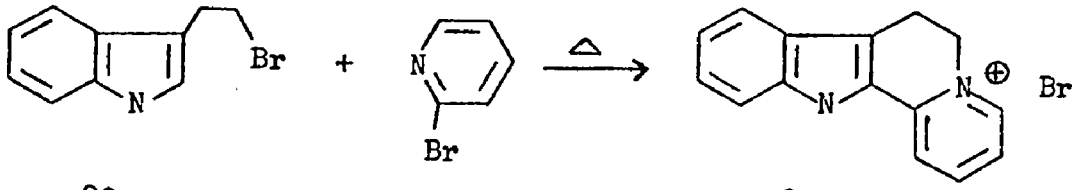
### A. APPROACH INVOLVING THE HAMMICK REACTION

It was our purpose to devise a facile synthetic route for the introduction of the ABCD ring system of the alkaloid by starting with a simple pyridine derivative. If such a model could be made then utilizing a properly substituted pyridine would provide a means to the E (lactone) ring. A cyclization reaction that can convert 77 to 78 is sought since oxidation[108] of 78 would be expected to yield readily the desired ring system 79.

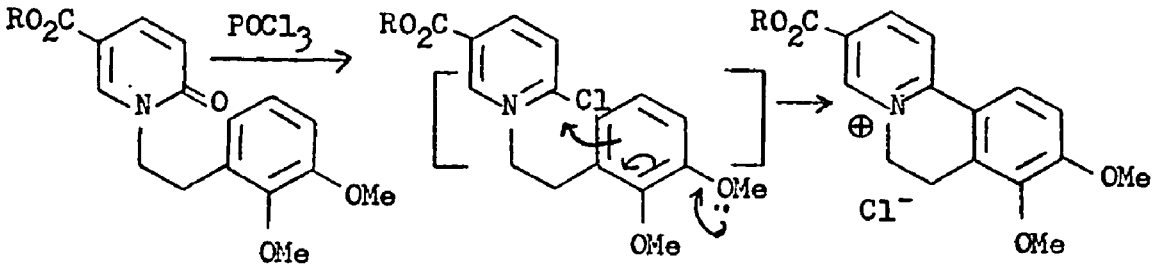


77 78 79

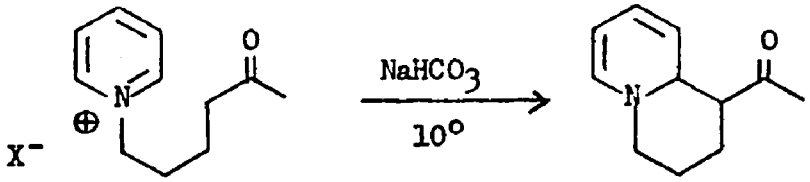
The reactivity of the 2-position of the pyridinium ion to various nucleophiles, such as Grignards ,[106] indoles,[107] and enolates,[109] is well documented. A few examples, such as the conversions 80 to 81, 82 to 83, and 84 to 85 clearly illustrate the point. If in compound 77 substituent Z were chosen such that its elimination generated an anion at that position, then it would appear that the cyclization 77 to 78 would be realizable.



80 81



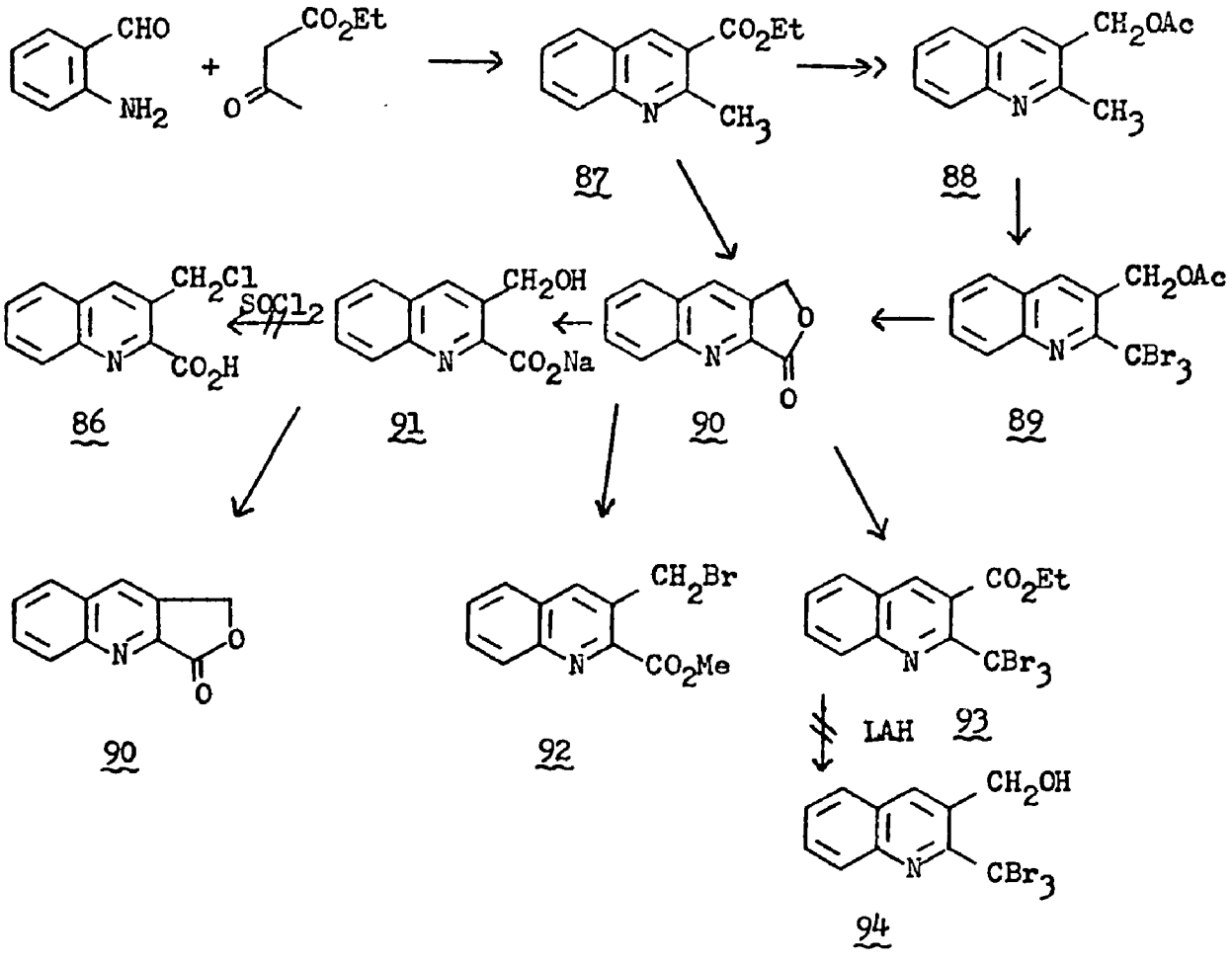
82 83



84 85

One such compound with a built in nucleophile would be 77, if Z is MgX of a Grignard reagent; however, preparation of quinolyl Grignard reagents is difficult. Indeed it has not been achieved by direct interaction of a halogenoquinoline with magnesium,[110] but it can be prepared in low yield by submitting the halogenoquinoline to the entrainment method in the presence of ethyl magnesium bromide.[111] Furthermore, selective formation of the quinolyl Grignard in the presence of 2-halogenopyridinium ion (see 77) would be very difficult if not impossible.

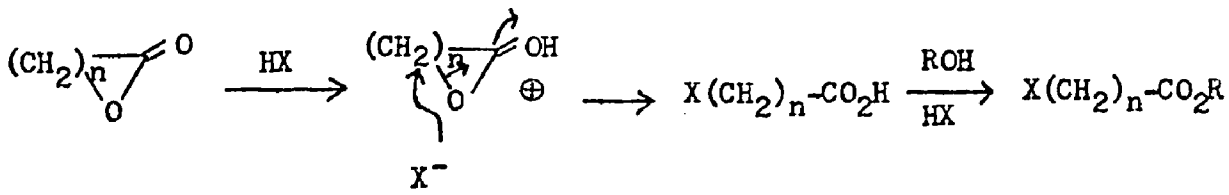
In 1934, Hammick[112] in a study of decarboxylation mechanisms of picolinic and quinaldic acids showed that these compounds decarboxylate through the generation of a carbanion intermediate which usually picks up a proton. The generation of the carbanion intermediate was demonstrated[113] by trapping it with several electrophiles such as diazonium salts, aldehydes, ketones and esters. It thus appeared reasonable to prepare 77, with Z = carboxylic group, since simple thermal decarboxylation would provide the needed nucleophile which hopefully undergo the desired cyclization to give 78. The first target compound was chosen to be 86 as it can subsequently be converted to 77 by standard procedures. The synthetic scheme used in attempts to obtain 86 is outlined below.



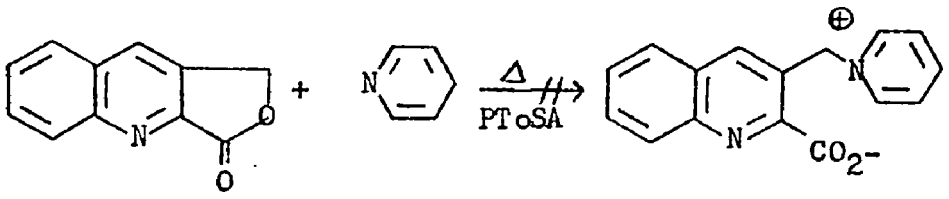
Freshly prepared o-aminobenzaldehyde[114] was condensed with ethyl acetoacetate in the presence of piperidine to yield 87 in 91% yield. Treatment of 87 with bromine in acetic acid readily gave the corresponding tribromo compound 93 in quantitative yield; however, attempts to reduce 93 to the tribromo alcohol 94 with lithium aluminium hydride led only to intractable tar. It was thus decided to reverse the order of bromination and reduction. Reduction of 87 with lithium aluminium hydride readily gave in 80% yield the corresponding alcohol which in turn was quantitatively acetylated in acetic anhydride and pyridine to provide 88. By use of the already mentioned bromination technique, 88 was converted to 89 in 90% yield, and hydrolysis thereof in 10% sulfuric acid led to the lactone 90 in 67% yield. This lactone proved to be very stable and unreactive. Refluxing it in 25% sodium hydroxide gave the sodium salt 91 as detected from the IR spectrum; 3500-3300 $cm^{-1}$ (OH) and 1690 $cm^{-1}$ ($\overset{O}{\overset{\|}{C}}-O^-$). Neutralization of the salt even in the mildest conditions caused it to cyclize back to the lactone 90. Treatment of 91 with thionyl chloride also clearly gave the lactone with no traces of chlorinated material. This is to be expected since, even if the alcohol was chlorinated, the carboxylate anion would nucleophilically displace the chlorine leading to the lactone 90.

Lactones are ordinarily converted to the corresponding halo esters[115] by refluxing them in the appropriate alcohol saturated with gaseous hydrogen chloride or hydrogen bromide. Various attempts, with different reaction times and different concentrations of acid, were made to convert 90 to 92; however, in all cases unchanged starting material was recovered. In the extreme case where gaseous hydrogen bromide was bubbled into the reaction mixture for over 24 hr and the reaction was refluxed for 3 days,

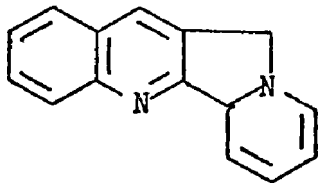
only trace amounts of 92 were detected spectroscopically. The mechanism of lactone opening in halo acids involves first protonation of the carbonyl oxygen, followed by nucleophilic attack of the halide ion as shown.



Based on the foregoing mechanism, it has been suggested[116] that one reason for the extreme unreactivity of the lactone 90 toward this type of acid catalyzed cleavage results from the adjacent quinoline nitrogen. The acid protonates the quinoline nitrogen first, making the subsequent protonation of the adjacent carbonyl group very difficult, since it is energetically very unfavorable for two positively charged species to exist adjacent one to another. However, unless the carbonyl oxygen gets protonated the lactone cannot be opened to give the desired product - at least not by the outlined mechanism. In a final effort to transform 90 to some useful intermediate, the lactone was refluxed in excess of pyridine with catalytic amount of p-toluenesulfonic acid in the hope of converting 90 to the pyridinium salt 95 or possibly the cyclized product 96. However, the reaction resulted only in the recovery of starting material as the major product, accompanied by some decomposition product. The 3-hydroxymethyl-2-quinoline carboxylic acid lactone 90, therefore, proved to be an unproductive intermediate in the proposed synthesis.

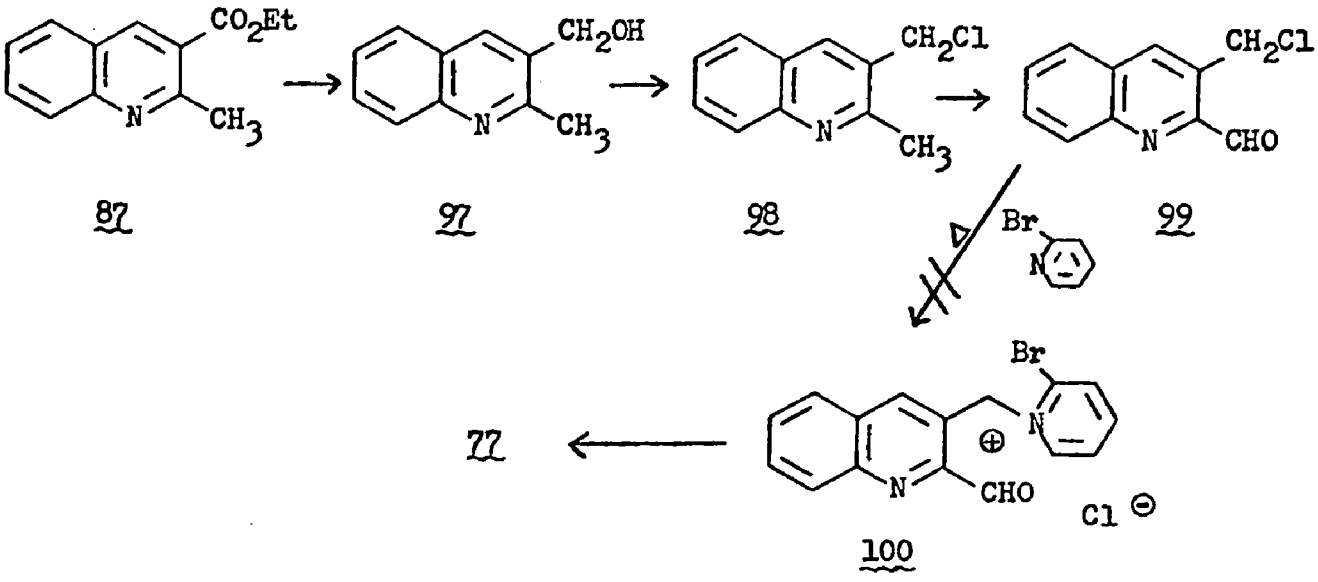


90 95



96

One way of avoiding the unreactive lactone intermediate in the synthesis of 77 would be to introduce the pyridinium derivative before the introduction of the carboxylic acid group. In an effort to do this, the previously described compound 87 was reduced to the alcohol 97 with lithium aluminium hydride. The halogen derivative 98 was then obtained from the reaction of 97 with thionyl chloride; oxidation of 98 by freshly sublimed selenium dioxide in wet dioxane[117] gave the aldehyde 99 in 67% yield. It is noteworthy that the selenium dioxide



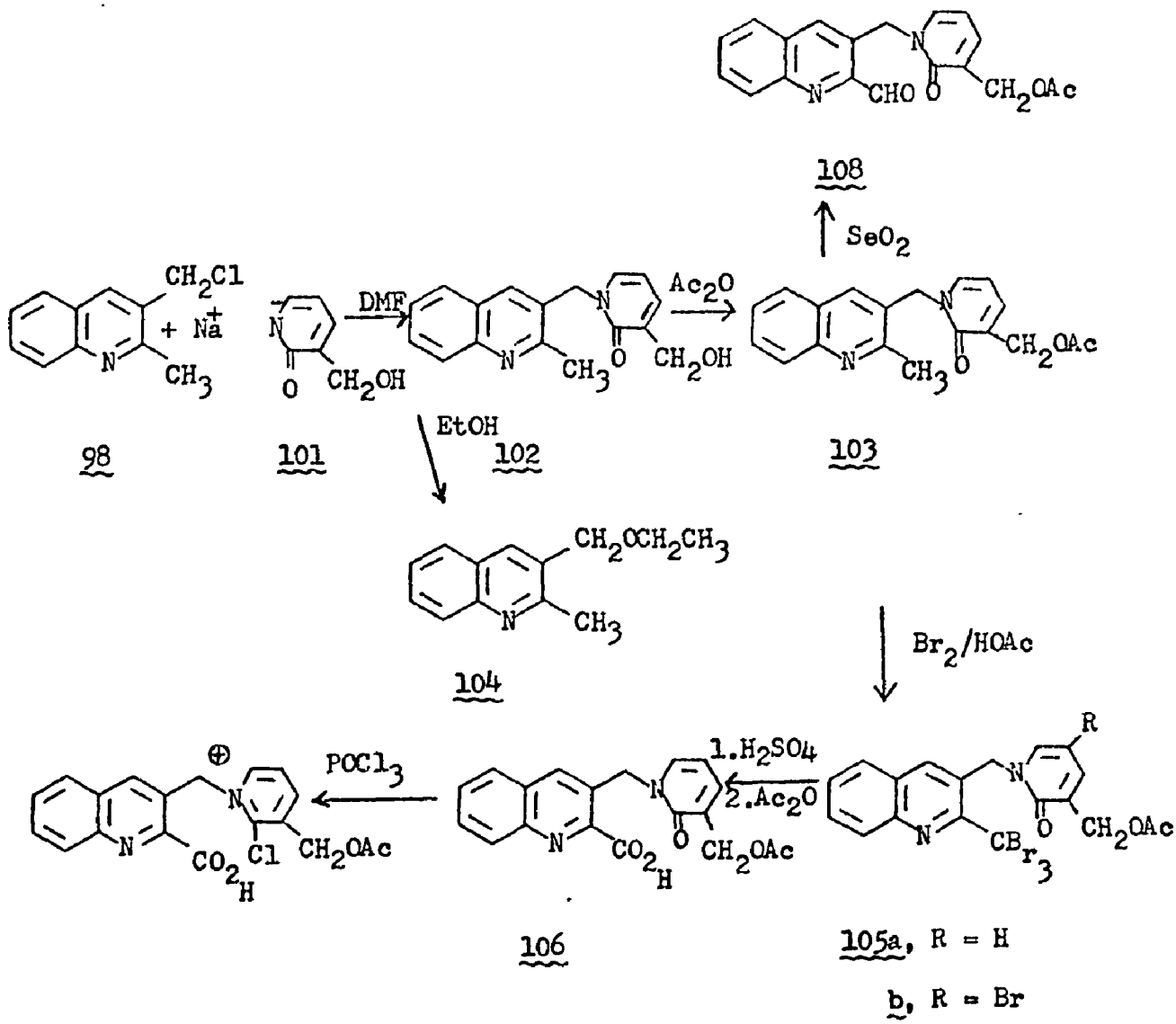
87 97 98 99

100 77

must be freshly sublimed, since aged selenium dioxide gives unreproducible results.[118] Refluxing the 3-chloromethyl quinaldehyde 99 in 2-bromopyridine as solvent gave an ether insoluble dark gum whose ir showed no aldehyde absorption, and nmr indicated no aliphatic protons. Attempts to purify this gum, by means of conventional methods of extraction, crystallization and chromatography, failed to yield any characterizable product.

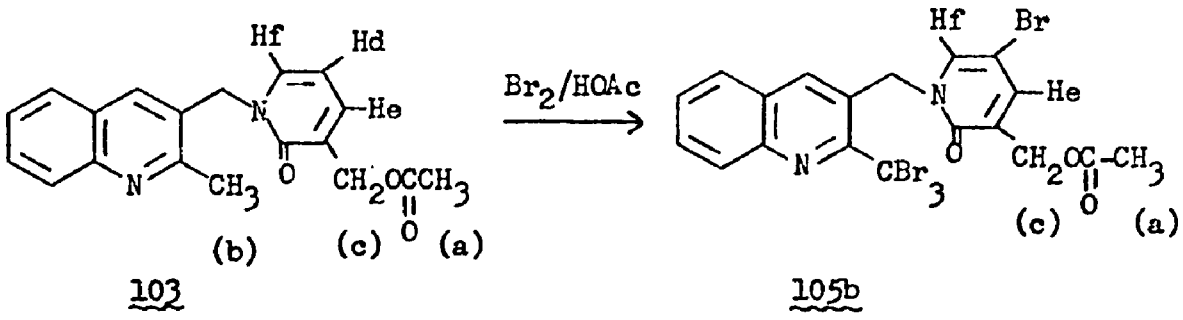
The pka of 2-bromopyridine is significantly lower than that of pyridine. Thus, alkylation at the nitrogen of 2-bromopyridine is expected to be slower than that of pyridine. Besides, the quinoline nitrogen in 99 exerts some competition through self-alkylation. Perhaps treatment of 99 with pyridine itself would have offered a better chance of forming a pyridinium derivative. Such an intermediate though would lead to a dihydropyridine derivative after decarboxylation and cyclization, but dihydropyridines are known to be unstable species[119] especially at the temperature of decarboxylation. Therefore, it was decided to substitute the 2-position of the pyridine derivative with a leaving group so that after the Hammick decarboxylation is performed, the product of cyclization would be a stable aromatic species.

Pyridones can ordinarily be converted to 2-chloropyridine using a variety of chlorinating agents, phosphorus oxychloride being the most common; furthermore, the pyridone nitrogen usually undergoes alkylation readily. In the hope of converting the pyridone moiety to the desired pyridinium derivative 107 at final stages of the synthesis a new synthetic scheme was pursued. 3-Hydroxymethyl-2[1H]-pyridone acetate (148) was prepared in 80% yield from rearrangement of the corresponding pyridine




N-oxide (see p. 59). The sodium salt of the pyridone was prepared by treatment of an ethanol solution of the pyridone with an equivalent amount of sodium metal. This, however, resulted in the hydrolysis of the acetate ester to the sodium salt of the pyridone 101, as confirmed by its nmr and ir. Treatment of 101 with 2-methyl-3-chloromethyl-quinoline 98 in ethanol gave a 1:1 mixture of the desired product 102 and the ether 104, which came from the displacement of chloride by

ethoxide ion. The composition of the mixture was determined from the analysis of the nmr spectrum. Conducting the reaction in dimethylformamide as the solvent, however, led to the desired product 102 in 88% yield. Acetylation of 102 in pyridine and acetic anhydride gave 103 in quantitative yield. Treatment of 103 with bromine in acetic acid gave a product which from a mere calculation of yield of product must contain at least three bromine atoms as in 105. The compound was very unstable and decomposed on standing. Attempts to recrystallize the crude product from ethanol, methanol, chloroform, acetone or combinations of them only caused the brownish compound to undergo further decomposition. Spectral data of the crude compound were revealing.



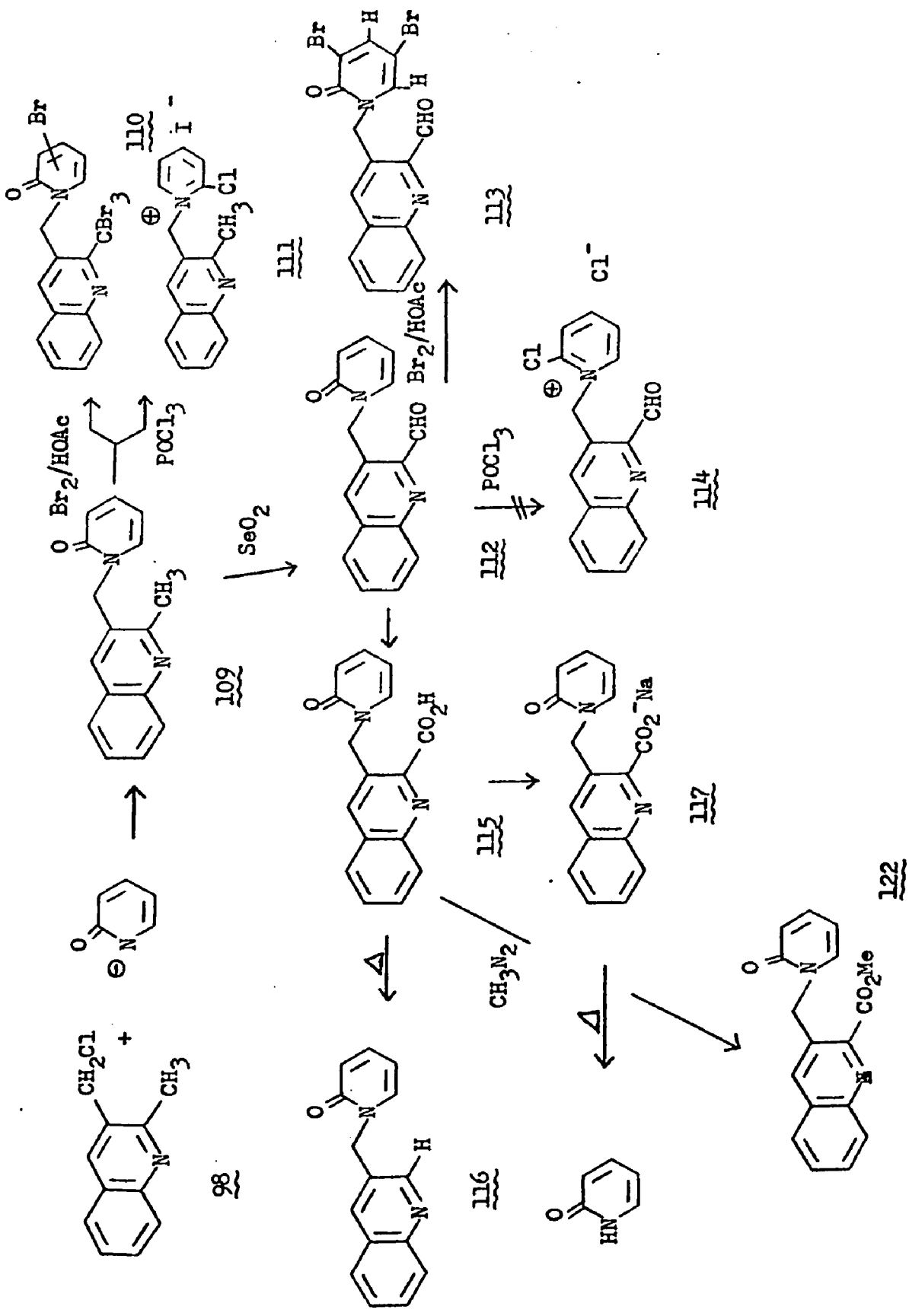

103 105b

The irs of the product and starting material (103) were quite similar in the carbonyl region. The product retained ester absorption at 1720 $cm^{-1}$ for the acetate and amide absorption at 1645 $cm^{-1}$ for the pyridone. In the nmr of the starting material (103), the quinoline methyl ($-CH_{3(b)}$) appears at 2.57 $\delta$ and the pyridone proton Hd appears at 6.30 $\delta$. The proton Hd though has a doublet of a doublet pattern but appears as more of a triplet owing to the closeness of Jfd and Jde, and the chemical shift of Hd, which is considerably upfield from the poorly resolved

aromatic protons, makes its detection very easy. The nmr of the crude product, compared to that of the starting material, showed the absence of both $-CH_{3(b)}$ and Hd; therefore, the structure 105b is assigned to the bromination product. The unexpected and undesired bromination of the pyridone ring is well documented in the literature[120-122] and probably occurs through a mechanism similar to the electrophilic bromination of phenoxide ion. The preparation of a more stable brominated pyridone derivative is described (see p. 52). Hydrolysis of the crude brominated product (105b) in 20% sulfuric acid for 10 hr gave a very dark brownish solid from which no characterizable product was obtained.

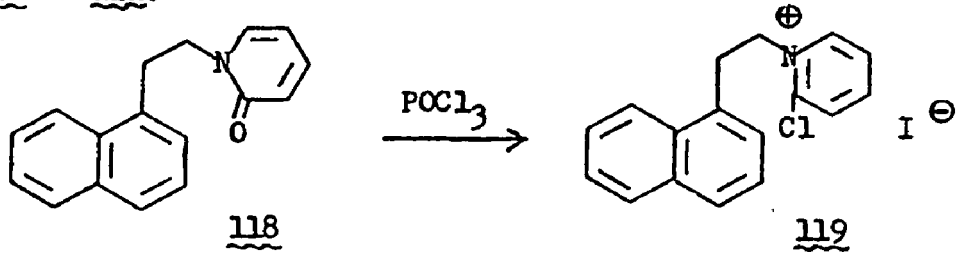
An efficient conversion of quinaldine to quinaldic acid via the tribromo intermediate did not seem too promising in the presence of a pyridone functionality; therefore, other means of oxidation were sought. The hydroxymethyl group in compound 103, though essential to the final synthesis of camptothecin, can be a source of complication in these exploratory reactions. The different oxidative studies were thus carried out with the more easily accessible unsubstituted pyridone derivative 109.

Treatment of 98 with commercially available thallium salt of 2-pyridone in dimethylformamide led to a 1:1 mixture of both the N- and O-alkylated products. The mixture was separated by column chromatography on alumina with benzene and methylene chloride as solvents. Hopkins[123] in a systematic study of both solvent and ion size on the N/O alkylation ratio concluded that the pyridone salts of large cations tend to undergo more O-alkylation, whereas salts of small cations undergo N-alkylation almost exclusively. Therefore, the sodium salt of 2-[1H]-pyridone was

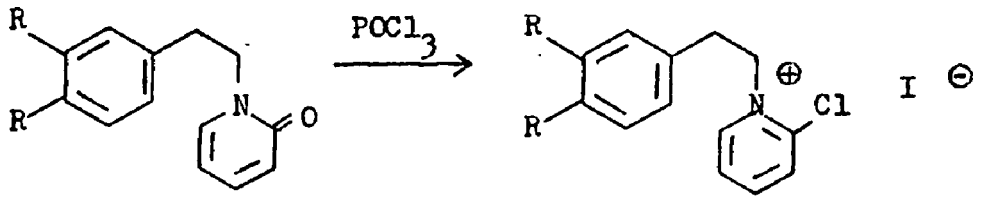

Br
CBr3
110
I-
CH3
Cl
Br2/HOAc
POCl3
109
CH3
SeO2
111
Br
H
CHO
Br2/HOAc
113
Cl-
POCl3
112
114
CO2H
CO2-Na
115
117
CH3N2
Δ
Δ
CH2Cl
98
116
HN
CO2Me
122

prepared,[123] and reacting it with 98 in dimethylformamide at r.t. gave 90% yield of pure N-alkylated product 109. As in the case of 103, attempts were made to study the bromination product of 109, but once again the product was found to be too unstable for proper characterization. Oxidation of 109 with freshly sublimed selenium dioxide in refluxing wet dioxane gave the aldehyde 112 in almost quantitative yield (> 90%). Separation of unreacted selenium dioxide and selenium acid from the organic products usually presents a problem; however, in the present case washing the chloroform solution of the product with saturated sodium bicarbonate, followed by 5% potassium cyanide solution, was found to be satisfactory. Furthermore, selenium dioxide oxidation of 103 with its hydroxymethyl group was also attempted. Again, the corresponding aldehyde 108 was produced in 90% yield.

The aldehyde 112 contains almost all the necessary functionalities to meet the requirements of the intermediate 77. The only two transformations needed are conversion of the 2-pyridone moiety to 2-halopyridinium salt and the aldehyde to an acid. Superficially both conversions appear to be trivial since numerous standard procedures are known that can effect transformations of these functionalities. Especially attractive precedence in the conversion of pyridone to pyridinium salts was seen in the work of Sugasawa,[124] Ban[125] and Paquette[126] who prepared several aromatic ethyl-2-chloropyridinium iodides, e.g. 118→ 119 and 120 → 121.




118 119




120 121

Conversion of the pyridone portion of 112 to the pyridinium ion by phosphorus oxychloride or thionyl chloride proved to be unfruitful. As short a time as ten minutes of reflux of the pale yellow solution of 112 in benzene with either phosphorus oxychloride or thionyl chloride caused the reaction mixture to turn dark brown. As the reaction progressed, it grew darker, eventually leading to a tarry decomposition product. Conducting the reaction at room temperature resulted mainly in recovery of starting material. No conditions suitable to produce the desired product could be found. Treatment of 109 with phosphorus oxychloride, however, gave the halogenated product (111) in poor yield (20%).

Oxidation of the aldehyde 112 to the carboxylic acid 115 - though not as troublesome as the chlorination reaction - was not a simple conversion; the aldehyde was surprisingly resistant to oxidation. The various oxidizing agents used are summarized in Table IV. Initially, such an oxidation was expected to occur so readily that it was attempted by simply bubbling - oxygen[127] into a carbon tetrachloride: acetic acid (1:1) solution of the compound, but even after three days the starting material was recovered quantitatively. Oxidation with acetic acid and hydrogen peroxide gave a very poor yield of the acid (15%), but since

this reagent can also form the N-oxide - thus increasing the propensity to decarboxylate - the low yield was not very surprising. Hydrogen peroxide oxidation with acetone[128] as a solvent gave the acid in 30% yield. It was then decided to use stronger oxidizing agents. Oxidation with Jones reagent gave a very poor yield (15%) of the desired product; however, the major difficulty seemed to be separation of the insoluble acid from the inorganic chromous salts. Base catalyzed potassium permanganate successfully oxidized the aldehyde 112 to the acid 115 in good yield ( > 70%). No efforts were made to maximize any of the tabulated yields. The acid was obtained as an amorphous pale yellow solid. Attempts to recrystallize it from various solvents were unsuccessful. Its identity was confirmed through spectral properties and a methyl ester derivative 122. The ir of the acid does not show strong O-H stretch from the acid since

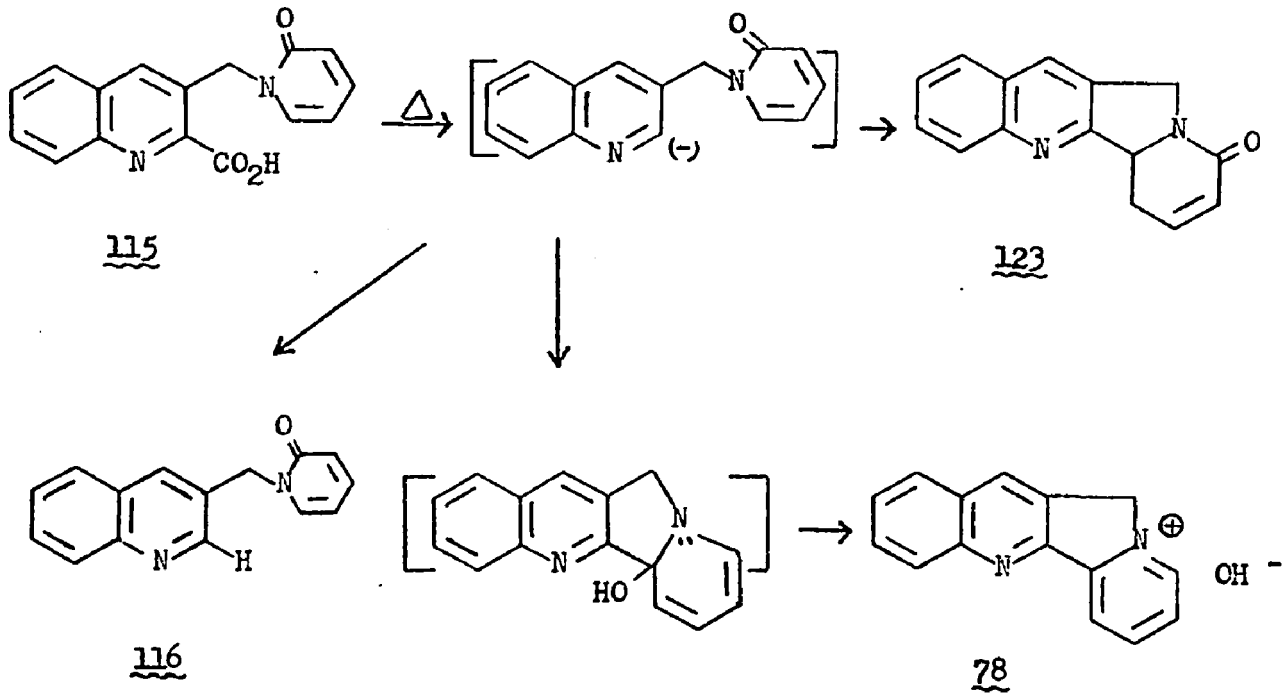
TABLE IV

Results of various oxidizing agents on 112

| Oxidizing Agent | % Yield of acid 115 |
|---|---|
| $O_2/CCl_4/HOAc$ | - |
| $H_2O_2/HOAc$ | 15 |
| $H_2O_2/CH_3\overset{O}{\overset{\|}{C}}CH_3$ | 30 |
| $CrO_3^{=}/CH_3\overset{O}{\overset{\|}{C}}CH_3$ | 15 |
| $KMnO_4/NaOH/H_2O$ | 70 |

it exists as a zuitterion. However, it showed salt bands at 2500 and 2300 $cm^{-1}$ and the carbonyl absorption of the carboxylate ion is shifted by about 30 $cm^{-1}$ and appears at 1695 $cm^{-1}$.

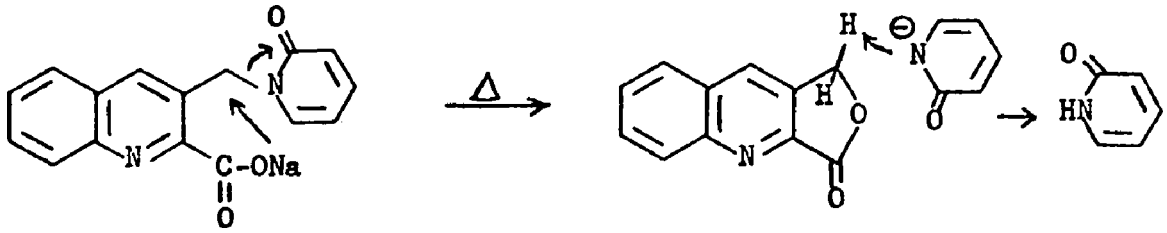
Failure to convert the pyridone moiety to the desired 2-halopyridinium salt was disappointing. Nevertheless, it was still felt that the pyridone might interact with the Hammick carbanion to give either of the intermediates 78 or 123, both of which could easily be converted the desired product 79. Therefore, the quinaldic acid



derivative 115 was heated to 130° for 2 hr under nitrogen without any solvent, and decarboxylation occurred at about 100-110° to yield a white crystalline material in 60% yield. The ir of the product showed no carboxyl absorption but had a sharp amide absorption at 1645 $cm^{-1}$; the nmr was very similar to the starting material except for the presence of low field proton at 9.15 $\delta$ for the proton at the 2-position of the quinoline moiety. Thus, the spectral data supplemented by elemental analysis established that the decarboxylation of 115 produced only 116 with no traces of any cyclized material.

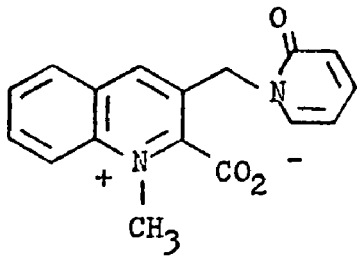
The mechanism of the Hammick decarboxylation is believed to involve first protonation of the quinoline nitrogen. This stabilizes the generation of the adjacent carbanion and also markedly reduces the temperature of decarboxylation in comparison to that of the salts. However, the carboxylic acid proton is a good competitor for the Hammick carbanion, for if the electrophile on hand (i.e., the pyridone in this case) is not reactive enough, only the protonated product may result. Such appears to be the case with 116.

In order to remove this competing protonation, the sodium salt of 115 was prepared by treating the acid 115 with an equivalent amount of sodium hydroxide. In an attempt to effect decarboxylation in an oil bath, the salt was heated to 285°, but it proved to be very stable. The salt was then transferred to a sublimator and in vacuo it was heated by open flame which resulted in much decomposition. Nevertheless, a yellowish compound was collected at the cold finger. The ir and nmr of the yellow compound were superimposable with that of 2-(1H)-pyridone. A possible mechanism for the undesired generation of 2-pyridone from the sodium salt of 115 is suggested as shown. 3-Hydroxymethyl-2-quinoline



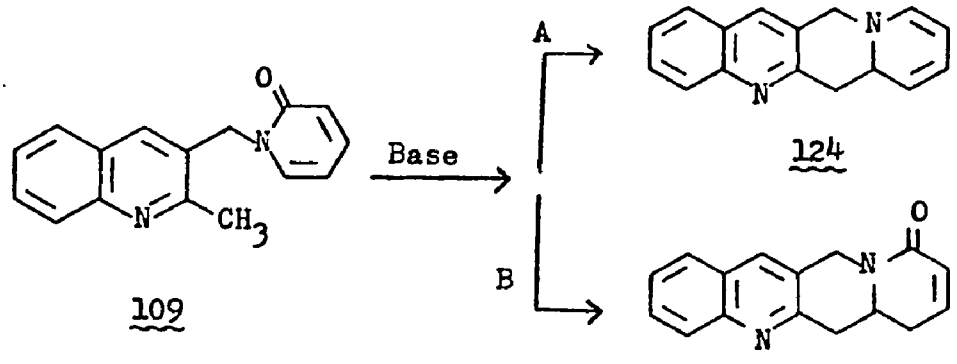
carboxylic acid lactone was prepared by a different route (see p. 36), and attempts were made to detect this lactone in the non-sublimed fraction; however, at the temperature of the reaction it seemed that all the non-sublimed portion had decomposed completely. Perhaps fragmentation at a carefully controlled temperature could result in the detection of the other product and clarify the mechanism of fragmentation.

Ratt[128] and Quast showed that betaines (i.e., 2-carboxypyridinium salts) undergo decarboxylation through the Hammick intermediate under very mild conditions namely, 60° (cf. picolinic acid decarboxylates at 175° C and its N-oxide[130] at 90°). The propensity to decarboxylate easily is attributed to the stabilization of the intermediate carbanion by the adjacent cation rendering the anion a form of stable ylide. Heterocyclic ylides in the azoles[131] governed by similar stabilizing factors are also known. Thus, an alternate way of removing the competition from the proton in the decarboxylation of 115, without increasing the reaction temperature, would be to convert the quinoline portion of 115 to an N-alkylated quinolinium ion as in 124. Such a compound would certainly not have the problem of a competing proton; furthermore, it should undergo decarboxylation under very mild conditions. The question of whether the pyridone is a good enough electrophile to undergo the desired cyclization remains unanswered. There is no precedence for the proposed cyclization reaction involving pyridones except for the Bischler-Napieralsky[132] reaction which involves amides. As compound 124 was not readily accessible, attempts to study the susceptibility of the pyridone nucleus toward nucleophilic attack was made with 109 which was already



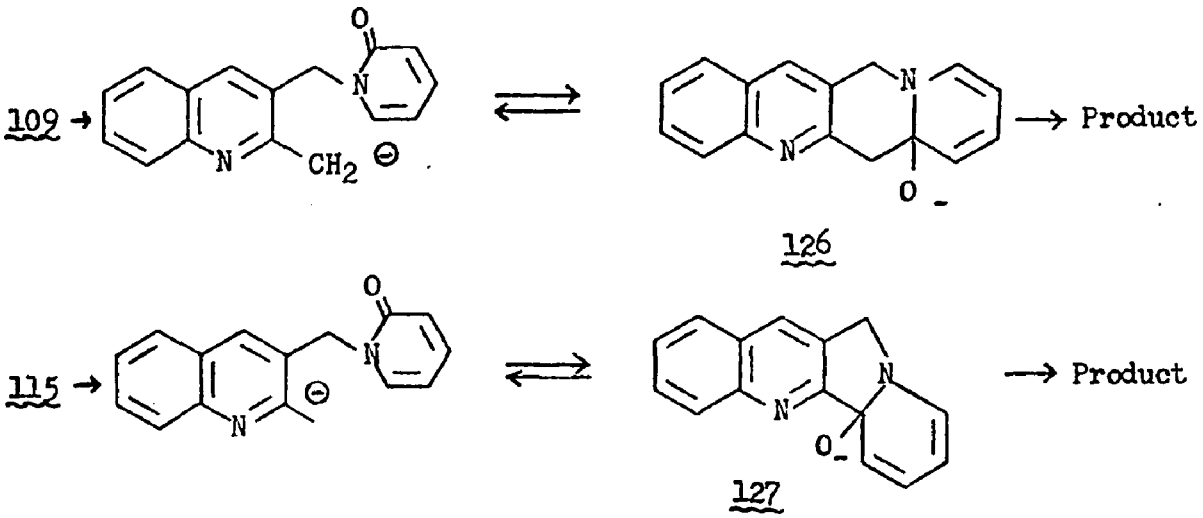
124

on hand. Treatment of 109 with diisopropyllithiumamide in tetrahydrofuran and a few drops of hexamethylphosphoramide would be expected to yield 124 or 125 if the pyridone cyclized in one of the proposed modes (see p. 47). Treatment of 109 with the base at -78° generated the anion, as evidenced by the solution's deep blue coloration. The reaction mixture was then allowed to warm to room temperature and was kept there overnight, during which time the solution became decolorized. After 18 hours of standing at room temperature, it was refluxed for one hour. But only starting material was recovered. The pyridone appears to be a poor electrophile.



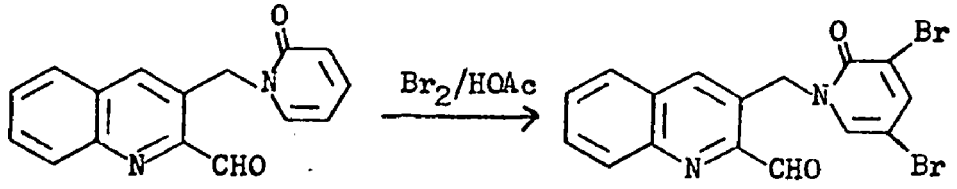

109 124 125

It seems unlikely that the carbanion would attack the pyridone in a Michael fashion (pathway B) since it would need sizeable energy to disrupt the aromatic character of the pyridone. However, nucleophilic attack at the pyridone carbonyl probably occurs by generating the intermediate 126 in the base treatment of 109 and 127 in the decarboxylation of 115. Neither 126 nor 127 hold efficient enough leaving groups to shift the equilibrium in favor of the product. It




seems in the case of 109 that the anion eventually abstracted a proton either during work up or from the atmosphere, whereas in the decarboxylation of 115 such a proton is readily furnished by the carboxylic acid group. It does, therefore, seem imperative to introduce a good leaving group into the pyridone nucleus, such as a 2-halopyridinium salt, for such a cyclization to be effective. The failure to convert the pyridone to a 2-halopyridinium ion was crucial in the failure of the first approach. Based on the Bishler-Napieralsky mode of cyclization, perhaps the replacement of the pyridone moiety by a glutarimide in 115 may have offered a better chance of cyclization.

With aldehyde 112 on hand the bromination reaction was once again attempted and refluxing 112 with a slight excess of bromine in acetic acid gave a white crystalline material which was both stable and easy to characterize. The ir revealed the presence of aldehyde at 1710 $cm^{-1}$ and the nmr showed the absence of two upfield aromatic protons which are ortho and para to the pyridone carbonyl, clearly indicating the

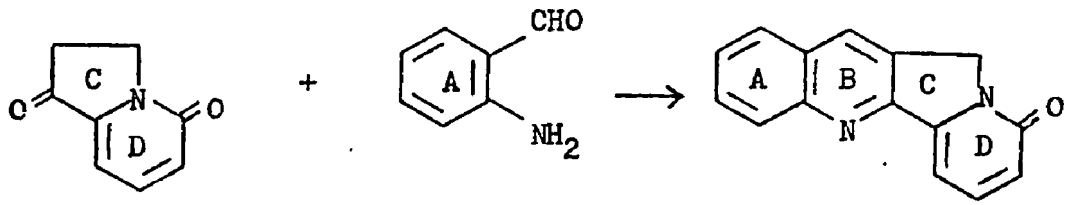



112 113

formation of a dibromo compound. This was further confirmed by elemental analysis and mass spectral data with $M^+$ at m/e 420.

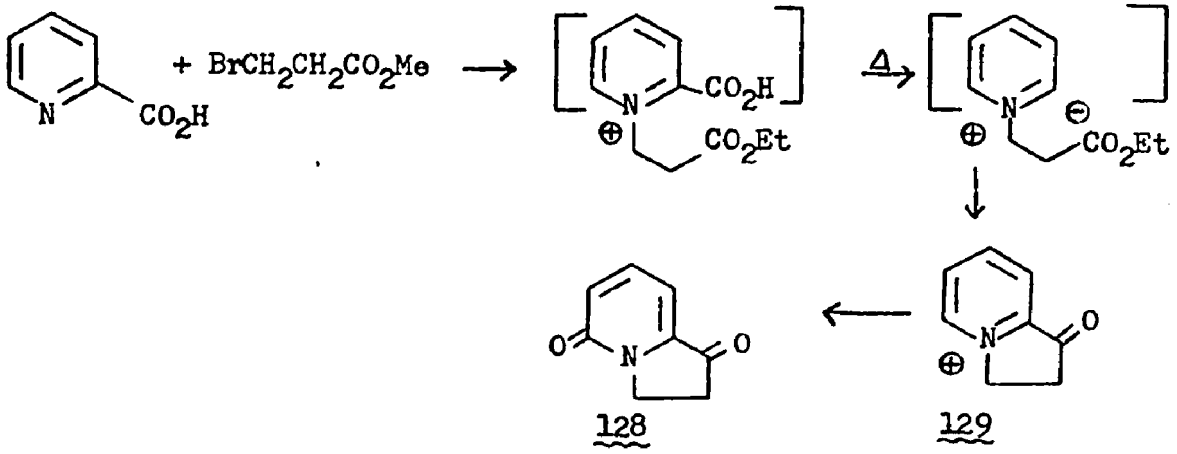
## B. APPROACH INVOLVING N-OXIDE REARRANGEMENT

The second approach attempted to construct the C and D rings first and build on to them the A and B rings as shown below. The condensation of 128 with o-aminobenzaldehyde through the Friedlander synthesis should lead directly to the desired ring system 79. Since



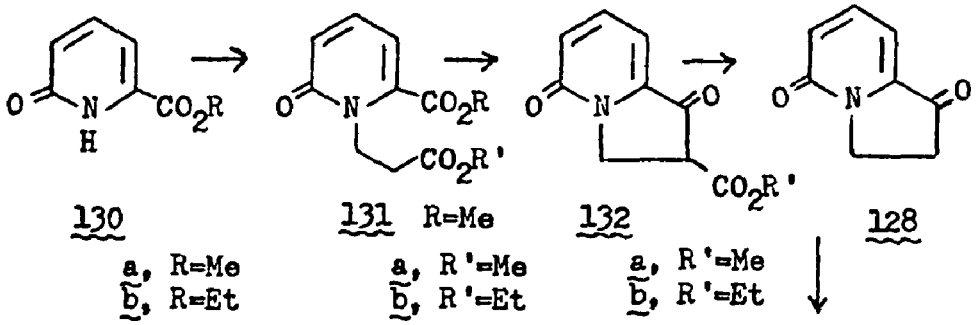
128 79

o-aminobenzaldehyde is readily made from the reduction of o-nitrobenzaldehyde, this approach focused on the synthesis of bicyclo compound 128. Here too attempts were made to utilize the Hammick decarboxylation. As pointed out earlier, 2-carboxypyridinium salts undergo decarboxylation with great ease; thus, if picolinic acid is alkylated with a group containing an electrophile a decarboxylation cyclization leading to the bicyclo ring system can be envisaged.

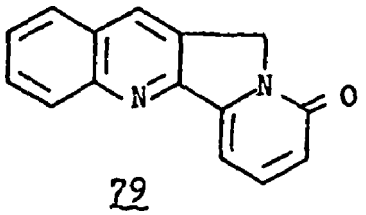


Refluxing methyl β-bromopropionate with an equimolar amount of picolinic acid in various solvents (e.g. acetonitrile, benzene, toluene or xylene) resulted only in the quantitative recovery of starting materials. The nitrogen of picolinic acid is poorly nucleophilic, since it tends to exist in the zwitterionic form. In order to increase its basicity, it was esterfied to ethyl picolinate. Attempts to N-alkylate this ester with methyl β-bromopropionate in refluxing toluene led to recovery of unchanged ethyl picolinate and polymerization of the alkylating agent. Presumably, the bromo ester first alkylates the ethyl picolinate giving the desired salt which rapidly eliminates HBr and methyl acrylate, which under the reaction conditions easily polymerizes.

An alternate route to the synthesis of 128 was thus sought, and the Dieckmann type of cyclization of an intermediate such as 131 appeared to be an attractive route to 128, especially since 131 could probably be formed from 130 and methyl β-bromopropionate or methyl acrylate. The pyridine derivative 130a was prepared as described by



130 (a, R=Me; b, R=Et) → 131 (R=Me; a, R'=Me; b, R'=Et) → 132 (a, R'=Me; b, R'=Et) → 128



29

Boekelheide[133] through acetic anhydride rearrangement of the methyl picolinate N-oxide. Picolinic acid N-oxide was esterified to the methyl ester by Newbold's method.[134] This operation proved to be very sensitive to moisture and extreme care was taken to exclude moisture from both the gaseous hydrogen chloride and the methanol. Warming methyl picolinate N-oxide with acetic anhydride at 120-125° under nitrogen for 6 hours gave 130 in 60-65% yield. This is marked improvement from the reported 35% conversion.[133] The currently accepted mechanism of the acetic anhydride catalyzed rearrangement

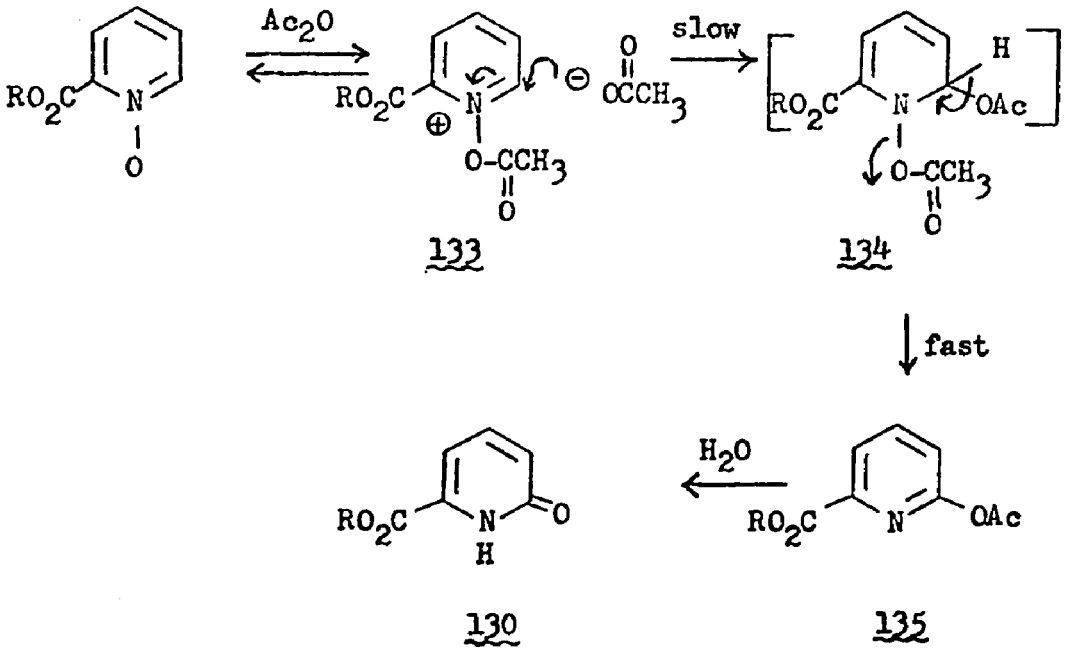



Figure 6. Acetic anhydride catalyzed rearrangement of pyridine N-oxides

of pyridine N-oxides is outlined (Figure 6).[135] Acetic anhydride undergoes nucleophilic attack by pyridine N-oxide to produce the 1-acetoxypyridinium ion 133 and acetate ion in an equilibrium reaction. The ion

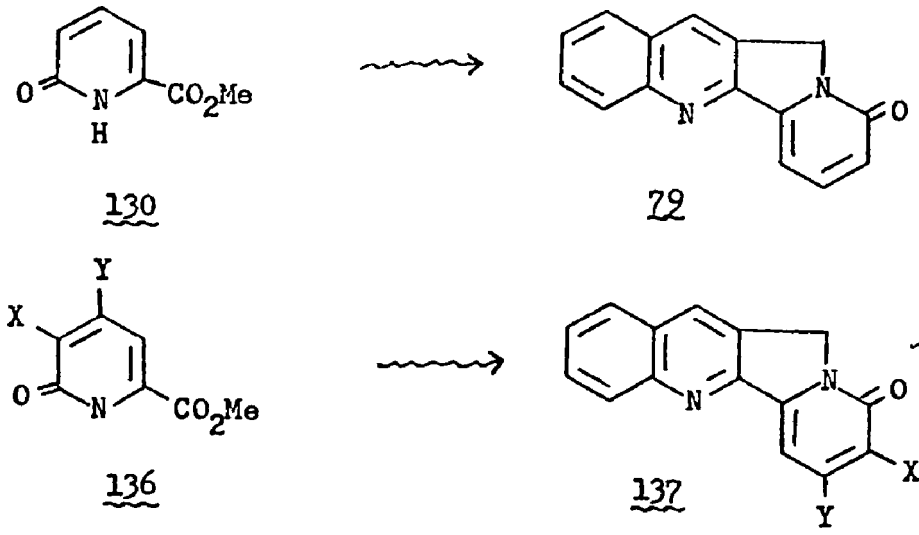
133 then slowly undergoes nucleophilic attack by acetate in generating 134, which rapidly eliminates acetic acid and produces 135, which is readily hydrolyzed during work up to give the pyridone derivative 130. The rearrangement of ethyl picolinate N-oxide under the outlined conditions led to a disappointingly low yield (5%) of 130b. The reaction was accompanied by a great deal of decomposition; however, when the rearrangement was carried out in the presence of acetic anhydride and dry sodium acetate the yield of 130b was improved to 20%.

Refluxing 130a with ethyl or methyl acrylate under nitrogen for 6 hours in dimethyl formamide, with anhydrous sodium carbonate as base, allowed both the Michael addition and the Dieckmann cyclization to occur in the same flask. Here again methyl acrylate led to 132a in 70% yield, whereas ethyl acrylate gave 132b in 25% yield. The reason for such differences in yields is not apparent. Compound 132a was treated with o-aminobenzaldehyde in 20% hydrochloric acid in the hope of effecting both decarboxylation and condensation; however, such conditions caused the acid catalized dimerization and polymerization of o-aminobenzaldehyde with no traces of condensed product. Refluxing 132a in concentrated hydrochloric acid for one hour gave 128 in 75% yield. The ir spectrum showed strong absorption at 1745 $cm^{-1}$ for the 5-membered carbonyl, 1645 $cm^{-1}$ for the pyridone carbonyl with no ester absorption at 1720 $cm^{-1}$. The nmr ($CDCl_3$) had a poorly resolved aromatic region but with two clean triplets from the two methylene protons, at 4.2 $\delta$ for those adjacent to the nitrogen and at 2.9 $\delta$ for those adjacent to the carbonyl. The Friedlander condensation of 128 with o-aminobenzaldehyde in sodium hydroxide and ethanol[3] gave the desired product 79 albeit in only 10% yield, owing to the extreme instability of 128 under basic conditions.*

* While this final conversion was being investigated a publication by Kametani[14] appeared detailing the same approach.

## C. APPROACHES TO RING E

The first-stated problem of construction of the ABCD ring system has now been solved. This success can be summarized by the transformation of 130 to 79. Application of the reaction sequences that were performed in this model transformation would be expected to convert 136 to 137. If the substituents X and Y were chosen appropriately,




130 79

136 137

137 could then be converted to camptothecin.

Key intermediate 136 might be obtained by a modification of an available pyridine derivative or from total synthesis. The latter was considered first after a review of the use of oxazoles[136-138] in the synthesis of pyridoxine derivatives. The oxazole nucleus can serve as a dienophile and thus react with various olefins to yield substituted pyridines. The proposal outlined in Figure 7 initially appeared to be a reasonable source of the properly functionalized pyridone 142. Diels-Alder addition between the oxazole 138 and the olefin 139 would be

expected to lead to mixtures of the intermediate adducts 140. Treatment of the adducts 140 with base would be expected to lead to the desired pyridone 142 and its position isomer 141.

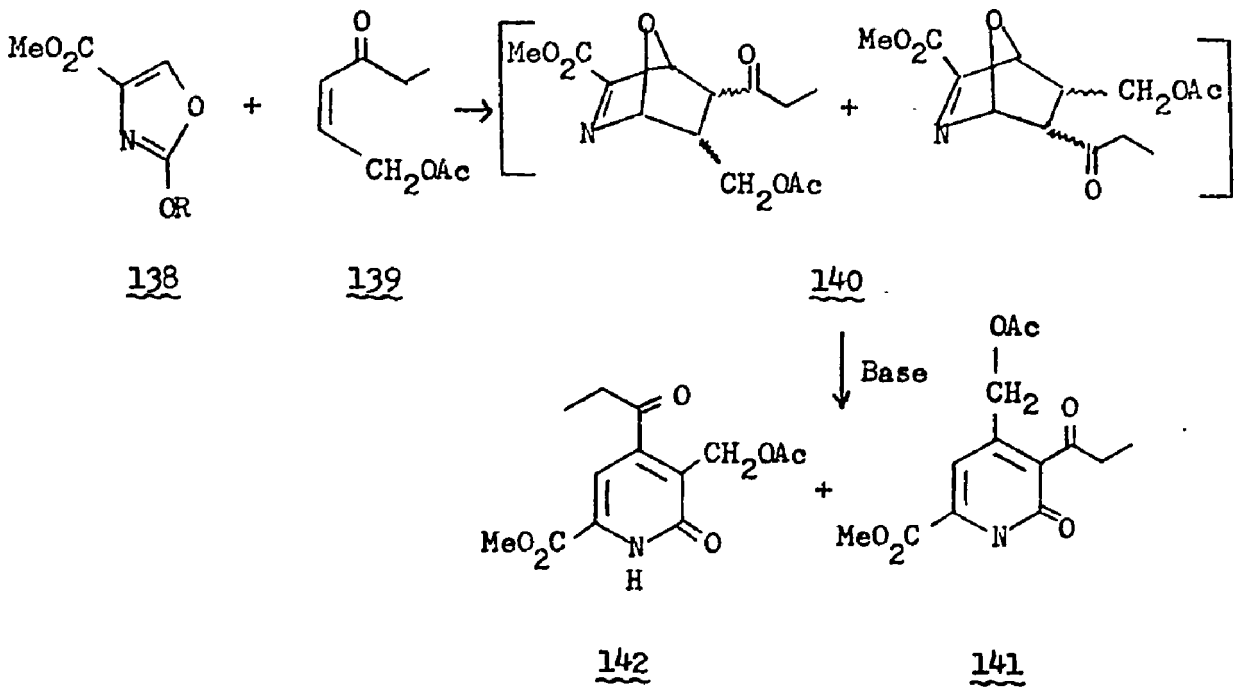
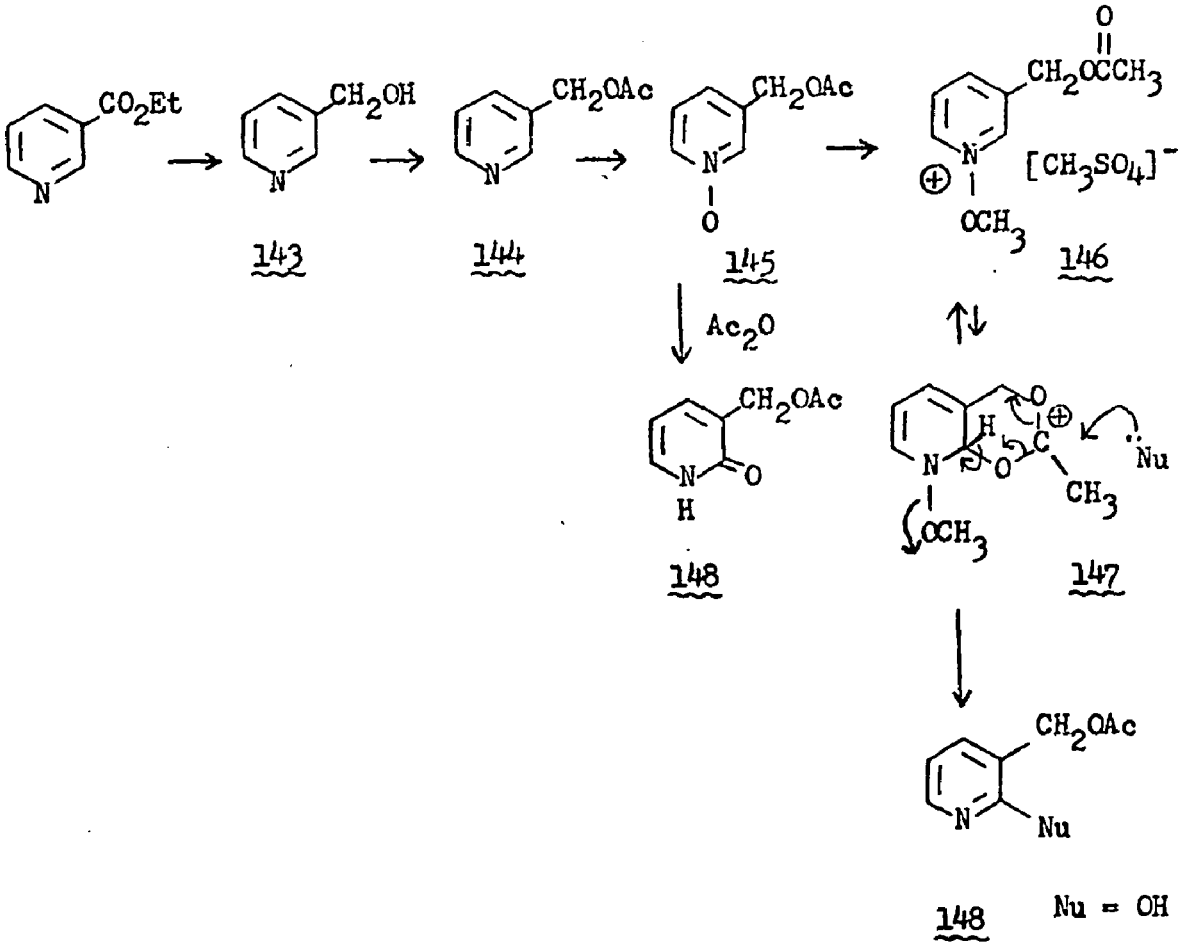



Figure 7. Proposed synthesis of substituted pyridone 142.

The high concentration of hydrogen cyanide involved in the synthesis of the oxazole[139] coupled with the poor dienophilic character of this oxazole, owing to the electron withdrawing carboxyl group, resulted in a major effort to modify an available pyridine derivative. This problem was subdivided into three parts: 1) efficient conversion of a pyridine to pyridone; 2) synthesis of the lactone E ring; 3) introduction into a pyridine nucleus of a 6-carboxyl group to allow construction of the C ring.

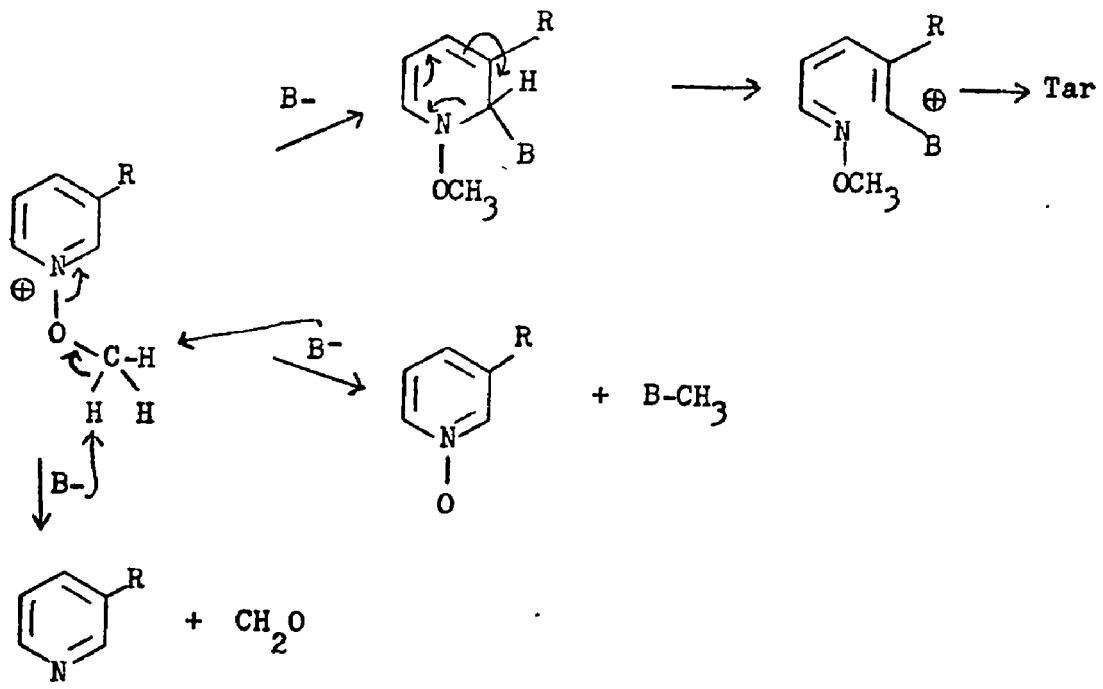
The conversion of pyridine N-oxides to pyridone via the acetic anhydride rearrangement has already been described (p. 55). This rear-

rangement, however, is usually accompanied by excessive decomposition with yields of rearranged products ranging from 30 to 60%. One of the groups that constitute the lactone portion of camptothecin is a hydroxymethyl group, and attempts were made to facilitate the pyridine to pyridone conversion through neighboring group participation of the hydroxymethyl group. In a model system, ethyl nicotinate was reduced to 3-hydroxymethyl pyridine[140] 143, which was acetylated to 144. It in




turn was converted to the N-oxide 145 by treatment with hydrogen peroxide in acetic acid. Treatment of the N-oxide 145 with dimethyl sulfate produced the 1-methoxypyridinium ion 146, which probably exists to some measure as 147 owing to participation of the acetate group. The salt 146 was treated with aqueous sodium hydroxide solution at pH 8 and also

at pH 14, under varying temperatures ranging from -10° to 40°. This resulted in an unattractive decomposition product with no traces of desired product. Occasionally the starting material was recovered when the reaction was conducted at -10° and pH 8. Where the nucleophile is also a strong base, some of the types of decompositions that methoxypyridinium ions undergo have been described[141] as shown. A

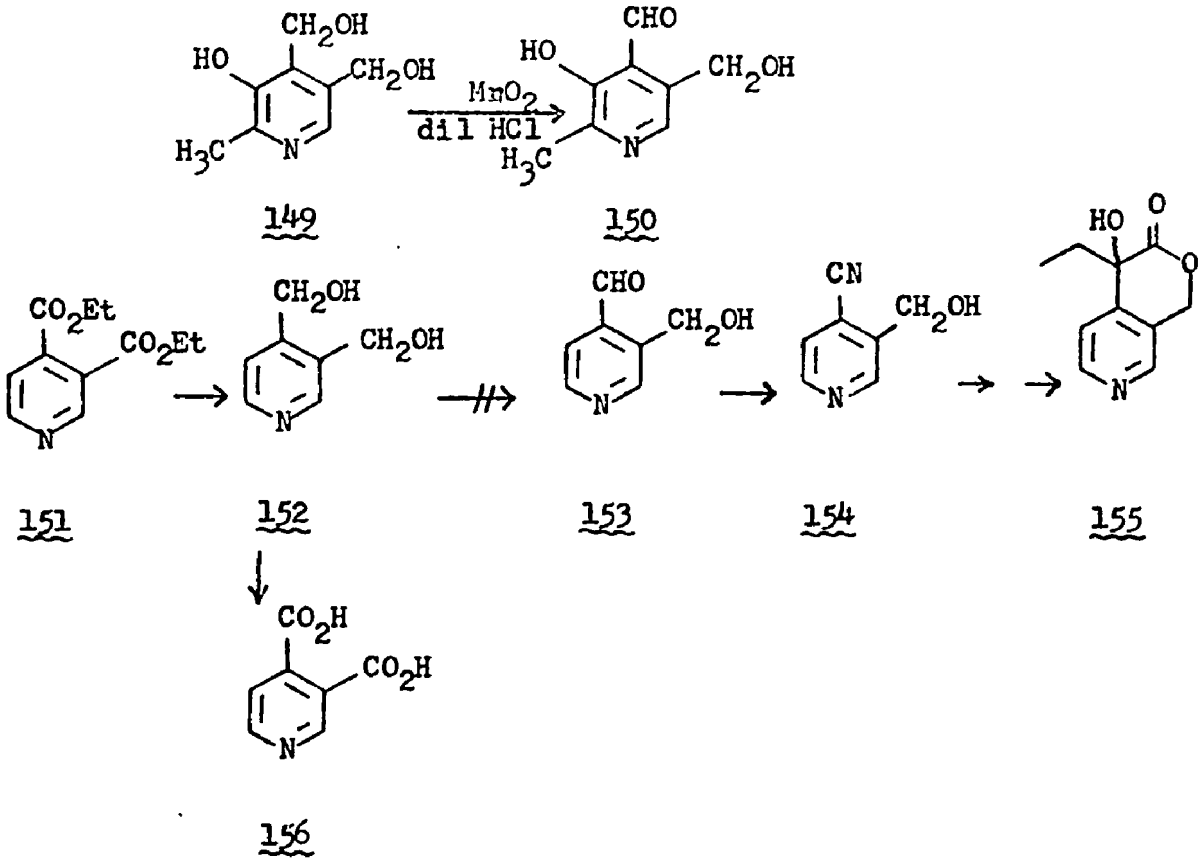



saturated aqueous solution of sodium acetate was added to the salt 146, first at room temperature (12 hr) and then at reflux (8 hr); however, most of the starting material was recovered yielding less than 5% of the desired pyridone. Warming the N-oxide 145 in a large excess of acetic anhydride at 100° for a little over an hour resulted in 80% conversion of the pyridine derivative to the desired pyridone. TLC analysis showed that the reaction resulted in only one major product with trace amounts of impurities. The identity of the major product was determined spectro-

scopically to be 148. The nmr of the aromatic portion of 148 consisted of two doublets and a doublet of doublets typical for three adjacent protons.

The next problem approached involved attempted formation of the E ring. Based on the precedent of the efficient oxidation of 149 to pyridoxal 150 by manganese dioxide efforts were made to oxidize the alcohol 152 to 153. The aldehyde 153 was expected to be transformed to the desired lactone as outlined in the scheme below.

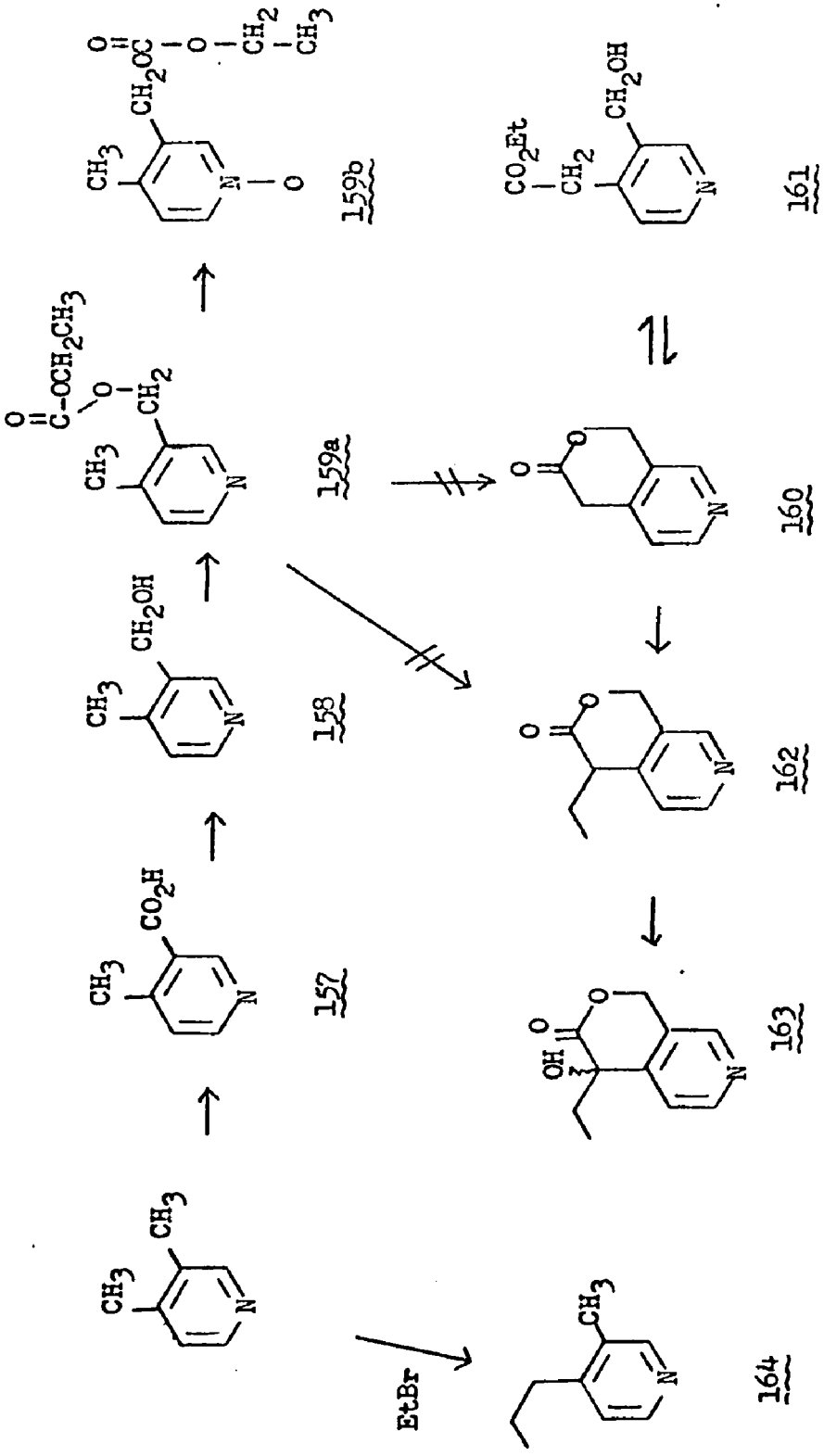
Various preparations of active manganese dioxide were used. Obtained by various procedures,[143,144] the preparations were checked for



activity by oxidation of benzyl alcohol to benzaldehyde. In dilute hydrochloric acid, none of the manganese dioxide preparations oxidized the alcohol 152. When dimethyl sulfoxide was used as a solvent, manganese dioxide oxidized 152 all the way to the diacid 156. Attempts to effect selective oxidation by reducing the amount of manganese dioxide in dimethyl sulfoxide resulted only in decreased yields of 156, and no selective oxidation was observed. In the case of the successful oxidation of 149 to 150, the 3-hydroxyl group may play a part in orienting $MnO_2$ in such a manner that the 4-hydroxy methyl is oxidized more readily than the 5-hydroxymethyl group.

3,4-Dimethylpyridine was then chosen as an alternate pyridine derivative allowing addition of the E ring. The crucial reactions in modifying this compound were the selective oxidation of the 3-methyl group and ethoxycarbonylation at the activated 4-methyl group. These reactions would lead to the basic lactone ring, and the alkylation and hydroxylation necessary to convert this lactone to the final E ring were viewed as reasonably realizable. Plattner[145] oxidized 2-methyl-5-ethylpyridine with potassium permanganate hoping to oxidize the more acidic 2-methyl group and obtain a picolinic acid derivative; however, he observed that the 5-ethyl group was oxidized instead, and 6-methylnicotinic acid was isolated. Furthermore, efficient carbomethoxylation of 3-ethyl-4-methylpyridine by means of dimethyl carbonate and diisopropyl lithium amide reported by Uskokovic[146] served as a good precedent for proposed reactions outlined as follows.

Potassium permanganate treatment of 3,4-dimethylpyridine gave the desired 4-methylnicotinic acid 157 in 60% yield. The acid was esterified with ethanolic hydrogen chloride, and the ester was reduced with lithium-


EtBr
164
157
158
159a
159b
160
161
162
163

alluminiumhydride to give the alcohol 158 in 75% yield from 157. Treatment of the alcohol with ethyl chloroformate in triethylamine and THF gave a 50% yield of a 3:1 mixture of product and starting material as analyzed by vpc. However, conduct of the reaction in cold aqueous sodium hydroxide under Schotten-Baumann conditions gave a 90% yield of the desired carbonate ester 159a; as determined by vpc, the product was > 90% pure. The carbonate ester 159a was obtained as a yellowish oil, and attempts to distill it led to complete decomposition. Even on standing the yellowish oil turned brown. Treatment of 159a with m-chloroperbenzoic acid in chloroform afforded the N-oxide in good yield. The various efforts that were made to effect intramolecular ethoxycarbonylation of both 159a and its N-oxide 159b are summarized in Table V.

TABLE V

Summary of reactions of 159a and its N-oxide with various bases

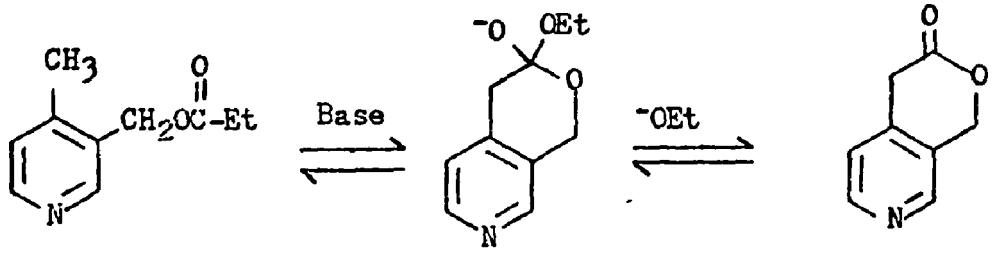
| Reagent | Solvent | Base | Temperature, °C | Product |
|---|---|---|---|---|
| 159a | THF | LDiPA+ | -78 | 159 + 158 |
| 159a | THF | LDiPA | 25 | 158 |
| 159a | THF | LDiPA | reflux | 158 |
| 159a | THF/HMPA+ | LDiPA | reflux | 158 |
| 159a | t-Butanol/HMPA | KOtBu | reflux | 158 + tar |
| 159a | diethyl carbonate | LDiPA | reflux | 159 + 158 |
| 159a | ØH | NaOEt | reflux | 158 |
| 159b | THF | LDiPA | reflux | 158 N-oxide |
| 159b | ØH | NaOEt | reflux | 158 N-oxide |
| 159 + EtBr | THF/HMPA | LDiPA | reflux | 158 |
| 159 + EtBr | t-Butanol | KOtBu | 25 | 158 |

+ LDiPA, lithiumdiisopropylamide; HMPA, hexamethylphosphoramide.

All attempts to convert 159a to 160 by varying solvents, base or temperature proved unsuccessful. Invariably, under various reaction conditions, hydrolysis of the ethyl carbonate ester occurred giving 158. The reason for this hydrolysis is not yet clear. Initially, the reactions were quenched by dilute hydrochloric acid. To minimize hydrolysis during work up, glacial acetic acid was used; however, the results remained the same. When the reaction was run at low temperatures (-78°), the product isolated consisted of a 1:1 mixture of starting material and the hydrolysis product 158, as determined by vpc. To increase the chances of ethoxycarbonylation, the reaction was run in diethyl carbonate as a solvent with a few drops of HMPA added to solvolyze the base. But, after work up, no desired product could be detected. VPC analysis revealed that a mixture of both 158 and 159a was the only product. The major fraction was the starting material 159a.

In order to show that the base used in the reaction was sufficiently strong to generate the desired anion, 3,4-dimethylpyridine was treated with ethyl bromide in the presence of lithiumdiisopropylamide in tetrahydrofuran and 80% of alkylated product was isolated. VPC analysis of the product showed that 90% was 3-methyl-4-propylpyridine (164) and 5% was 3-propyl-4-methylpyridine. The remaining fraction which had a long retention time was probably polyalkylated material. Attempts were thus made to treat equimolar amounts of the carbonate ester 159a and ethyl bromide with lithiumdiisopropylamide or potassium t-butoxide in the hope of effecting both ethoxycarbonylation and alkylation to yield 162. However, neither ethoxycarbonylation nor alkylation occurred since, after work up, only the hydrolysis product 158 and traces of the starting ester 159a were obtained.

The simple and only reason that could be found to explain the formation of 158 in all the attempted reactions described is hydrolysis of unreacted ester 159a during work up. However, a good reason that will explain the failure of 159a to undergo intramolecular ethoxycarbonylation is yet to be found. It was suggested[147] that treatment of 159a with base probably generates the intermediate 165, which is in equilibrium with the starting material 159a. Thus, the driving force in the conversion of 159 to 160 is elimination of ethanol from 165. If the ethanol were distilled from the reaction mixture, the reaction could theoretically be driven to completion. The base lithiumdiisopropylamide can not cause the elimination of ethanol since only ethoxide and diisopropylamine are generated. Hence, the ester 159a was treated with sodium ethoxide in benzene with provision made for the continuous distillation of benzene and any formed ethanol. At different intervals of time, aliquots were quenched with acetic acid and analyzed by vpc to follow the progress of the reaction. After 30 minutes, all the 159a was

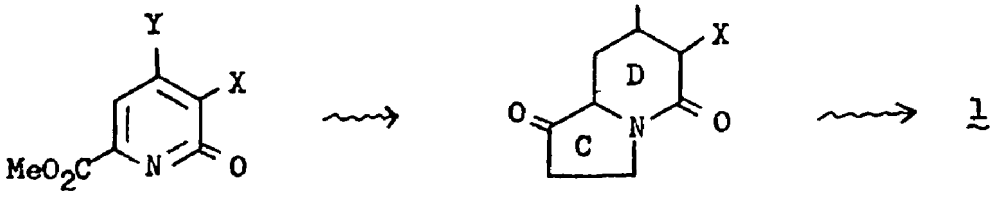



159 165 160

converted to 158. It seems in this case that conversion of 159a to 158 was even faster than the previous cases, presumably owing to the ethoxide nucleophile attacking at the carbonate ester generating diethyl carbonate and the sodium salt of 158.

Finally to increase the acidity of the 4-methyl group of 159a and, hopefully, to increase its chances of undergoing ethoxycarbonylation, 159a was converted to its N-oxide 159b. The N-oxide is a yellowish gummy solid that is very hygroscopic. Attempts to crystallize it as a picrate was unsuccessful, probably because of instability of the picrate. Treatment of 159b with sodium ethoxide and benzene, with continuous distillation of the solvent, led only to the hydrolyzed product 158 N-oxide. Similarly, conducting the reaction with lithium-diisopropylamide in tetrahydrofuran and hexamethylphosphoramide also led to 158 N-oxide with traces of starting material. The well precedented and mechanistically straight forward method of ethoxycarbonylation in our hands proved to be more complicated than can be explained. Furthermore it proved useless in providing the E ring of camptothecin.

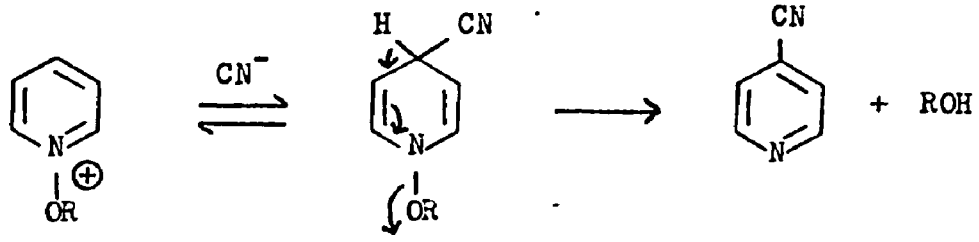
While efforts to modify X and Y so as to construct the lactone ring were in progress, the last problem of the pyridine modification - namely the selective introduction of the 6-carbomethoxyl group - was also being investigated. This group is essential for the introduction of the C-ring 166 (see p. 54).



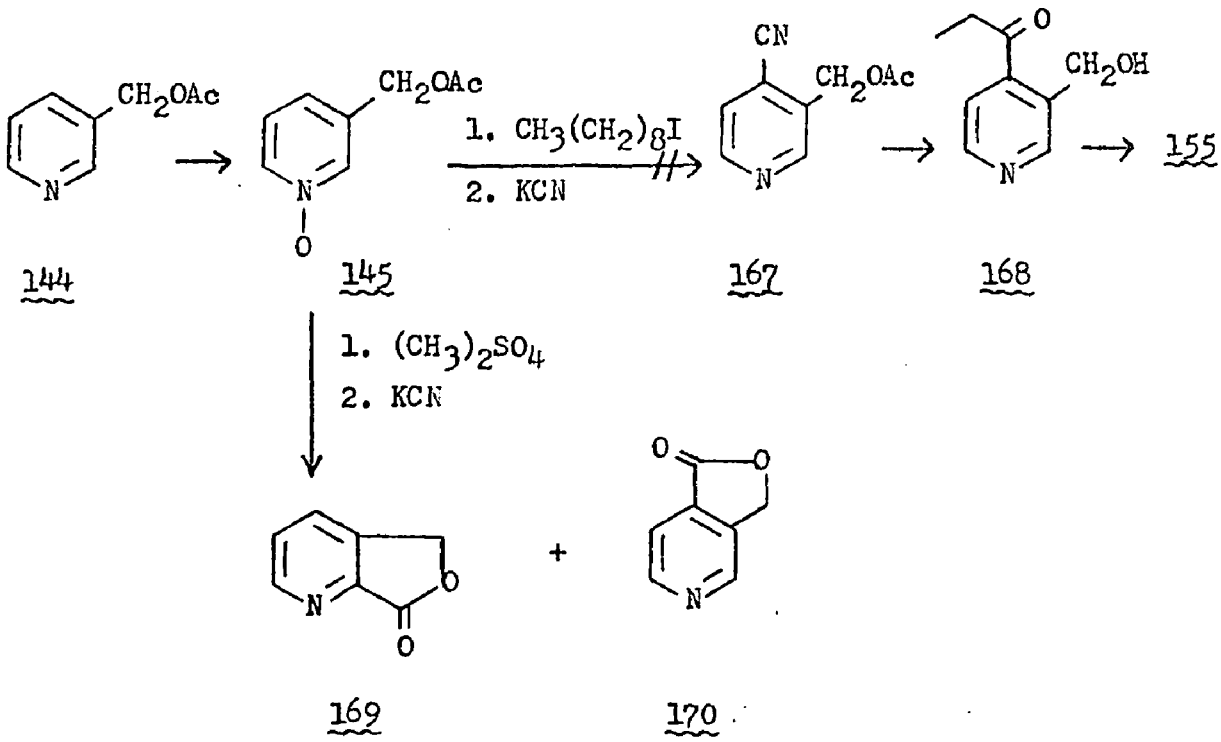

136 166 1

One method of introducing the carboxylic acid group at the pyridine is via cyanation. There are several procedures that can lead to cyanation of the pyridine nucleus. Reactions of pyridine N-oxides with

benzoyl halides and silver cyanide (Reissert reaction)[148] lead to small yields of cyanopyridines. Treatment of pyridyl diazonium salts[149] with potassium cuprous cyanide also leads to poor yields of cyanopyridines, usually with better yields for the 3 and 5 diazonium salts than for the 2, 4 and 6 salts. Cyanopyridines can also be prepared by heating halogenopyridines[150] with cuprous cyanide. The reaction tends to be erratic, but under well worked out procedures good to moderate yields of cyanopyridines may be obtained. Of practical importance are the reactions of quaternary salts of pyridine N-oxides with aqueous potassium cyanide. The 1-methoxypyridinium methosulfates have usually been used, and with aqueous potassium cyanide the 2-, 4- and 6-cyanopyridines result. The quaternary function is eliminated as an alcohol and its ready elimination makes the reaction a practical source of cyanopyridines.[151] Change of the quaternizing group from methoxy to nonyloxy changes the substitution pattern from predominantly 2-cyano to purely 4-cyanopyridine, respectively.



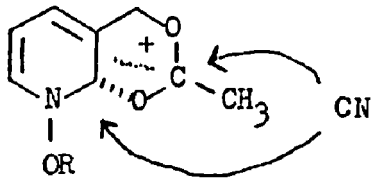

Ethyl nicotinate was taken as a model compound owing to its ready availability. It was reduced and acetylated as described previously (p. 59), and the acetate 144 was converted to its N-oxide 145 by hydrogen peroxide in acetic acid. p-Nonyl iodide was prepared from nonyl alcohol by Wood's[152] procedure in 89% yield. Attempts were made to react this




iodide with the N-oxide 145 to prepare the corresponding 1-nonyloxypyridinium derivative, since this salt could make a good source of the 4-cyano derivative 167. Treatment of 145 with nonyl iodide in refluxing acetonitrile resulted in a very dark brown oil which could not be characterized; it was used as such in the next reaction. Treatment of this crude dark oil with aqueous potassium cyanide gave no desired product. Instead, a very unattractive tarry product, from which no characterizable product could be identified, resulted.

Dissolving the N-oxide 145 in equimolar amount of dimethylsulfate resulted in an exothermic reaction and gave the methoxypyridinium salt 146 as a yellow oil. Treatment of the oil with an equimolar amount of potassium cyanide in alcohol-water solution, kept at -10°, gave only a 2% yield of a crystalline material which was separated from the major tar by distillation. The ir of the compound has carbonyl absorption at

1750 $cm^{-1}$ for the $\gamma$-lactone. The splitting pattern of the nmr indicate the product to be mainly 3-hydroxymethyl-2-pyridine carboxylic acid lactone (169) with possibly slight contamination by 170. The reason for the decomposition observed in these reactions is not apparent, but the poor yield in the cyanation of the pyridine nucleus could perhaps result from participation of the acetate group making the pyridine nucleus poorly electrophilic, as in 171. Also, such participation directs the cyano group into the undesired position.

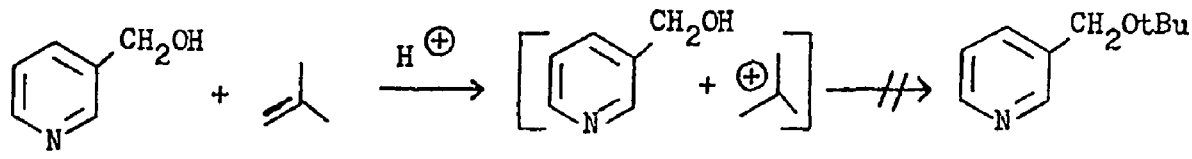



171

It was deemed necessary that the 3-hydroxymethyl group not only have a protecting group which does not undergo neighboring group participation as in 171, but at the same time the group needs to be sufficiently bulky to hinder nucleophilic substitution at the 2- and 4-positions. A protecting group that satisfies both criteria is a t-butyl group. The most common methods[153,154] of preparing t-butyl ethers involve: (1) reaction of the alcohol with isobutylene in the presence of strong acid, and reaction of an alkyl halide with potassium t-butoxide. As will be shown later, the pyridine nitrogen is a source of complication in both procedures. In any case, efforts to prepare the t-butyl ether of 3-hydroxymethylpyridine were carried out.

3-Hydroxymethylpyridine was first treated with isobutylene in ether and concentrated sulfuric acid at -78° in a pressure bottle. As the

reaction mixture was allowed to warm up to room temperature, its color turned brownish. After work up, no desired ether was observed. Instead, a brownish glass-like gum was obtained which is a result of polymerization of the pyridine in the presence of t-butylcarbonium ion. Treatment of the alcohol with thionyl chloride

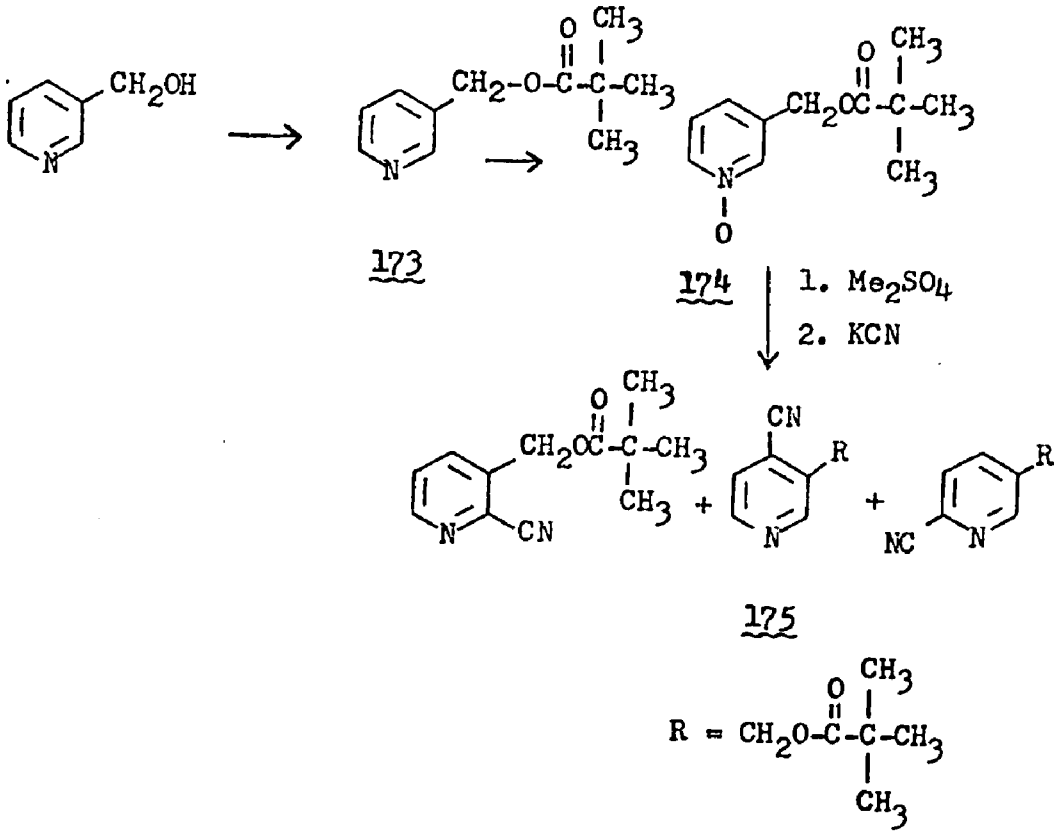


172

under mild conditions provided 3-chloromethylpyridine as a hydrochloride in quantitative yield. Attempts to liberate the free base even under low temperature failed in all cases; self-alkylation and polymerization resulted. Similarly, treatment of the hydrochloride with potassium t-butoxide also failed to give the desired ether. With these results, the idea of preparing ethers as protecting groups was abandoned.

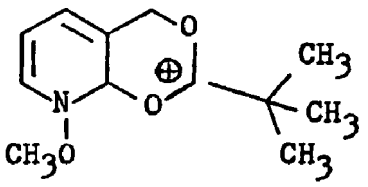
Pivaloyl esters are used as blocking groups in amino acid chemistry. However in the present case, it did not seem likely that the t-butyl group would hinder the pivaloyl ester carbonyl from participating. Nevertheless, a priori it was not possible to predict what sort of directing effect it might have on the cyanide ion. Therefore, efforts were made toward the preparation of the pivaloyl ester of 3-hydroxymethylpyridine. Parish[156] developed a convenient method for the esterification of very hindered acids involving the use of trifluoroacetic anhydride. But, repeated efforts to make the pivaloyl ester of the alcohol by Parish's

method proved unsuccessful. Only starting material was obtained in each case. But, refluxing the alcohol in excess pivaloyl anhydride for 6 hours produced the desired ester 173 in 70% yield. Subjecting the ester to the usual hydrogen peroxide in acetic acid treatment cleanly afforded the N-oxide 174 in 70% yield. Treatment of 174 first



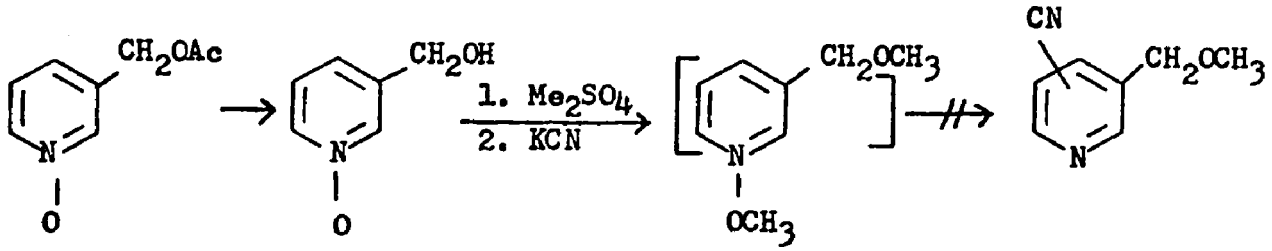

with dimethyl sulfate followed by potassium cyanide in water gave a mixture of cyano pyridines 175 in 60% yield. The boiling points of the various isomers were so close that separation by vacuum distillation was unsuccessful, but vpc analysis showed that the mixture contained three fractions. Separation of these fraction and spectral characterization of each fraction revealed the composition of the mixture to be 54% of 2-cyano-, 21% of 4-cyano-, and 25% the desired

6-cyanopyridine. The chemical shift of the pyridine protons, coupled with their splitting pattern, was instrumental in the identification of each isomer. The ratio of cyanopyridines obtained clearly shows that the ester participates facilitating the entry of the cyanide ion at the 2-position of 176.



176

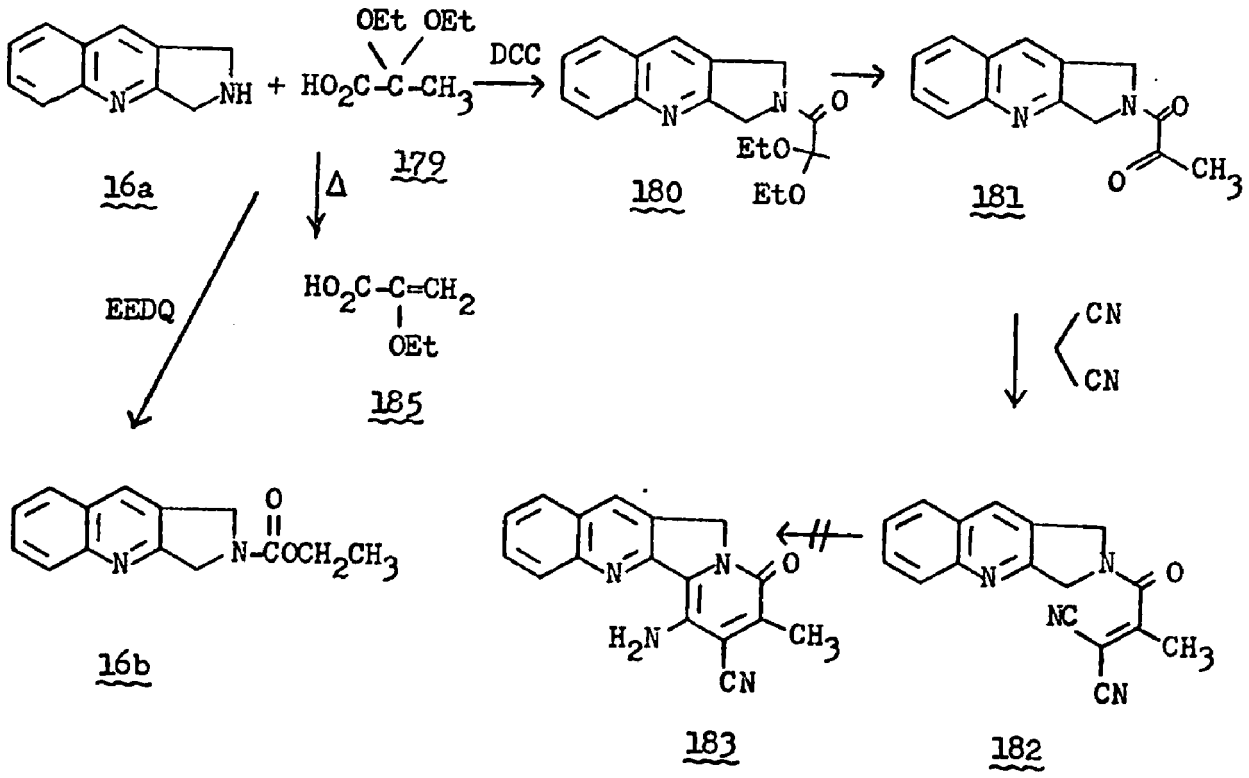
As a final effort to introduce the cyanide group at the 6-position of pyridine derivatives, the N-oxide 145 was quantitatively hydrolyzed with dilute hydrochloric acid to give 177. Treatment of 177 with two equivalents of dimethyl sulfate gave a thick oil. Without any further purification or characterization, this oil was treated with potassium cyanide, but no traces of cyano pyridine was obtained. The reaction resulted in excessive decomposition similar to that observed in the case of 145. Thus, the problem of modifying a pyridine derivative to furnish the D and E rings of camptothecin remained unsolved owing to the failure to ethoxycarbonylate 159 and also to failure of selective introduction of the cyano group into the pyridine nucleus.




145 177 178

## D. APPROACH INVOLVING PYRUVIC ACID

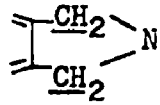
The failure to ethoxycarbonylate 109 and thus construct the E ring coupled with the failure to selectively introduce a 6-carbomethoxy group into the pyridine derivative 145, greatly limited the effectiveness of the second approach. Thus, in developing a third approach efforts were made to build the D ring with appropriate substituents from which the E ring may be constructed.

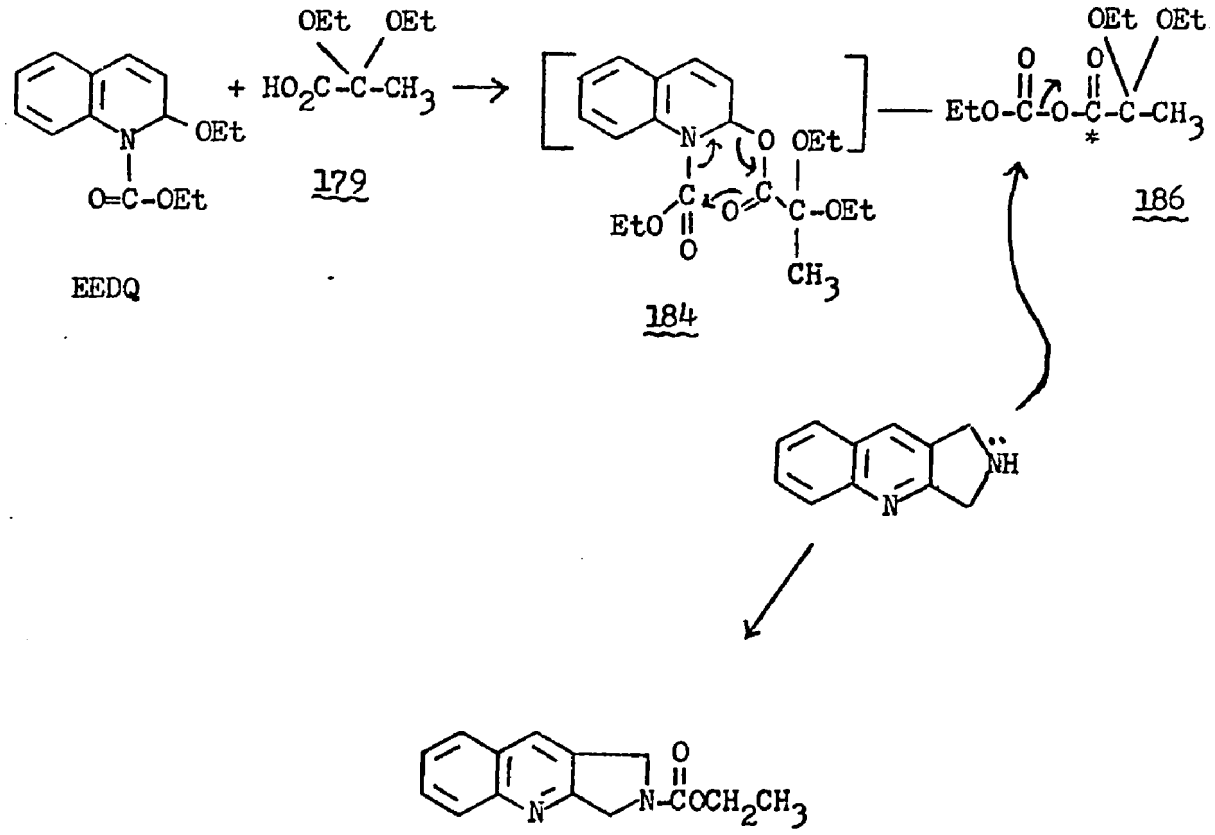



Ethyl pyruvate was treated with triethylorthoformate and p-toluenesulfonic acid in ethanol to give ethyl pyruvate diethyl ketal in 96% yield.[157] The ester was hydrolyzed using ethanolic potassium hydroxide at room temperature to give the corresponding acid 179 in 90% yield. Attempts to purify the acid 179 by distillation caused the compound to

eliminate ethanol and afford the enol ether 185 as a white solid in 50% yield. Hence, the acid 179 was kept cold soon after preparation and was used in the reactions outlined without purification.

Condensation of the acid 179 with the unstable amine[158] 16a was studied in the presence of various coupling agents (Table VI). In the presence of dicyclohexylcarbodiimide, the reaction of 179 with 16a produced the amide 180 in very low yield; tarry material being the major product. The amide 180 was contaminated by dicyclohexylurea and attempts to purify it by means of chromotography (Alumina) or recrystallization was unsuccessful. In the nmr, the integration of the aromatic protons and the downfield aliphatic protons was consistent with the assigned structure 180. Furthermore, hydrolysis of 180 in 90% acetic acid yielded the keto amide 181 as a crystalline compound. The ir showed strong absorption at 1710 and 1645 $cm^{-1}$ for the ketone and amide group respectively; and the nmr showed a methyl peak at 2.55 $\delta$ for the methyl adjacent to the ketone. The overall yield of 181 as calculated from 16a was 5%. Attempts to maximize the yield by varying reaction times and temperatures was fruitless.

Belleau[159] developed a reagent EEDQ which is excellent for coupling peptide bonds and the side products, namely ethanol and quinoline are easy to remove. Conducting the reaction of 179 and 16a in the presence of EEDQ led to a high yield (80%) of a compound whose spectral properties were inconsistent with the expected product 180. The ir showed absorption at 1680 $cm^{-1}$ (amide C=O) and nmr ($CDCl_3$) $\delta$ 8.20-752 (m, 5, aromatic protons), 4.90 (broad s, 4, ), 4.30 (m, 2, J=7 Hz, $OCH_2CH_3$), 1.33 (t, 3, J=7 Hz, $CH_2$-$CH_3$) were consistent with structure 16b, and were identical to that reported by Nabors.[9] The initial reaction of EEDQ with



EEDQ

179

184

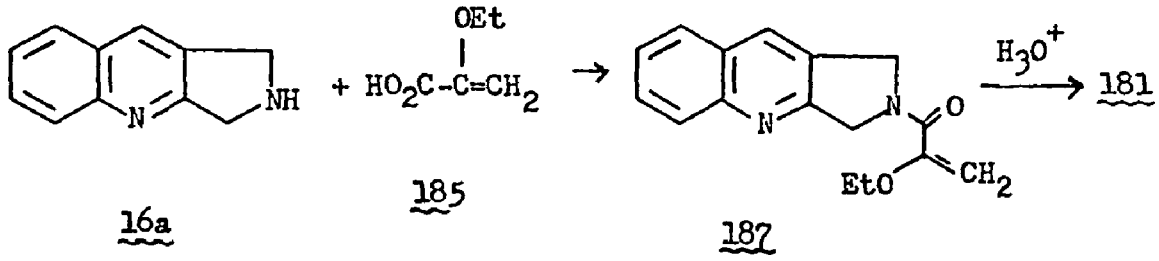
186

16b

the acid 179 is believed to involve displacement of ethoxide ion from EEDQ to yield the intermediate 184; this collapses rapidly to yield the mixed anhydride 186. This reactive anhydride has two electrophilic centers, the carbonyl with asterisk being the more reactive, nucleophilic attack ordinarily occurs at this carbonyl to give the expected amide. In the present case, however, the reactive carbonyl is highly hindered and the amine attacks the less reactive carbonyl leading to 16b.

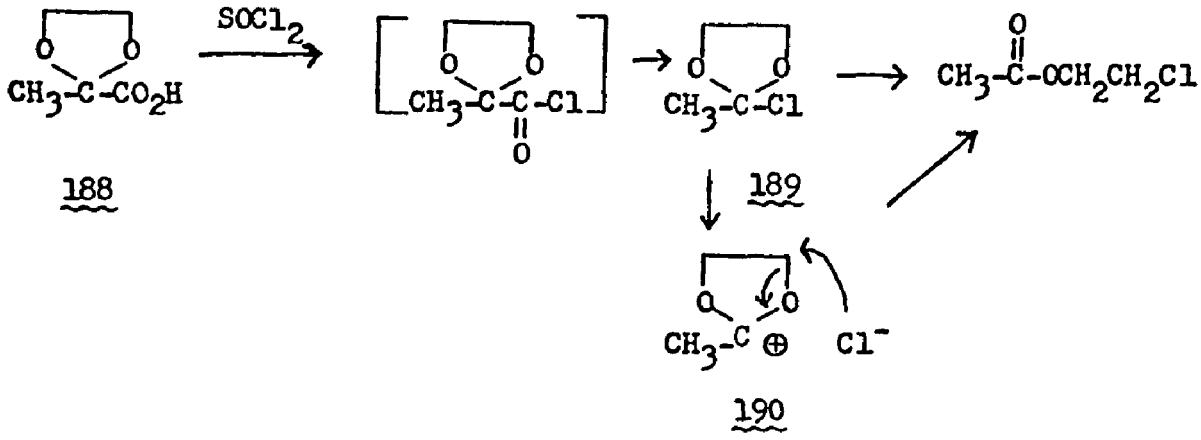
Sheehan[160] developed another peptide coupling reagent, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (DAEC). This reagent is readily soluble in dilute acid and therefore does not offer the problem of separation experienced with dicyclohexylcarbodiimide. Attempts to condense 179 with

16a in the presence of this reagent also led to excessive decomposition and no characterizable product could be isolated. The enol ether of pyruvic acid 185 lacks steric bulk $\alpha$ to the carboxyl group hence better yields of amide formation with the amine 16a were expected. Conducting the reaction of 185 and 16a in the presence of each of the three coupling reagents described led to decomposition and polymerization and only in the case of dicyclohexylcarbodiimide was there a 2% yield of the desired material 187. This was characterized by hydrolysis to the known compound 181.




16a 185 187

In connection with efforts toward the synthesis of acyl halides of $\alpha$-keto acids, the treatment of 2-carboxy-2-methyl-1,3-dioxolane (188) with thionyl chloride was reported[161] to yield 2-chloro-2-methyl-1,3-dioxolane (189). Additional work has now revealed[162,163] that even though 189 can be detected at -60° the product isolated is 2-chloro-




188 190

ethyl acetate. The proposed mechanism of this rearrangement is believed to involve initial formation of acyl halide which decarbonylates rapidly to give 189 which has the character of close ion pair as in 190. Attack of chloride ion at the dioxolane methylene then leads to 2-chloroethyl acetate. The ease of decarbonylation is attributed to the marked stability of the carbonium ion in 190. The unreactivity of 179 in the amide forming reactions can partly be explained simply on steric grounds, since the carboxyl group is very hindered by bulky substituents. But, it has been suggested[164] that the excessive decomposition observed in these reactions could perhaps be caused by some decarbonylative decomposition, through a mechanism related to that shown in the halogenation of ketals of α-keto acids.

Condensation of 2,4-DNPH pyruvyl chloride[165,166] (191b) with 16a in benzene and triethylamine led to very poor yield reaction mainly because of solubility problems. Best results were obtained when 2,4-DNPH pyruvic acid (191a) was treated with 16a in the presence of N-(dimethylaminopropyl)-N'-ethylcarbodiimide, in which 192a was obtained in 50% yield. Various attempts were made to convert 192a to the desired intermediate 181. Hydrolysis of 192a in either refluxing aqueous acetic acid or dilute hydrochloric acid resulted in recovery of unchanged starting material; whereas refluxing it in 30% sulfuric acid caused the cleavage of the amide bond yielding 2,4-DNPH pyruvic acid. Refluxing 192a in large excess of levulinic or pyruvic acid failed to cause exchange of hydrazones as unchanged 192a was recovered. 192c was also prepared by the treatment of 16a and 191c in 60% yield and once again Sheehan's imide proved to be the most suitable coupling agent for this reaction. Hydrolysis conditions used with 192a failed to convert 192c to 181. The oxime

192b is more unstable than the hydrazone 192a. The oxime undergoes slow decomposition in aqueous or ethanol solution, furthermore the amide bond cleaves even in dilute hydrochloric acid.

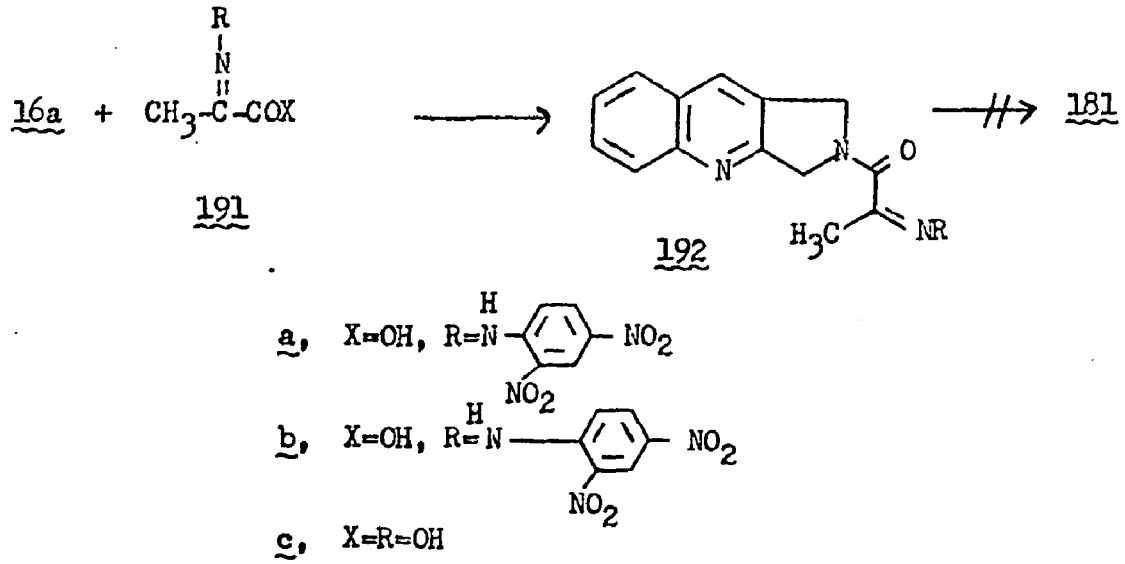
TABLE VI

Summary of reactions of 16a with various pyruvic acid derivatives in the presence of coupling agents - products isolated or expected are given figures in parenthesis represent yields

| Pyruvic acid derv. | Products and % yields | | |
|---|---|---|---|
| | DCC | EEDQ | DAEC |
| 179 | 181 (5) | 184 (80) | 181 (-) |
| pyruvic acid | 181 (5) | 181 (-) | 181 (-) |
| 185 | 181 (2) | 187 (-) | 187 (-) |
| 191a | 192a (10) | 192a (-) | 192a (50) |
| 191c | 192c (20) | 192c (-) | 192c (60) |

The synthesis of pyruvyl chloride was first reported by Carré[155] in 1936, but efforts to reproduce this early work were met with difficulties;[161,167] however, a recent modification of Carré's method converted pyruvic acid to pyruvyl chloride in 25% yield.[168] Because of the low yield and difficulty in handling it, the use of pyruvyl chloride to prepare 181 was not given serious consideration. Treating pyruvic acid with 16a in the presence of each of three coupling agents resulted mainly in the formation of intractable tar. But, in the case where dicyclohexylcarbodiimide was the coupling agent 5% of the desired pure 181 was isolated. Treatment of 181 with malanonitrile in the presence of β-alanine as a

catalyst gave an amorphous product in 20% yield whose spectral data




are consistent with structure 182. The ir has strong absorption at 2250 $cm^{-1}$ (CN) 1660 (C=O amide); nmr ($CDCl_3$) $\delta$ 8.33-7.27 (m, 5, aromatic protons), 5.33-4.50 (broad s, 4, Ar$\langle_{CH_2}^{CH_2}\rangle$N), 2.1 (s, 3, C(=N)-$CH_3$). The methyl adjacent to the carbonyl in 181 appears at $\delta$ 2.55 whereas the methyl in 182 appears at 2.1. This upfield shift is caused because the methyl in 182 lies above the shielding cone of the nitrile group. Owing to the scanty supply of 181 and difficulty in preparing it, the cyclization of 182 to 183 was not studied.

## E. APPROACH INVOLVING A FURAN CLEAVAGE

The acid catalyzed cleavage of the furan nucleus to give γ-oxo-butyric acid derivative was first studied by Marckwald[169] and its first ingenuous application to organic synthesis was demonstrated by Robinson[170] in his synthesis of ± isoequilenin (Figure 8). Surprisingly, this reaction, like the Hammick reaction, has not found much application in organic syntheses. Very recently, Johnson has utilized such a cleavage for biogenetically pattern synthesis of steroids.[171] Based on

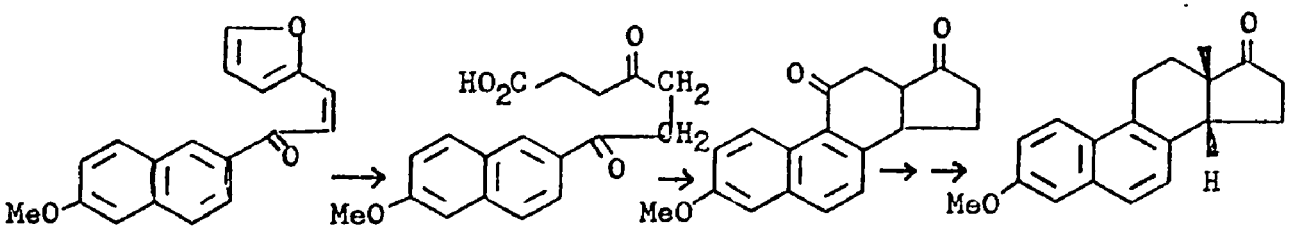
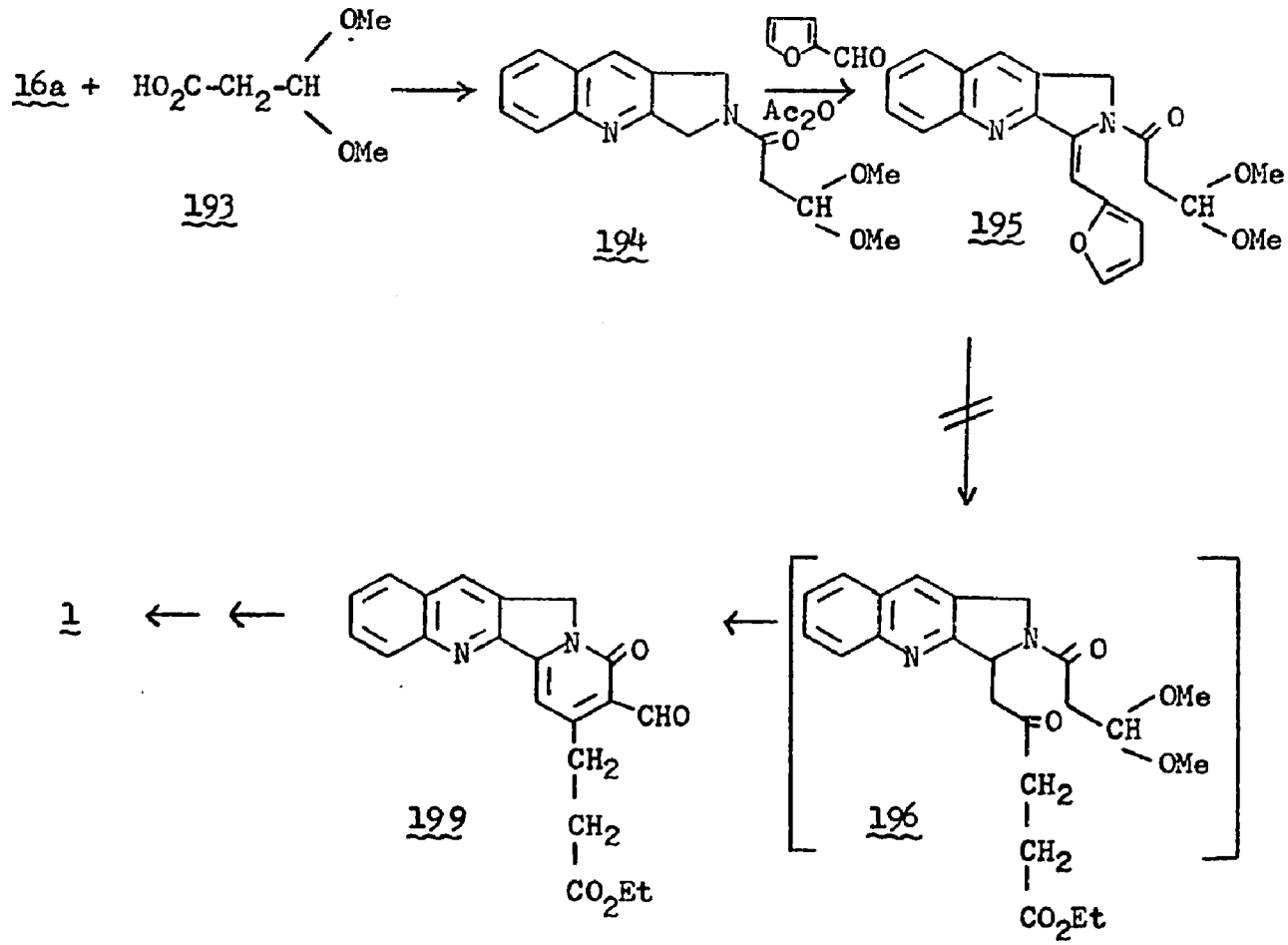



Figure 8. Robinson's synthesis of ± isoequilenin

these analogies attempts were made to utilize similar cleavage of the furan nucleus in the construction of the ABCD ring system and eventually to camptothecin.

Condensation of the dimethylacetal of formylacetic acid (193) with the amine 16a in the presence of dicyclohexylcarbodiimide gave the amide 194 in good yield[103] (85%). Both the work up procedure and the yield was improved by using Sheehan's imide as the coupling agent. Treatment of the amide 194 with furaldehyde in refluxing acetic anhydride gave a bright yellow compound in 40% yield. The ir ($CHCl_3$) showed amide absorp-

tion at 1650 $cm^{-1}$; nmr ($CDCl_3$) $\delta$ 8.05-7.25 (m, 8, aromatic protons), 6.41 (s, 1, vinyl proton), 5.11 (s, 2, Ar-$CH_2$-N), 4.70 (t, 1, J=5.8 Hz, $CH_2$-CH(O)-O), 3.23 (s, 6, CH($OCH_3$)$_2$), 2.75 (d, 2, J=5.8 Hz, -C(=O)-$CH_2$-CH) is all consistent with the desired structure 195. Com-




pound 195 proved to be extremely sensitive to acid since all attempts to cleave the furan nucleus under a variety of conditions: e.g., methanolic hydrogen chloride, p-toluenesulfonic acid in benzene in the presence of ethylene glycol, acetic acid with catalytic amount of p-toluenesulfonic acid, were all unsuccessful. Under short reaction time, it was possible to recover starting material in low yields, but in all other cases charred compound was isolated. The reason for the

extreme reactivity of 195 though not definitely known is probably caused by the presence of a nitrogen atom in conjugation to the furan system. The nitrogen probably exerts some enamine character thus increasing the reactivity of the furan nucleus. Other methods of cleaving the furan nucleus by means of bromine and anhydrous alcohol are also known,[172] however, these have not been investigated in the present case.

## IV. EXPERIMENTAL

Melting points were taken in open capillary tubes on a Mel-Temp electric block and are uncorrected. The ultraviolet spectra were determined in ethanol solution with a Cary-14 recording spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 337 spectrophotometer. The nuclear magnetic resonance spectra were obtained with a Varian A-60A spectrometer with tetramethyl silane as internal standard. Vapor phase chromotographic analysis were carried out with a Varian Model 90-P Gas chromotograph with $\frac{1}{4}$" x 5', 5% SE30 column. Mass spectrometry were carried with a DuPont mass spectrometer, Model 21-490. Elemental analysis were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

3-Hydroxymethyl-2-quinolinecarboxylic acid lactone (90).- During a period of 18 hr, 4.0 g (0.0088 mol) of 89 was refluxed in 10% $H_2SO_4$. The compound slowly went into solution. Refluxing was continued for 6 more hr. The solution was then cooled and poured onto ca 20 g of crushed ice, and it was neutralized with conc $NH_4OH$ to pH 7-8. A reddish precipitate was collected which was recrystd from benzene to yield 0.98 g (67%) of colorless crystals: mp 232-233°, ir ($CHCl_3$) 1750 $cm^{-1}$(strong, lactone C=O); nmr ($CDCl_3$) $\delta$ 8.5-8.0 (m, 5, aromatic hydrogens), 5.9 (s, 2, Ar-$CH_2$-O).

Anal. Calcd for $C_{11}H_7NO_2$: C, 71.35; H, 3.81; N, 7.56. Found: C, 71.25; H, 3.79; N, 7.70.

3-Hydroxymethyl-2-quinolinecarboxylic acid sodium salt 91.- A solution of 9.85 g (0.0049 mol) of the lactone (90) in 10% NaOH (10 ml) was refluxed for 5 hr and then allowed to sit at rt for 5 more hr. The solution was concd in vacuo, and solid residue was extracted with 3 x 10 ml abs ethanol. The combined ethanol extract was concd to yield 0.75 g (80%) of an amorphous white solid that was resistant to crystn; ir (nujol mull) 3600-3200 $cm^{-1}$ (strong, H-bonded OH), 1695 (strong, carboxylate ion C=O); nmr (DMSO-$d_6$) $\delta$ 8.32-7.53 (m, 5, aromatic hydrogens), 4.82 (s, 2, Ar-$CH_2$-OH), 3.53 (broad s, 1, O-H). This material cyclized very ready to 90 under mild acid conditions.

2-Tribromomethyl-3-carbethoxyquinoline (93).- To a mixture of 5.0 g (0.06 mol) of anhyd NaOAc, 10 ml of glacial acetic acid and 2.15 g (0.01 mol) of 2-methyl-3-carbethoxyquinoline[173] kept at 70°, was added dropwise a soln of 4.8 g (0.01 mol) of $Br_2$ in 10 ml of glacial acetic acid. Soln was stirred mechanically and after addition was completed, it was refluxed for 30 min. The solution was then cooled and poured onto ca 30 g of crushed ice, and product precipitated out as a greyish solid which after recrystn from acetic acid gave 4.04 g (90%) of colorless needles: mp 127-128°; ir (nujol mull) 1725 $cm^{-1}$ (strong ester C=O); nmr ($CDCl_3$) $\delta$ 8.30-7.47 (m, 5, aromatic hydrogens), 4.47 (m, 2, J=7 Hz, $-\overset{O}{\overset{\|}{C}}-OCH_2-CH_3$), 1.43 (t, 3, J=7 Hz, $-CH_2CH_3$).

Anal. Calcd for $C_{13}H_{10}Br_3NO_2$: C, 34.53; H, 2.21; Br, 53.07; N, 3.10. Found: C, 34.30; H, 2.21; Br, 53.19; N, 3.07.

Attempted cleavage of the lactone (90) with HBr.- Dry HBr was continuously passed through refluxing solution of 1 g (5.4 mmol) of 3-hydroxymethyl-2-quinolinecarboxylic acid lactone (90) in dry methanol (20 ml) for 12 hr. It was then cooled and allowed to stand at rt for 12 more hr before it was evaporated to dryness. A suspension of the residue and sodium bicarbonate in benzene was shaken thoroughly and filtered, and the residue was washed with 2 10 ml-portions of benzene. The combined extracts were dried ($MgSO_4$) and concentrated to give greyish solid which after recrystn from benzene afforded 0.85 g (85%) of colorless crystals which were identical to the starting lactone (90) in every respect.

Attempted cleavage of the lactone (90) with pyridine.- A solution of 1 g (5.4 mmol) of 3-hydroxymethyl-2-quinolinecarboxylic acid lactone (90) in pyridine (10 ml) was refluxed for 5 hr. After being cooled, the solution was diluted with 40 ml of anhyd ether and the precipitate was collected. The crude material (0.42 g, mp 217-225°) was contaminated with some decomposition material. Its ir was superimposable with that of the starting lactone.

3-Hydroxymethyl-2-pyridone sodium salt (101).- To a cooled solution of 1.5 g (0.01 mol) of 3-hydroxymethyl-2[1H]-pyridone acetate (148) in abs ethanol (10 ml) was added 0.23 (0.01 mol) of metallic sodium. The solution was stirred at rt for 6 hr and then was refluxed for 30 min after which it was evaporated to dryness to give 1.2 g of greyish solid; ir (mujol mull) 3500-3200 $cm^{-1}$ (H-bonded O-H), 1660 (amide C=O); nmr ($D_2O$) $\delta$ 7.88-6.47 (m, 3, aromatic hydrogens), 4.62 (s, 2, Ar-$CH_2$-O). This crude product was used in the next reaction without purification.

3-[[3-(Hydroxymethyl)-2-oxo-1(2H)-pyridyl]methyl]-quinaldine (102). (A) Alkylation in ethanol.- To a solution of 230 mg (1 mmol) of $Na^{\circ}$ in 25 ml of abs ethanol was added 1.26 g (0.75 mmol) of 3-hydroxymethyl-2[1H]-pyridone acetate (148) in 10 ml of abs ethanol. After being stirred for 15 min, a solution of 1.44 g (0.75 mmol) of 2-methyl-3-chloromethylquinoline[114] in 10 ml of abs ethanol was added dropwise. The solution was refluxed for 4 hr. It was then cooled and was filtered. The filtrate was evaporated to dryness and the residue was heated with 15 ml of water and then was extracted with chloroform several times. The chloroform layer was dried ($K_2CO_3$) and concentrated to yield 2.01 g of a gummy oil. The nmr of the crude product indicated that the oil was composed of a 1:1 mixture of the desired 102 and 2-methyl-3-hydroxymethylquinoline ethyl ether (104). Extraction of the crude oil with acetone removed the ether 104 leaving 102 as a greyish solid residue (0.75 g). Recrystn from a mixture of ethanol and water gave colorless prisms: mp 155-157°; ir ($CHCl_3$) 3500-3200 $cm^{-1}$ (O-H), 1645 (C=O amide); nmr (DMSO-$d_6$) $\delta$ 8.13-7.50 (m, 7, aromatic hydrogens), 6.30 (t, 1, J=6.5 Hz, proton para to the pyridone carbonyl), 5.40 (s, 2, Ar-$CH_2$-OAc), 4.50 (s, 2, Ar-$CH_2$-N), 3.38 (s, 3, Ar-$CH_3$).

Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 69.53; H, 5.97; N, 9.55.

(B) Alkylation in DMF.- To a stirred suspension of 0.95 g (5 mmol) of 3-hydroxymethyl-2-pyridone sodium salt (101) in 15 ml of dry DMF was added dropwise a solution of 1.03 g (5.4 mmol) of 2-methyl-3-chloromethylquinoline[114] in 10 ml of dry DMF. The solution was filtered and

the filtrate was concentrated in vacuo to yield a yellowish thick oil. On addition of ice water (30 ml), the oil solidified to give 1.34 g (84%) of a white precipitate. Recrystn from ethanol and water gave colorless prisms: mp 156-157°, identical in all respects with the sample 102 obtained in part A.

3-[[3-Hydroxymethyl)-2-oxo-1(2H)-pyridyl]methyl]-quinaldine acetate (ester) (103).- A solution of 500 mg (1.78 mmol) of the alcohol 102 in acetic anhydride (10 ml) and pyridine (5 ml). The solution was stirred at room temperature for 12 hr before it was diluted with 20 ml of ice water. It was then neutralized by the addition of solid $NaHCO_3$ and was extracted with 3 20 ml-portions of $CHCl_3$. The combined $CHCl_3$ extracts were consecutively washed with 2% HCl and water before it was dried ($K_2CO_3$) and concentrated to yield greyish solid. The crude product was passed through a short column (alumina) with methylene chloride as eluent. After evaporation of the solvent, 0.57 g (~~ 100%) of the ester 103 was obtained. Analytical sample was prepared by sublimation of the crude product at 195° (0.01 mm), followed by recrystn from ethanol: mp 122-123°; ir ($CHCl_3$) 1720 (C=O ester), 1650 $cm^{-1}$ (C=O amide); nmr ($CDCl_3$) $\delta$ 8.18-7.20 (m, 7, aromatic hydrogens), 6.27 (t, 1, J=6.5 proton para to the pyridone carbonyl) 5.35 (s, 2, Ar-$CH_2$-OAc), 5.17 (s, 2, Ar-$CH_2$-N), 2.73 (s, 3, Ar-$CH_3$), 2.13 (s, 3, O-$\overset{O}{\overset{\|}{C}}$-$CH_3$).

Anal. Calcd for $C_{19}H_{18}N_2O_3$: C, 70.79; H, 5.63; N, 8.69. Found: C, 70.80; H, 5.68; N, 8.66.

3-[[3-(Hydroxymethyl)-2-oxo-4-bromo-1(2H)-pyridyl]methyl]-2-tribromethylquinoline acetate (ester) (105b).- To mixture of 500 mg (6.1 mmol) of anhyd powdered NaOAc, 15 ml of glacial acetic acid and 265.5 mg

(0.82 mmol) of 103 kept at 70°, was added dropwise 400 mg (2.5 mmol) of bromine in 1.5 ml glacial acetic acid. The solution was stirred at 70° for 1 hr and was then boiled for 30 min. After being cooled, it was poured onto 20 g of crushed ice and brown solid precipitated, which after filtration and drying yielded 494 mg of brownish crude material. Attempts to prepare analytical sample by recrystn from ethanol, methanol, chloroform, acetone or combinations of them were unsuccessful. The crude material had mp 80-100° dec; ir ($CHCl_3$) 1720 (C=O ester), 1645 $cm^{-1}$ (C=O amide); nmr (TFAA) $\delta$ 8.84-7.8 (m, 7, aromatic hydrogens), 5.92 (s, 2, Ar-$CH_2$-OAc), 5.28 (s, 2, Ar-$CH_2$N), 2.24 (s, 3, O-$\overset{O}{\overset{\|}{C}}$-$CH_3$).

Because of instability, the compound was not analyzed.

3-[[3-(Hydroxymethyl)-2-oxo-1(2H)-pyridyl]methyl]-quinaldehyde acetate (ester) (108).- To a stirred solution of 400 mg (1.25 mmol) of 103 in 10 ml of dioxane and 0.5 ml of water was added 400 mg (3.60 mmol) of freshly sublimed $SeO_2$. The mixture was refluxed for 3 hr, was cooled, and was filtered. The residue, obtained after evaporation of the filtrate, was extracted with 5 x 20 ml of chloroform. The combined $CHCl_3$ extract was washed consecutively with 5% $NaHCO_3$, 5% KCN and water before it was dried ($K_2CO_3$) and concentrated to give 410 mg (~100%) of amorphous solid. Recrystn from benzene and pet ether gave colorless prisms: mp 156-7° ; ir ($CHCl_3$) 2870-2820 (aldehyde bands), 1720 (C=O ester), 1700 (C=O aldehyde), 1650 $cm^{-1}$ (C=O amide); nmr ($CDCl_3$) $\delta$ 10.77 (s, 1, aldehyde proton), 8.42-6.28 (m, 8, aromatic hydrogens), 5.80 (s, 2, Ar-$CH_2$-O), 5.12 (s, 2, Ar-$CH_2$-N), 2.1 (s, 3, O-$\overset{O}{\overset{\|}{C}}$-$CH_3$).

Anal. Calcd for $C_{19}H_{16}N_2O_4$: C, 67.85; H, 4.80; N, 8.33. Found: C, 67.92; H, 4.89; N, 8.22.

3-[[2-oxo-1(2H)-Pyridyl]methyl]-quinaldine (109).- To stirred suspension of 590 mg (5 mmol) of the sodium salt of 2-[1H]-pyridone in 10 ml of dry DMF (distilled over $CaH_2$) was added a solution of 960 mg (5 mmol) of 2-methyl-3-chloromethylquinoline[114] in 10 ml of DMF. After 30 hr of stirring at rt, the reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give a yellowish oil, which on addition of 20 ml of ice water solidified to afford 1.22 g of white amorphous product. The crude product was passed through a short column of neutral alumina (activity II). From the methylene chloride eluate was obtained 1.05 g (88%) of white solid. Analytical sample was prepared by sublimation at 200° (0.01 mm) followed by recrystn from ethanol water mixture to give colorless crystals: mp 160-162° ir ($CHCl_3$) 1660 (amide C=O), 1600 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 8.22-7.17 (m, 7, aromatic hydrogens), 6.72 (d, 1, J=6.5 Hz, proton ortho to pyridone carbonyl), 6.22 (t, 1, J=6.5 Hz, proton para to pyridone carbonyl), 5.30 (s, 2, Ar-$CH_2$-N), 2.70 (s, 3, Ar-$CH_3$).

Anal. Calcd for $C_{16}H_{14}N_2O$ : C, 76.78; H, 5.64; N, 11.19. Found: C, 76.80; H, 5.59; N, 11.24.

When the sodium salt of 2-[1H]-pyridone was replaced in just described experiment by the thallium salt, the product isolated showed two spots on tlc. The crude product was passed through neutral alumina (Woelm neutral grade 1). The column was eluted successively with benzene and chloroform. From the benzene eluate was obtained a white solid (0.167 g): ir ($CHCl_3$) 1600 $cm^{-1}$ C=C. Since amide C=O was absent, this product was

assigned o-alkylated structure. The chloroform eluate gave 0.185 g (45%) of white solid which after sublimation and recrystn from ethanol and water mixture gave crystals: mp 160-162°. Identical in every respect to 109 prepared by the previous procedure.

3-[[2-Chloropyridinium]methyl]-quinaldine iodide (111).- A solution of 238 mg (1 mmol) of 109 in 20 ml of anhyd toluene was refluxed with 3.07 g (20 mmol) of $POCl_3$ for 2.5 hr. The reaction mixture was then concentrated to 5 ml and treated with large excess of pet ether. After 1 hr in the ice box, the solvent was decanted from the dark red oil which settled to the bottom. The oil was dissolved in 5 ml of 0.2 N HCl and the resulting clear solution was warmed and treated with an excess of saturated KI solution. The precipitate was filtered, was washed well with cold water to yield 80 mg (20%) of a reddish compound: mp (crude) 251-252° dec, ir (nujol mull) 1625 (C=$\overset{\oplus}{N}$H salt), 1595 $cm^{-1}$ (C=C); nmr (DMSO-$d_6$/TFAA) $\delta$ 8.75-6.75 (m, 9, aromatic protons), 5.60 (s, 2, Ar-$CH_2$-N), 3.17 (s, 3, Ar-$CH_3$).

3-[[2-oxo-1(2H)-Pyridyl]methyl]-quinaldehyde (112).- To a stirred solution of 1.5 g (6 mmol) of 109 in 20 ml of dioxane and 1 ml of water was added 1.0 g (9 mmol) of freshly sublimed $SeO_2$. The mixture was refluxed for 3 hr, was cooled, and was filtered. The residue, obtained after evaporation of the dioxane, was extracted with 5 x 30 ml of chloroform. The combined chloroform extract was successively washed with 5% $NaHCO_3$, 5% KCN and water before it was dried ($K_2CO_3$) and concentrated to yield 1.60 g of crude amorphous product. The crude material was passed through a short column of silica gel (100 mesh, grade 923) and was washed with acetone and recrystn from 50% chloroform in ether to provide analytical sample: mp 204-205°; ir ($CHCl_3$) 1701 (aldehyde C=O), 1650 (amide C=O), 1595 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 10.46 (s, 1, aldehyde proton), 8.32-7.34 (m, 7, aromatic protons), 6.70 (d, 1, J=6.5 Hz, proton ortho to pyridone carbonyl) 6.28 (t, 1, J=6.5 Hz, proton para to pyridone carbonyl), 5.80 (s, 2, Ar-$CH_2$-N).

Anal. Calcd for $C_{16}H_{12}N_2O_2$: C, 72.71; H, 4.58; N, 10.6. Found: C, 72.59; H, 4.64; N, 10.57.

3-[[2-Oxo-3,5-dibromo-1(2H)-pyridyl]methyl]-quinaldehyde (113).- To a mixture of 500 mg (6.1 mmol) of anhyd powdered NaOAc, 10 ml of glacial acetic acid, and 132 mg (0.5 mmol) of aldehyde 112 kept at 70°, was added dropwise 160 mg (1.0 mmol) of bromine in 3.0 ml of glacial acetic acid. Solution was stirred at 70° for 1 hr and was refluxed for 30 min. It was cooled and was poured onto 20 g of crushed ice and a greyish solid precipitated out which after filtration and drying afforded 179 mg (85%) of crude product. Sublimation followed by recrystn from chloroform and ether provided white crystals: mp 206-207°; ir

($CHCl_3$) 1701 (aldehyde C=O), 1650 (amide C=O), 1600 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 10.43 (s, 1, aldehyde proton), 8.52-7.75 (m, 7, aromatic protons), 5.80 (s, 1, Ar-$CH_2$-N); m/e 420 ($M^+$, parent peak).

Anal. Calcd for $C_{16}H_{10}Br_2N_2O_2$: C, 45.52; H, 2.38; Br, 37.86; N, 6.64. Found: C, 45.50; H, 2.38; Br, 37.77; N, 6.65.

Attempted chlorination of 112.- To a solution of 132 mg (0.5 mmol) of the aldehyde 112 in 20 ml of anhyd benzene was added 500 mg (3.3 mmol) of $POCl_3$. The solution was refluxed for 2 hr and was allowed to stand at room temperature for 18 hr. After dilution with large excess of pet ether (40-60°), the precipitate was collected by titration to yield 143 mg of dark brown powder that decomposes at 120°. The crude material was dissolved in 10 ml of 0.2 N HCl and after filtration, filtrate was treated with excess of saturated KI solution. The precipitate was filtered, washed with cold water to give 15 mg of brown powder; ir (nujol) showed no absorption for aldehyde nor for pyridone; nmr (DMSO-$d_6$) showed very poorly resolved aromatic protons only.

3-[[2-Oxo-1(2H)-pyridyl]methyl]-quinaldic acid (115). A. Potassium permanganate oxidation of 112.- To a suspension of 132 mg (0.5 mmol) of the aldehyde 112 in 20 ml of distilled water was added 90 mg (0.57 mmol) of potassium permanganate and 4 drops of 1N NaOH solution. The mixture was well stirred and kept at 80° for 1 hr and allowed to stand at room temperature for 15 hr. The solution was filtered and the dark brown manganese dioxide residue was extracted with 3 x 15 ml of boiling water. The combined aqueous layer was concentrated to 5 ml, and the solution was acidified by the addition of 3 ml of concentrated

HCl. The acidified solution was concentrated to dryness and the remaining residue was extracted with abs ethanol. Evaporation of the ethanol gave 100 mg (70%) of an amorphous powder. Ir (KBr) 3400-2300 (acid $\overset{O}{\overset{\|}{C}}$-O-H), 1700 (acid C=O), 1645 $cm^{-1}$ (amide C=O); nmr (DMSO-$d_6$ $\delta$ 8.33-7.50 (m, 7, aromatic protons), 6.63-6.30 (m, 2, protons ortho and para to the pyridone carbonyl), 5.60 (s, 2, Ar-$CH_2$-N$\langle$ ).

The compound 115 is very hygroscopic, therefore it was characterized as its methyl ester as in 122.

B. Jones oxidation of 112.- A solution of 264 mg (1 mmol) of the aldehyde 112 in 55 ml of acetone was treated with Jones reagent (0.4 ml) added dropwise. After a few drops, precipitation began and after the addition was completed the mixture was stirred for 1 hr at 0°. Dilution with saturated brine caused the mixture to separate into a greenish lower layer and the top acetone layer. The acetone layer was extracted into 10% $Na_2CO_3$ solution. After acidification with 2N $H_2SO_4$ and saturation with sodium chloride, it was extracted with 4 x 20 ml of $CHCl_3$. The combined $CHCl_3$ extract was dried ($MgSO_4$) and concentrated to yield 42 mg (15%) of greyish solid. Physical and spectral properties were identical to that prepared by procedure A.

C. Hydrogen peroxide oxidation of 112.- A stirred solution of 66 mg (0.25 mmol) of the aldehyde 112 and 0.5 ml of 30% hydrogen peroxide in 10 ml of glacial acetic acid was kept at 90° for 3 hr. Additional portion of 0.5 ml of hydrogen peroxide was added and heating and stirring was continued for additional 8 hr. The reaction mixture was allowed to remain at room temperature for 12 hr before it was concentrated in vacuo; 5 ml of water was added to the residue and the solution was again con-

centrated and extracted into 25 ml of chloroform. The chloroform layer was extracted into 10% $Na_2CO_3$ and after acidification with 2N $H_2SO_4$ it was evaporated to dryness. The residue was extracted with abs ethanol and evaporation of the ethanol gave 10 mg (15%) of light yellow solid whose physical and spectral properties were identical to that prepared by procedures A and B.

D. Hydrogen peroxide/acetone oxidation of 112.- A solution of 100 mg (0.38 mmol) of the aldehyde 112 in 20 ml of acetone was refluxed for 1 hr with 1.5 ml of 30% hydrogen peroxide. The solution was evaporated at reduced pressure, and to the residue was added 4 ml of 10% NaOH, and allowed to stir for 15 min. After acidification with glacial acetic acid, the solution was concentrated and residue was extracted with 3 x 30 ml of abs ethanol. Evaporation of the ethanol extract gave 33 mg (30%) of the acid 115, identical in all respects to that prepared by procedures A, B and C.

3-[[2-Oxo-1(2H)-pyridyl]methyl]-quinoline (116).- The acid 115 (70 mg, 0.25 mmol) was heated to 175° under innert atmosphere ($N_2$) and kept at that temperature for 8 hr. After about 1 hr of heating, decarboxylation was essentially complete and shortly thereafter decarboxylated product began to sublime to the sides of the reaction vessel. The mixture was cooled and was extracted several times with ether and, except for small portion of charred material, everything was taken into ether. The etheral extract was concentrated to yield 40 mg (67%) of crystalline material which after recrystaln from ether provided white prisms: mp 134-135°; ir ($CHCl_3$) 1655 (amide C=O), 1595 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 9.21 (d, 1, J=2 Hz, $C_2$ proton of the quinoline), 8.25-

7.30 (m, 7, aromatic protons), 6.72 (d, 1, J=6.5 Hz, proton ortho to pyridone carbonyl), 6.25 (t, 1, J=6.5 Hz, proton para to pyridone carbonyl), 5.40 (s, 2, Ar-$CH_2$-N$\langle$).

Anal. Calcd for $C_{15}H_{12}N_2O$ : C, 76.25; H, 5.12; N, 11.86. Found: C, 75.40; H, 5.23; N, 11.88.

Thermal fragmentation of the sodium salt of 115.- A solution of 140 mg (0.5 mmol) of the acid 115 in 10 ml of 0.1N NaOH was stirred for 5 min and then evaporated to dryness. The residue was extracted with abs ethanol to give 140 mg (93%) of the salt as a greyish solid. Part (100 mg, 0.33 mmol) of the salt was transferred to a sublimator and was heated with a gentle flame at 0.015 mm pressure for 30 min. At the cold finger was collected 15 mg of light yellow compound which after recrystn from benzene gave colorless crystals: mp 105-106°; and the ir and nmr were superimposable with that of authentic 2-pyridone. The non-sublimed fragment, however, was decomposed completely and could not be characterized.

Methyl 3-[[2-oxo-1(2H)-pyridyl]methyl]-quinaldate (122).- To a solution of 140 mg (0.5 mmol) of the crude acid 115 in 5 ml of absolute methanol was added 5.0 ml of 0.3 m solution of diazomethane in ether (1.5 mmol). The solution was stirred for 15 min and was then evaporated under reduced pressure to yield 103 mg (70%) of a syrup: ir ($CHCl_3$) 1720 (ester C=O), 1650 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) $\delta$ 8.33-7.30 (m, 7, aromatic protons), 6.73 (d, 1, J=6.5 Hz, $C_3H$ of pyridone), 6.27 (t, 1, J=6.5 Hz, $C_5H$ of pyridone), 5.70 (s, 2, Ar-$CH_2$-N), 4.10 (s, 3, $\overset{O}{\overset{\|}{C}}$-O-$CH_3$).

Analytical sample was prepared by recrystn from chloroform.

Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.37; H, 4.80; N, 9.52. Found: C, 70.62; H, 5.25; N, 9.84 .

Attempted cyclization of 109. A.- To solution of 1.2 mmol of lithiumdiisopropylamide (prepared by the addition of equimolar amounts of n-butyl lithium and diisopropylamine in THF) in 20 ml of dry THF (distilled over $CaH_2$) cooled at -78° was added dropwise a solution of 125 mg (0.5 mmol) of 109 in 10 ml of THF. When the addition was complete, the solution had deep blue coloration which persisted for ca. 6 hr. After 2 hr at -78°, the reaction mixture was allowed to warm to room temperature and was stirred at that temperature for 12 hr. The solution was concentrated in vacuo and to the residue was added ca. 15 g of crushed ice. After initial acidification with acetic acid followed by neutralization with solid $NaHCO_3$; the solution was extracted with 3 x 20 ml of chloroform. The combined chloroform extracts were dried ($K_2CO_3$) and concentrated to give a greyish solid (120 mg) whose physical and spectral properties were identical to the starting material 109.

B.- When the lithiumdiisopropylamide was replaced by potassium t-butoxide and 50% t-butanol in hexamethylphosphoramide was used as a solvent at reflux temperature for 3 hr, neither starting material nor product could be isolated. After work up which was identical to that described in procedure A, only intractable tar was obtained.

4-Carbomethoxy-3-oxopyrrolidino[2,1-f]-2-pyridone (132a).- A mixture of 1.53 g (0.01 mol) of methyl 6-oxo-1,6-dihydropicolinate (130a), 4.5 ml of methyl acrylate and 1.2 g of anhyd $Na_2CO_3$ in 30 ml of dry dimethylformamide was stirred at 100° under nitrogen for 6 hr. The reaction mixture was then allowed to stand at room temperature for 18 hr and the mixture was filtered. The newly formed precipitate was

thoroughly washed with methanol. Acidification of a solution of the solid in just sufficient amount of water gave 1.47 g (70%) of a yellowish precipitate when refrigerated. Recrystn from methanol gave colorless prism: mp 210-211° dec, ir (nujol) 1700 (ester C=O), 1650 $cm^{-1}$ (amide C=O); nmr (DMSO-$d_6$) $\delta$ 7.80-6.38 (m, 3, aromatic protons) 4.57 (s, 2, O-$CH_2$-N), 3.76 (s, 3, $\overset{O}{\overset{\|}{C}}$-O-$CH_3$).

Anal. Calcd for $C_{10}H_9NO_4$: C, 57.97; H, 4.38; N, 6.76. Found: C, 57.61; H, 4.41; N, 6.92.

4-Carboethoxy-3-oxopyrrolidino[2,1-f]-2-pyridone (132b).- A mixture of 1.53 g (0.01 mol) of methyl 6-oxo-1,6-dihydropicolinate (130b), 4.0 ml of ethyl acrylate and 1.0 g of anhyd $Na_2CO_3$ in 25 ml of dry DMF was stirred at 100° under nitrogen for 6 hr. The reaction mixture was then allowed to stand at room temperature for 18 hr. Treatment and work up as described for 132a; 0.55 g (25%) of a brownish crystalline material was obtained. Recrystn from methanol gave colorless prisms: mp 173-176°; ir ($CHCl_3$) 1725 (C=O), 1700 (ester C=O), 1650 $cm^{-1}$ (amide C=O); nmr (DMSO-$d_6$) $\delta$ 7.80-6.40 (m, 3, aromatic protons), 5.50 (s, 2, N-$CH_2$-C), 3.89 (m, 2, J=6.0 Hz, O-$CH_2CH_3$), 1.21 (t, 3, J=6.0 Hz $CH_2CH_3$).

Anal. Calcd for $C_{11}H_{11}NO_4$: C, 59.72; H, 5.01; N, 6.33. Found: C, 59.60; H, 5.01; N, 6.18.

3-Oxopyrrolidino[2,1-f]-2-pyridone (128).- A solution of 1.03 g (5.0 mmol) of 132a in 50 ml of concentrated HCl was refluxed for 1 hr under nitrogen. The solution was evaporated to dryness under reduced pressure and the residue was suspended in methylene chloride. Solid $NaHCO_3$ was added to neutralize the acid, and the solid was removed by filtration. The residue was washed with fresh methylene chloride.

The combined methylene chloride filtrates were dried ($Na_2SO_4$) and concentrated to yield a light yellow solid 0.62 g (80%). Recrystn from benzene gave colorless crystals: mp 127-128°; ir ($CHCl_3$) 1745 (5-membered C=O), 1660 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) $\delta$ 7.38-6.73 (m, 3, aromatic protons), 4.33 (t, 2, J=7 Hz, N-$CH_2$-$CH_2$), 2.90 (t, 2, J=7 Hz, $CH_2$-$CH_2$-C=O).

Anal. Calcd for $C_8H_7NO_2$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.27; H, 4.68; N, 9.48.

1-Oxo-indolizino-11-[1,2-b]quinoline (79).- To a stirred solution of 80 mg (2 mmol) of NaOH in 7 ml of ethanol was added simultaneously 300 mg (2 mmol) of 128 and 250 mg (2.1 mmol) of o-aminobenzaldehyde. The solution was allowed to stand at room temperature for 24 hr. The precipitate formed was filtered and washed with methanol to yield 46 mg (10%) of crude product. Recrystn from methanol gave light yellow crystals: mp 261-263° [lit[14] 265°].

2-Oxo-3-acetoxymethyl-1,2-dihydropyridine (148). A. Acetic anhydride.- A solution of 3.0 g (18 mmol) of 3-acetoxymethylpyridine-N-oxide (145) in 25 ml of acetic anhydride was heated to 120° under nitrogen for 2 hr. The acetic anhydride was removed in vacuo, and the residue was made alkaline by the addition of saturated $NaHCO_3$ solution. Then 15 ml of water was added to the alkaline solution and the mixture was extracted with 4 x 50 ml of benzene. The combined benzene extract was dried ($Na_2SO_4$) and concentrated to give light brown solid 2.42 g (80%). The crude product was subjected to column chromotography on neutral alumina (Woelm activity I) and was eluted first with benzene

followed by 20% methanol in benzene. The methanol benzene eluate gave a white solid which after recrystn from benzene gave colorless crystals: mp 144-146°; ir ($CHCl_3$) 1720 (ester C=O), 1660 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) $\delta$ 7.63-7.32 (m, 2, pyridone $C_4\underline{H}$ and $C_6\underline{H}$), 6.32 (t, 1, J=6.5 Hz, pyridone $C_5\underline{H}$), 5.10 (s, 2, Ar-$C\underline{H}_2$-O), 2.10 (s, 3, O-$\overset{O}{\overset{\|}{C}}$-$C\underline{H}_3$).

Anal. Calcd for $C_8H_9NO_3$: C, 57.48; H, 5.43; N, 8.38. Found: C, 57.62; H, 5.44; N, 8.27.

B. Attempted rearrangement of 146 to 148 by base.- Compound 146 was prepared by the slow addition of 2.34 g (19 mmol) of technical dimethyl sulfate to 3.0 g (18 mmol) of 3-acetoxymethylpyridine N-oxide (145). The mixture was warmed to 95° for 2 hr to ensure complete addition and was then cooled. The resulting gum was dissolved in 10 ml of water and was cooled to -5° while 3 ml of 25% NaOH was added slowly over a period of 45 min. After 1 hr the reaction mixture was allowed to warm to room temperature and was stirred for 10 hr. The solution was acidified with 10% HCl and 1.0 g of dark precipitate was collected. This material was mainly decomposition product and thus could not be characterized. The mother liquor was extracted with 2 x 50 ml of chloroform. Evaporation of the combined and dried ($MgSO_4$) chloroform extract also gave intractable tar.

When the amount of NaOH added was regulated so as to maintain pH 8 throughout the duration of the reaction (11 hr), some of the starting material was identified in the aqueous layer. The chloroform extract as analyzed by tlc showed no traces of the desired compound 148; however, two spots were observed but their nature has not been determined.

C. Attempted rearrangement of 146 to 148 by NaOAc.- To a stirred solution of 3.17 g (11 mmol) of 146 (prepared according to procedure described in B) in 10 ml of 30% alcohol was added dropwise a solution of 2.67 g (33 mmol) of NaOAc in 10 ml of 30% alcohol. After the addition was completed (15 min), the solution was refluxed for 8 hr before it was concentrated to dryness under reduced pressure. The residue was dissolved in 1 ml of water and was extracted with 3 x 20 ml of chloroform. The combined chloroform extract were dried ($Na_2SO_4$) and concentrated to yield 90 mg (5%) of brownish solid. Recrystn from benzene gave colorless crystals: mp 143-145°. Identical in all respects with the sample obtained in part A.

Attempted $MnO_2$ oxidation of 152 to 153.- To a solution of 1.0 g (7.2 mmol) of 3,4-dihydroxymethylpyridine (152) in 20 ml of DMSO was added 10.0 g (140 mmol) of activated $MnO_2$ (prepared according to Goldman's procedure[143]). The mixture was stirred and refluxed for 20 hr. It was cooled and was filtered, and the recovered $MnO_2$ was washed with a small amount of DMSO. The combined DMSO filtrate was concentrated to dryness, and the residue was extracted with 3 x 50 ml of chloroform followed by 25 ml of ethanol. The chloroform layer gave mainly starting material; however, the ethanol fraction gave 0.24 g (20%) of greyish solid which after recrystn from ethanol gave colorless prisms of 3,4-pyridinedicarboxylic acid, mp 267° dec (lit[174] 265° dec). There was no depression with an authentic sample. Ir was superimposable.

When solvent was changed to 0.6 N HCl, $MnO_2$ failed to oxidize 152 and unchanged starting material was recovered.

4-Methylnicotinic acid (157).- Mixture of 5.0 g (0.047 mol) of 3,4-dimethylpyridine and 24 g (0.15 mol) of potassium permanganate in 500 ml of water was stirred at 35-40° for 2 hr. The reaction mixture was allowed to stand at room temperature for 16 hr until most of the color of the solution faded. A few drops of 20% $H_2SO_4$ was then added to catalyze the conversion of unreacted excess of $KMnO_4$ to $MnO_2$. The brown solution was filtered and the residue was suspended in 2 x 100 ml of hot water, and the combined aqueous filtrate was concentrated to 20 ml. The solutions were made slightly acidic by the addition of 40% $H_2SO_4$ and the resulting $K_2SO_4$ was removed by filtration. The filtrate was heated to boiling with a solution of 8.5 g of copper acetate in 100 ml of water. The resulting copper salt was dissolved in 50 ml of water and 20 ml of concentrated HCl. A stream of $H_2S$ was bubbled through the solution for 20 min. The solution filtered and filtrate was evaporated to dryness. The residue was pulverized and dried at 120° to give 4.0 g (53%) of white crystalline compound. Recryst from ethanol gave colorless prisms: mp 248-250° dec [lit[175] 215-216° for the free base]; ir (nujol) 3300-2600 (acid O-H), 1735 (acid C=O), 1600 $cm^{-1}$ (C=C); nmr (TFAA) $\delta$ 9.48 (s, 1, $C_2\underline{H}$), 8.90 (d, 1, J=5.5 Hz, $C_6\underline{H}$), 8.01 (d, 1, J=5.5 Hz, $C_5\underline{H}$).

4-Methyl-3-pyridylmethyl ethyl carbonate (159a). A.- To a cooled and stirred solution of 1.25 g (10 mmol) of 3-hydroxymethyl-4-methylpyridine (158) and 1.5 g (15 mmol) of triethylamine in 20 ml of THF was added dropwise a solution of 1.5 g (14 mmol) of ethyl chloroformate in 5 ml of THF. The solution was refluxed for 7 hr. It was cooled and was filtered. The filtrate was concentrated to give a thick reddish oil

which was extracted with 3 x 50 ml of ether. The ethereal extract was dried ($MgSO_4$) and concentrated to give 0.98 g (50%) of a yellowish oil. Vpc analysis of the crude product showed it to contain 3.5:1 of 159 : 158. The oil decomposed on heating, thus distillation was avoided. An analytical sample was collected from the vpc: ir (neat), 1740 (ester C=O), 1600 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 8.60 (s, 1, $C_2\underline{H}$), 8.50 (d, 1, J=5.0 Hz, aromatic $C_6\underline{H}$); 7.17 (d, 1, J=5.0 Hz, aromatic $C_5\underline{H}$), 5.23 (s, 2, Ar-$C\underline{H}_2O$), 4.23 (m, 2, J=7 Hz, O-$C\underline{H}_2$-$CH_3$), 2.40 (s, 3, Ar-$C\underline{H}_3$), 1.32 (t, 3, J=7 Hz, O-$CH_2C\underline{H}_3$).

Anal. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.61; H, 6.10; N, 7.81.

B.- A mixture of 1.25 g (10 mmol) of 3-hydroxymethyl-4-methylpyridine (158), 10 ml of ice water and two drops of phenolphthalein in 100 ml 3-necked flask, fitted with magnetic stirrer and two additional funnels was cooled in an ice bath. To the stirred solution was added simultaneously in a dropwise fashion 2.0 g (18 mmol) of ethyl chloroformate and 10% NaOH over a period of 30 min at such a rate that the solution remained slightly basic. The temperature was maintained at 10-15° throughout. After the addition was completed, stirring was continued for 45 min. The solution was extracted with chloroform. Drying ($K_2CO_3$) and evaporation of the chloroform extract gave 1.72 g (95%) of a clear oil. By vpc analysis, the crude product was found to contain 95% of the carbonate ester 159a and 5% of the starting alcohol 158. The ester 159a obtained by this procedure was identical in all respects with the sample obtained in part A.

4-Methyl-3-pyridylmethyl ethyl carbonate N-oxide (159b).- To a cooled (-5°) solution of 1.0 g of 85% m-chloroperbenzoic acid (4.9 mmol) in 20 ml of dry chloroform was added dropwise a solution of 900 mg (4.6 mmol) of the carbonate ester 159a in 5 ml of chloroform. The temperature was allowed to rise to room temperature and the solution was stirred at room temperature for 30 hr. The solution was washed with saturated solution of $NaHCO_3$ and then with water. Drying ($MgSO_4$) and concentration of the chloroform layer yielded 0.8 g (76%) of yellowish oil that solidified slowly: ir (neat) 1740 (ester C=O), 1690 (amide C=O) 1602 (C=C) 1165 $cm^{-1}$ (N-O); nmr ($CDCl_3$ $\delta$ 8.33 (s, 1, aromatic $C_2\underline{H}$), 8.15 (d, 1, J=6 Hz, aromatic $C_6\underline{H}$), 7.18 (d, 1, J=6 Hz, aromatic $C_5\underline{H}$), 5.17 (s, 2, Ar-$C\underline{H}_2$-O), 4.26 (m, 2, J=7 Hz, -O-$C\underline{H}_2CH_3$), 2.38 (s, 3, Ar-$C\underline{H}_3$), 1.33 (t, 3, J=7 Hz, O-$CH_2$-$C\underline{H}_3$).

Owing to instability, its picrate could not be obtained as analytical sample, the compound 159b was characterized as its hydrolytic product (158 N-oxide).

Hydrolysis of 159b.- To a solution of 138 mg (6 mmol) of sodium hydroxide in 10 ml of absolute methanol was added dropwise a solution of 316 mg (1.5 mmol) of 159b in 3 ml of methanol. The reaction mixture was stirred at room temperature for 12 hr and was refluxed for 2 hr. The solvent was removed in vacuo and the residue was dissolved in 10 g of ice water and the solution was acidified by the addition of 10% HCl. The solution was dried under reduced pressure and the residue was extracted with 50 ml of absolute ethanol. Evaporation of the ethanol gave 166 mg (80%) of a dark oil: ir ($CHCl_3$) 3400-3200 $cm^{-1}$ (OH); nmr (DMSO-$d_6$) $\delta$ 8.30 (s, 1, aromatic $C_1\underline{H}$), 8.13 (d, 1, J=5.5 Hz, aromatic $C_6\underline{H}$), 6.97 (d, 5, J=5.5 Hz, aromatic $C_5\underline{H}$), 4.65 (s, 2, Ar-$C\underline{H}_2$-OH), 2.30 (s, 3, Ar-$C\underline{H}_3$).

Attempted intramolecular ethoxycarbonylation of 159a. A. In lithiumdiisopropylamide.- To a stirred and cooled (-78°) solution of 175 mg (1.6 mmol) of lithiumdiisopropylamide [prepared by the addition of 150 mg (1.6 mmol) of diisopropylamine and 0.6 ml of 11.5% n-BuLi solution] in 10 ml of anhyd THF was added dropwise a solution of 220 mg (1.1 mmol) of the carbonate ester 159a in 5 ml of THF. The solution was kept under nitrogen at -78° for 3 hr, after which it was allowed to warm up to room temperature for 1 hr. The solution was concentrated in vacuo and the residue was dissolved in 10 ml of ice water. The aqueous solution was acidified with 10% HCl and after neutralization by the addition of solid $NaHCO_3$, it was extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and concentrated to give 180 mg of a yellowish oil which by nmr and vpc analysis was shown to consist of 1:1 mixture of starting material 159a and its hydrolytic product 158.

Various modifications of this basic procedure were attempted: 1. conducting the reaction at room temperature; 2. at reflux; 3. changing the solvent to 10% hexamethylphosphoramide in THF; 4. using diethyl carbonate as the solvent. In all cases, the base used was lithiumdiisopropylamide. After work up (reactions were quenched with acetic acid instead of 10% HCl), only 3-hydroxymethyl-4-methylpyridine (158) was identified by its nmr, ir and vpc retention time.

B. In potassium t-butoxide.- A solution of 100 mg (0.55 mmol) of 159a in 15 ml of 10% hexamethylphosphoramide in t-butanol was treated with 80 mg (0.71 mmol) of potassium t-butoxide at 10° under nitrogen. The reaction mixture was stirred at 10° for 2 hr and then was allowed

to remain at room temperature for 10 hr. It was then warmed to 100-110° for 2 hr. The solution was concentrated in vacuo and the residue was dissolved in 10 ml of water. After acidification with acetic acid followed by neutralization with $NaHCO_3$, it was extracted with chloroform. The chloroform extract was dried ($K_2CO_3$) and concentrated to give a dark thick oil which was purified by ether extraction. The ether extract gave 35 mg of a clear oil which by its ir and vpc was identified to be 158. The residue from the ether extract was uncharacterizable tar.

C. In sodium ethoxide.- A solution of 100 mg (0.5 mmol) of the carbonate ester 159a and 32 mg (0.5 mmol) of sodium ethoxide in 40 ml of anhyd benzene was distilled in an apparatus equipped with a Dean-Stark separator. The volume of benzene was replenished from an addition funnel during the course of the distillation. After 30 min, the solution was concentrated to dryness and the residue was dissolved in 10 ml of ice water. After acidification with acetic acid followed by neutralization by $NaHCO_3$, the aqueous layer was extracted with chloroform. The chloroform extract was dried ($K_2CO_3$) and evaporated to yield 0.60 g (95%) of the alcohol 158 as confirmed by its ir and nmr.

Attempted intramolecular ethoxycarbonylation of 159b.- Intramolecular ethoxycarbonylation of 4-methyl-3-pyridylmethyl ethyl carbonate N-oxide (159b) was also attempted by use of exact procedures described for 159a. Similar to the case of 159a, the product isolated after work up was mainly 4-methyl-3-hydroxymethylpyridine N-oxide as determined by its ir.

Attempted alkylation and ethoxycarbonylation of 159a.- To a stirred and cooled (-78°) solution of 175 mg (1.6 mmol) of lithiumdiisopropylamide in 10 ml of THF was added dropwise a solution of 220 mg (1.1 mmol) of 159a and 120 mg (1.1 mmol) of ethyl bromide in 5 ml of THF. The solution was kept under nitrogen at -78° for 1 hr and refluxed for 5 hr. It was then concentrated in vacuo and the residue was dissolved in 10 ml of ice water. After acidification with acetic acid followed by neutralization with $NaHCO_3$, it was extracted with chloroform. The chloroform extract was dried ($MgSO_4$) and concentrated to give oil which was identified by its ir as 158.

Repeating foregoing procedure with potassium t-butoxide as base and t-butanol as the solvent also gave 158 as the only product after work up.

3-Methyl-4-propylpyridine (164).- To a stirred solution of 3.42 g (32 mmol) of lithiumdiisopropylamide (prepared from 23 ml of 11.5% solution of n-BuLi and 3.23 g of diisopropylamine) in 30 ml of dry THF kept at -78° and under nitrogen, was added at a fast dropping rate a solution of 3.21 g (30 mmol) of 3,4-dimethylpyridine in 10 ml of THF. The reaction mixture changed color from colorless to deep blue. Stirring was continued for 15 min and a solution of 3.27 g (30 mmol) of ethyl bromide in 10 ml of THF was added dropwise over a period of 30 min. After addition was completed, the reaction mixture lost its intense color and stirring was continued for 1 additional hr. The solution was concentrated and the residue was dissolved in 25 ml of ice water. After acidification with 10% HCl followed by neutralization with solid $NaHCO_3$, the aqueous solution was extracted with chloroform. The chloroform ex-

tract was dried ($MgSO_4$), concentrated and distilled to yield 3.20 g (80%) of alkylated product. Vpc analysis showed the product to be 90% 3-methyl-4-propylpyridine: bp 84-85° (20 mm) [lit[176] 205°; yield 32.9%]; nmr ($CDCl_3$) $\delta$ 8.40 (s, 1, aromatic $C_2\underline{H}$), 8.40 (d, 1, J=6.0 Hz, aromatic $C_6\underline{H}$), 7.05 (d, 1, J=6.0 Hz, aromatic $C_5\underline{H}$), 2.57 (t, 2, J=9.0 Hz, Ar-$C\underline{H}_2$-$CH_2$-), 2.23 (s, 3, Ar-$C\underline{H}_3$), 1.60 (m, 2, -$CH_2$-$C\underline{H}_2$-$CH_3$), 0.95 (t, 3, J=7.0 Hz, -$CH_2$-$CH_2$-$C\underline{H}_3$).

Nonyl iodide.- The general procedure of Woods[152] was used. To a cooled mixture of 23 ml of phosphoric acid and 11.0 g (0.077 mol) of $P_2O_5$ was added 55.0 g (0.35 mol) of finely divided potassium iodide and 24.0 g (0.17 mol) of nonyl alcohol. The mixture was mechanically stirred, was heated in an oil bath at 110-120° for 5 hr and was cooled. It was diluted with 70 ml of water and the resulting solution was extracted with ether. The ethereal extract was successively washed with aqueous sodium thiosulfate and water, and was then dried over $MgSO_4$. The solvent was evaporated and the residual brown oil was distilled to give 37.5 g (89%) of nonyl iodide: bp 58° (0.25 mm) [lit[177] 245° (760 mm)].

3-Hydroxymethyl-2-pyridinecarboxylic acid lactone (169).- To a cooled (0° C) and stirred colution of 7.4 g (0.15 mol) of NaCN in 20 ml of water was added dropwise over a period of 1 hr a solution of 8.8 g (0.033 mol) of 1-methoxy-3-acetoxymethylpyridinium methyl sulfate (prepared by warming 3.8 g of dimethyl sulfate and 5.0 of the N-oxide 145). The reaction mixture was allowed to stand in a refrigerator overnight (12 hr) and was finally stirred at room temperature for 6 hr. The solution was extracted with 3 x 30 ml of chloroform. The extract was dried

($MgSO_4$), was concentrated, and was distilled to give 89 mg (2%) of 169 as an oil that solidified slowly. Recrystn from ethanol gave colorless needles: mp 163-165°; ir ($CHCl_3$) 1780 (5-membered lactone C=O), 1600 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 8.64 (d, 1, J=5.0 Hz, aromatic $C_6\underline{H}$), 7.90-7.34 (m, 2, aromatic $C_4\underline{H}$ and $C_5\underline{H}$), 5.30 (s, 2, Ar-$C\underline{H}_2$-O).

Anal. Calcd for $C_7H_5NO_2$: C, 62.22; H, 3.70; N, 10.37. Found: C, 62.34; H, 3.76; N, 10.31.

3-Methylpyridyl pivalate (173). A.- A solution of 1.0 g (9.3 mmol) of 3-hydroxymethylpyridine in 20 ml of pivalic anhydride was heated in an oil bath at 145-155° for 10 hr. It was cooled and 10% of NaOH was added until the solution became basic. It was extracted several times with chloroform. The chloroform extract was dried ($Na_2CO_4$) and concentrated to yield a thick dark brown oil. The crude product was then extracted with pet ether, and the pet ether layer gave 1.3 g (75%) of a clear yellowish oil. Vpc showed the compound to be 95% pure: ir (neat) 1735 $cm^{-1}$ (ester C=O), nmr ($CDCl_3$) $\delta$ 8.63-7.28 (m, 4, aromatic protons), 5.13 (s, 2, Ar-$C\underline{H}_2$-O), 1.23 (s, 9, $-\overset{O}{\overset{\|}{C}}-\overset{CH_3}{\overset{|}{C}}-C\underline{H}_3$ with $C\underline{H}_3$).

Anal. Calcd for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82; N, 7.25. Found: C, 68.19; H, 7.95; N, 7.16.

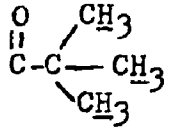
B. Parish's procedure.- To a solution of 1.02 g (10 mmol) of pivalic acid, 8.0 g (38 mmol) of trifluoroacetic anhydride in 20 ml of dry benzene was added dropwise a solution of 1.08 g (10 mmol) of 3-hydroxymethylpyridine[140] in 5.0 ml of benzene. The reaction mixture was allowed to stir at room temperature for 5 hr, and was refluxed for 30 min, and was cooled. The solution was extracted with 10% NaOH. The benzene layer was washed

with water, was dried ($Na_2SO_4$) and was concentrated to give trace amounts of the starting alcohol as confirmed by its ir. The sodium hydroxide layer was acidified with 10% HCl and neutralized by the addition of solid $NaHCO_3$. Extraction with chloroform, drying ($MgSO_4$) and evaporation of the chloroform yielded 0.75 g of the starting 3-hydroxymethylpyridine as confirmed by its ir.

3-Methylpyridyl pivalate N-oxide (174).- A solution of 3.83 g (20 mmol) of 173 and 4 ml of 30% hydrogen peroxide in 15 ml of glacial acetic acid was heated to 90° for 2.5 hr. An additional portion of 3.0 ml of hydrogen peroxide was added and heating and stirring continued for further 8 hr. The reaction mixture was then allowed to stand at room temperature for 24 hr. The solution was concentrated in vacuo to 15 ml, and after the addition of 10 ml of water, it was concentrated once again to 15 ml. Chloroform (25 ml) was added to the solution, and it was treated with a slurry of $K_2CO_3$ until it was neutralized. The mixture was filtered and aqueous and chloroform layers in the filtrate were separated. The aqueous layer was extracted with 3 x 30 ml of chloroform. The combined chloroform extract was dried ($Na_2SO_4$) and concentrated to give oil that slowly solidified, 3.5 g (85%): ir ($CHCl_3$) 1735 $cm^{-1}$ (ester C=O); nmr ($CDCl_3$) $\delta$ 8.27-7.27 (m, 4, aromatic protons), 5.10 (s, 2, Ar-$CH_2$-O-), 1.23 (s, 9, $-C(=O)-C(CH_3)_3$).

Analytical sample was prepared as its picrate which recryst from ethanol as colorless prisms: mp 95-97°.

Anal. Calcd for $C_{17}H_{18}N_4O_{10}$: C, 46.58; H, 4.14; N, 12.78. Found: C, 46.45; H, 4.08; N, 12.62.

Cyanation of 174.- To 7.0 g (33.5 mmol) of 174 was slowly added equimolar quantity of dimethyl sulfate (4.50 g) at room temperature. After the initial exothermic reaction, the solution was heated on a steam bath for 2 hr to ensure complete reaction. It was cooled, was dissolved in 15 ml of 35% ethanol and was added dropwise over a period of 1 hr to a cooled (-5°) solution of 5.60 g (86 mmol) of KCN in 20 ml of 35% ethanol. The reaction mixture was allowed to stand in the refrigerator for 12 hr and was stirred at room temperature for 6 hr. The solution was extracted with chloroform several times. The extract was dried ($MgSO_4$), concentrated, and distilled to yield 4.1 g (60%) of a clear oil: bp 115-135° (0.3 mm); ir (neat) 2215 (C≡N), 1735 $cm^{-1}$ (ester C=O); nmr ($CDCl_3$) $\delta$ 8.73-7.77 (m, 3, aromatic protons), 5.2 (s, 2, Ar-$CH_2$-O-), 1.2 (s, 9,  ). Vpc separated the crude cyano product into three isomers, the nmrs of the aromatic section of each isomer were: Nmr ($CDCl_3$) $\delta$ 8.70 (m, 1, $J_{5,6}$=5 Hz, $J_{4,6}$=2 Hz, $C_6H$), 7.57 (m, 1, $J_{4,5}$=8.5 Hz, $J_{4,6}$=2.0 Hz, $C_4H$), 7.37 (m, 1, $J_{4,5}$=8.5 Hz, $J_{5,6}$=5.0 Hz, $C_5H$), 2-cyano derivative 54%. $\delta$ 8.87 (s, 1, $C_2H$), 8.80 (d, 1, $J_{5,6}$=5.0 Hz, $C_6H$), 7.60 (d, 1, $J_{5,6}$=5 Hz, $C_5H$), 4-cyano derivative 21%. $\delta$ 8.73 (broad s, 1, $C_2H$), 7.77 (broad s, 2, $C_4H$ and $C_5H$) for 6-cyano derivative 25%.

Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.03; H, 6.47; N, 12.84. Found: C, 65.99; H, 6.39; N, 12.92.

Pyruvic acid diethyl ketal (179).- A mixture of 11.6 g (0.1 mol) of ethyl pyruvate, 26 ml (0.15 mol) of triethyl orthoformate, 1.5 g of p-toluenesulfonic acid monohydrate and 24 ml of anhyd ethanol was allowed

to stand at room temperature for 24 hr and was refluxed for 8 hr. The low boiling components were removed in the Rinco evaporator and the residue was poured into ice water containing 40 ml of 5% $NaHCO_3$. After extraction with methylene chloride, the organic phase was dried ($MgSO_4$), was concentrated and distilled to yield 15.6 g (89%) of ethyl pyruvate diethyl ketal: bp 78-79° (10 mm), [lit[179] 81.5-82.5° (14 mm)]; 10.8 g (0.057 mol) of the distillate was added to a stirred and cooled solution of 6.40 g (0.114 mol) of potassium hydroxide in 40 ml of anhyd ethanol. The resulting reaction mixture was stirred at room temperature for 20 hr. After the remaining potassium hydroxide used was neutralized with a methanolic solution of hydrogen chloride and the solvent was evaporated *in vacuo*, the residue was dissolved in 20 ml of water and was made slightly acidic by the addition of 10% HCl. The solution was extracted with ether and the ethereal extract was dried ($MgSO_4$) and concentrated to yield 4.20 g (90%) of a clear oil: ir (neat) 3500-2800 (carboxylic acid O-H), 1745 $cm^{-1}$ (acid C=O); nmr ($CDCl_3$) $\delta$ 3.55 (m, 4, J=7 Hz, $-OCH_2-CH_3$), 1.53 (s, 3, $CH_3-C(=O)(-O)-CO_2H$), 1.22 (t, 6, J=7 Hz, $-O-CH_2CH_3$).

Attempted vacuum distillation led to loss of ethanol and generation of α-ethoxyacrylic acid as described under 185. The compound was not submitted for elemental analysis.

2,3-Dihydro-2-pyruvoyl-1H-pyrrolo[3,4-b]quinoline diethyl ketal (180).- To a stirred suspension of 6.64 g (0.02 mol) of the dihydrobromide salt of 16a in 35 ml of dry chloroform, was added 4.04 g (0.04 mol) of triethylamine, with ice cooling. To the resulting mixture was added a solution of 4.10 g (0.02 mol) of dicyclohexylcarbodiimide

(DCC) in 15 ml of chloroform, followed by the addition of 3.20 g (0.02 mol) of 179 in 15 ml of chloroform. The stirred reaction mixture was kept at room temperature for 12 hr. The solution was then successively washed with water, 2% HCl, 5% $NaHCO_3$ and finally with water. The chloroform layer was dried ($Na_2SO_4$) and was concentrated. The residue was dissolved in 20 ml of benzene and the insoluble urea was filtered off. The benzene layer was concentrated to give a thick brown oil. The brown oil was extracted into pet ether and passed over a short column (alumina). Evaporation of the pet ether gave 700 mg of a greyish solid which after recrystn from ethanol-water gave white crystals: mp 85-87°; ir ($CHCl_3$) 1655 $cm^{-1}$ (broad amide, C=O); nmr ($CDCl_3$) $\delta$ 8.12-7.33 (m, 5, aromatic protons), 5.33 (s, 2, Ar-$CH_2$-N), 5.10 (s, 2, Ar-$CH_2$-N), 3.67 (m, 4, J=7 Hz, -O-$CH_2CH_3$), 1.77-1.60 (envelope, dicyclohexyl urea protons), 1.47 (t, 6, J=7 Hz, O-$CH_2CH_3$).

An analytical sample could not be prepared because of difficulty of separating the product from contamination of dicyclohexyl urea. The compound 180 was characterized by its hydrolysis product 181.

2,3-Dihydro-2-pyruvoyl-1H-pyrrolo[3,4-b]quinoline (181). A. From hydrolysis of 180.- A solution of 500 mg (1.6 mmol) of 180 and a catalytic amount of p-toluenesulfonic acid in 30 ml of 90% acetic acid in water was stirred at room temperature for 10 hr and was refluxed for 3 hr. The solution was concentrated in vacuo and the residue was taken up in 20 ml of ethanol and was treated with decolorizing charcoal. After evaporation of the ethanol solution, the residue was extracted with 10 ml of chloroform. Concentration of the extract afforded 238 mg (5% from 16a) of a

crystalline compound. Recrystn from ethanol gave colorless prisms: mp 191-193°; ir ($CHCl_3$) 1710 (C=O) 1645 (amide C=O), 1600 $cm^{-1}$ (C=C); nmr ($CDCl_3$) $\delta$ 8.20-7.31 (m, 5, aromatic protons), 5.27 (s, 2, Ar-$CH_2$-N), 5.01 (s, 2, Ar-$CH_2$-N), 2.55 (s, 3, $-\overset{O}{\overset{\|}{C}}-CH_3$).

Anal. Calcd for $C_{14}H_{12}N_2O_2$: C, 69.99; H, 5.03; N, 11.66. Found: C, 70.03; H, 5.05; N, 11.73.

B. From condensation of 16a with pyruvic acid.

(1) With dicyclohexylcarbodiimide.- To a stirred and cooled (0°) suspension of 6.64 g (0.02 mol) of the dihydrobromide salt of 16a in 100 ml of dry chloroform, was added dropwise 4.04 g (0.04 mol) of triethylamine. To the resulting solution was added a solution of 4.10 g (0.02 mol) of DCC in 30 ml of chloroform followed by a solution of 1.76 g (0.02 mol) of pyruvic acid in 30 ml of chloroform. The reaction mixture was allowed to stand at room temperature for 12 hr. The solution was filtered and the filtrate was successively washed with 2% HCl, 5% $NaHCO_3$, and water. The chloroform layer was dried ($MgSO_4$) and was concentrated, and the residue was taken up in 100 ml of warm benzene. The insoluble solid was removed by filtration. Evaporation of the benzene layer gave a dark gum which was passed over alumina with benzene as the eluent. The benzene eluate was concentrated to give 310 mg (5%) of crystalline material. Recrystn from ethanol gave colorless prisms: mp 191-192°, identical to the sample obtained in part A.

(2) Attempt with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.- The procedure described for part B (1) was repeated with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide substituting for DCC, but after work up only intractable tar was obtained.

(3) Attempt with EEDQ.- To a stirred and cooled (-78°) suspension of 996 mg (3 mmol) of the dihydrobromide salt of 16a in 15 ml of abs ethanol, was added dropwise 606 mg (6 mmol) of triethylamine. To the resulting solution was added 791 mg (3 mmol) of EEDQ followed by a solution of 264 mg (3 mmol) of pyruvic acid in 5 ml of ethanol. The reaction mixture was allowed to stir at -78° for 2 hr and at room temperature for 1 hr. The solution was concentrated and the residue was taken up in chloroform. After washing the chloroform layer with 2% HCl and 5% $NaHCO_3$, it was dried ($MgSO_4$) and was concentrated to give a dark amorphous solid. The product showed no migration on tlc (alumina) with acetone, benzene and methylene chloride as solvents; furthermore, the crude product gave no meaningful ir nor nmr.

C. From condensation of 16a with 185.- To a stirred and cooled (0°) suspension of 6.64 (0.02 mol) of the dihydrobromide salt of 16a in 100 ml of dry chloroform, was added 4.04 g (0.04 mol) of triethylamine. To the resulting solution was added a solution of 4.10 g (0.02 mol) of DCC in 30 ml of chloroform and 2.35 g (0.02) of α-ethoxyacrylic acid (185). The reaction mixture was allowed to stir at room temperature for 12 hr and, after work up (as described in part B (1) ), 1.70 g of very dark oil was obtained. Without purification, the dark oil was dissolved in 30 ml of 90% HOAc and was refluxed in the presence of a catalytic amount of p-toluenesulfonic acid for 5 hr and was left at room temperature for 8 hr. After work up (as described in part A), 100 mg (2% calcd from 16a) of 181 was obtained. Recryst from ethanol gave colorless prisms: mp 191-193°, identical in all respects to the sample obtained in part A.

This procedure was also tried in the presence of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide and EEDQ in exactly the same way as described in part B (2 and 3). However, only excessive decomposition and tarry material resulted.

{1-[(1,3-Dihydro-2H-pyrrolo[3,4-b]quinolin-2-yl)carbonyl]ethylidene} malononitrile (182).- In a 15 ml microflask were placed 50 mg (0.76 mmol) of malononitrile, 100 mg (0.41 mmol) of 181, 500 mg of anhyd $Na_2CO_3$, a catalytic amount of β-alanine, 2 ml of glacial acetic acid, and 10 ml of dry benzene. The mixture was stirred and was refluxed for 16 hr. The cooled reaction mixture was filtered and the residue was washed with chloroform. The combined benzene-chloroform layer was concentrated to give a greyish gum, and, after triturating it with ether, 23 mg (20%) of a greyish solid was obtained: ir ($CHCl_3$) 2235 (C≡N), 1650 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) δ 8.33-7.27 (m, 5, aromatic protons), 5.33-4.50 (broad s, 4, Ar-$CH_2$-N), 2.10 (s, 3, $\rangle{=}\langle_{CH_3}$).

No elemental analysis was made owing to smallness of sample.

N-Carbethoxy-2,3-dihydro-2H-pyrrolo[3,4-b]quinoline (16b).- To a stirred and cooled (0°) suspension of 996 mg (3 mmol) of the dihydrobromide salt of 16a in 15 ml of abs ethanol, was added 606 mg (6 mmol) of triethylamine. To the resulting solution was added 791 mg (3 mmol) of EEDQ and a solution of 486 mg (3 mmol) of pyruvic acid diethyl ketal (179). The reaction mixture was allowed to stand at room temperature for 10 hr, and the solution was concentrated in vacuo. The residue was dissolved in 20 ml of chloroform and was washed successively with 2% HCl, 5% $NaHCO_3$, and water. The chloroform layer was dried ($Na_2SO_4$) and con-

centrated to give 930 mg of brownish solid. The crude product was subjected to column chromatography (Alumina Merck) and was eluted with benzene: acetone (1:1) to yield 730 mg (78%) of a pale yellow solid. Recrystn from ethanol and water gave colorless crystals: mp 129-131° [lit[9] mp 134-135°]; ir ($CHCl_3$) 1670 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) $\delta$ 8.20-7.25 (m, 5, aromatic protons), 4.90 (s, 4, Ar-$CH_2$-N-), 4.30 (m, 2, J=7 Hz, O-$CH_2CH_3$), 1.33 (t, 3, J=7 Hz, O-$CH_2CH_3$).

2-Ethoxyacrylic acid (185).- Vacuum distillation of 10 g (0.062 mol) of the crude acid 179 gave two fractions with the minor fraction distilling at 55-60° (0.5 mm) and the major fraction at 85-87° (0.2 mm). The major fraction was obtained as 4.30 g (60% yield) of oil that solidified on cooling: mp 45-47°, ir ($CHCl_3$) 3400-2600 (carboxylic acid O-H), 1735 $cm^{-1}$ (acid C=O); nmr ($CDCl_3$) $\delta$ 5.53 (d, 1, J=2.5 Hz, vinyl proton), 4.73 (d, 1, J=2.5 Hz, vinyl proton), 3.90 (m, 2, J=7 Hz, $OCH_2CH_3$), 1.4 (t, 3, J=7 Hz, -$OCH_2CH_3$).

Anal. Calcd for $C_5H_8O_3$: C, 51.73; H, 6.94.
Found: C, 51.56; H, 6.77.

2,3-Dihydro-2-pyruvoyl-1H-pyrrolo[3,4-b]quinoline 2,4 DNPH (192a).

A. From pyruvic acid 2,4 DNPH (192a), 169, and DCC.- To a stirred suspension of 3.32 g (0.01 mol) of 16a dihydrobromide in 10 ml of methylene chloride, was added 2.02 g (0.02 mol) of triethylamine. To the resulting solution was added 2.05 g (0.01 mol) of DCC in 5 ml of methylene chloride and 2.68 g (0.01 mol) of 192a. The reaction mixture was allowed to stir at room temperature for 15 hr and was then filtered. The residue was

thoroughly washed with a saturated solution of $NaHCO_3$ and water. The residue was dried at 120° to give 436 mg (10%) of a yellow compound. Recryst from DMF gave yellow crystals: mp 255-257°; ir (nujol mull) 1660 $cm^{-1}$ (amide C=O). Owing to the insolubility of the compound nmr was not possible.

Anal. Calcd for $C_{20}H_{16}N_6O_5$: C, 57.14; H, 3.84; N, 19.99. Found: C, 56.44; H, 4.08; N, 20.19.

B. From pyruvic acid, 2,4 DNPH 192a, 16a and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.- The procedure described in part A was repeated with N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide substituted for DCC. After work up, a yellow crystalline material was isolated in 50% yield: mp 255-257°. It was identical to sample obtained in part A.

C. From 192b and 16a.- To a cooled (0°) and stirred suspension of 1.66 g (5 mmol) of 16a dihydrobromide in 25 ml of anhyd benzene, was added dropwise 1.55 g (15 mmol) of triethylamine; 1.28 g (5 mmol) of the acid chloride 192b was added in small portions over a period of 15 min. The color of the reaction mixture turned dark red instantaneously. After the completion of addition, the reaction mixture was stirred for 1 hr at 0° and for 4 hr at room temperature. The reaction mixture was filtered and the collected solid was first suspended in cold water and then was washed thoroughly with saturated $NaHCO_3$ solution. It was then dried at 120° to yield 108 mg (5%) of yellow solid; recryst from DMF: mp 255-257°. It was identical in all respects with the sample obtained in part A.

2,3-Dihydro-2-pyruvoyl-1H-pyrrolo[3,4-b]quinoline oxime (192c).

A. With N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide.- A suspension of 6.64 g (0.02 mol) of 16a dihydrobromide in 15 ml of methylene chloride was a treated consecutively with 4.04 g (0.04 mol) of triethylamine, 5.0 g (0.02 mol) of the diimide in 10 ml of methylene chloride, and 2.06 g (0.02 mol) of pyruvic acid oxime (191c). The reaction mixture was stirred at 0° for 2 hr and was allowed to stand at room temperature for 10 hr. The solution was filtered and the residue was washed with cold water before drying (120°) to yield 2.60 g (55%) of a greyish solid: mp 179-181°; ir (nujol mull) 3100-2800 (oxime OH), 1650 (amide C=O), 1630 $cm^{-1}$ (C=N); nmr (TFAA + DMSO-$d_6$) $\delta$ 8.53-7.78 (m, 5, aromatic protons), 4.97 (s, 2, Ar-$CH_2$N), 4.92 (s, 2, Ar-$CH_2$-N), 2.03 (s, 3, $-\overset{\overset{N-OH}{\|}}{C}-CH_3$ ).

Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found:

Attempted hydrolysis of 192a. A. In sulfuric acid.- A suspension of 1.00 g (2.3 mmol) of 192a in 50 ml of 20% $H_2SO_4$ was refluxed for 4 hr. The solution was concentrated to 20 ml and the precipitate was collected by filtration. It was washed with cold water and was dried to yield 0.50 g (88%) of yellow crystalline material: mp 201-206° dec. It showed no depression on mixed melting with pyruvic acid 2,4 DNPH (191a).

B. In levulinic acid.- To a solution of 3.24 g (30 mmol) of levulinic acid in 0.5 ml of 1.0N HCl was added 430 mg (1 mmol) of 192a. The mixture was heated on a steam bath for 5 hr. It was cooled and was diluted with 5 ml of water. The solution was neutralized with saturated

$NaHCO_3$ solution and the precipitate was collected by filtration to yield 250 mg of a yellow solid. It was identified as the starting 192a by its mp 254-256°. The filtrate was extracted with methylene chloride, but drying ($MgSO_4$) and concentration gave no organic compound.

Attempted hydrolysis of 192c.- The procedures employed in attempted hydrolysis of 192c are those described for 192a. Analogous to results with 192a, attempted hydrolysis of 192c in 20% $H_2SO_4$ led to the production of pyruvic acid oxime (191c), whereas hydrolysis in levulinic acid gave back unchanged 192c.

1,3-Dihydro-β-oxo-2H-pyrrolo[3,4-b]quinoline-2-propionaldehyde dimethylacetal (194).- To a stirred and cooled (0°) suspension of 996 mg (3 mmol) of 16a dihydrobromide in 15 ml of dry chloroform, was added 606 mg (6 mmol) of triethylamine. To the resulting solution was added 465 mg (3 mmol) of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide in 5 ml of chloroform and 457 mg (3 mmol) of formylacetic acid dimethylacetal (193)[178] in 5 ml of chloroform. The reaction mixture was stirred at room temperature for 15 hr and was washed consecutively with 2% HCl, 5% $NaHCO_3$, and water. The chloroform layer was dried ($MgSO_4$) and concentrated to give 770 mg (~ 90%) of a white solid. Recryst from benzene gave colorless needles: mp 151-153° [lit[102] mp 150-152°].

3-Furfurylidene-1,3-dihydro-β-oxo-2H-pyrrolo[3,4-b]quinoline-2-propionaldehyde dimethylacetal (195).- A stirred mixture of 143 mg (0.5 mmol) of 194, 240 mg (2.5 mmol) of furfural and 0.5 g of acetic anhydride was heated at 120° under nitrogen atmosphere for 7 hr. The solution was

concentrated in vacuo and the remaining dark brown oil was subjected to column chromatography on alumina (Woelm, grade I activity II) with methylene chloride as eluent. Evaporation of the eluate gave 73 mg (40%) of a bright yellow solid. Recrystn from ethanol gave yellow crystalline flakes: mp 133-135°; uv max (EtOH) 394 mμ (ε 13100), 288 (14700), 247 (29300); ir ($CHCl_3$) 1650 $cm^{-1}$ (amide C=O); nmr ($CDCl_3$) δ 7.97-7.23 (m, 8, aromatic protons), 6.43 (s, 1, vinyl proton), 5.10 (s, 2, Ar-$CH_2$-N), 4.87 (t, 1, J=5.5 Hz, $-CH_2-CH(OMe)(OMe)$), 3.23 (s, 6, $-CH(OMe)(OMe)$), 2.77 (d, 2, J=5.5 Hz, $-C(=O)-CH_2-CH<$); $M^+$ at m/e 364 (calcd 364).

Anal. Calcd for $C_{21}H_{20}N_2O_4$: C, 69.21; H, 5.53; N, 7.69. Found: C, 69.01; H, 5.47; N, 7.76.

# V SUMMARY

The remarkable activity of camptothecin in L1210 leukemia and its promising results in preliminary clinical trials, coupled with the difficulty in obtaining it, generated great deal of interest to develop laboratory synthesis of the alkaloid. The chemistry, biochemistry, and pharmacology of the alkaloid has been reviewed so as to emphasize its medicinal potential. Four basic approaches to the ABCD ring system and a combination of approaches to the lactone E ring of camptothecin have been discussed.

In the first approach, the key intermediate 3-[[2-oxo-1(2H)-pyridyl]methyl]quinaldic acid (115) and its sodium salt (117) were prepared through a seven-step pathway. Attempts to effect thermally induced decarboxylative cyclization to the desired ring system via the Hammick anion intermediate were unsuccessful. In the case of 115, decarboxylation resulted at 120° to give 3-[[2-oxo-1(2H)-pyridyl]-quinoline whereas, when 117 was heated to > 400°, thermal fragmentation leading to 2(1H)-pyridone and possibly to 3-hydroxymethyl-2-quinoline carboxylic acid lactone (90) was observed.

In the second approach, the desired ABCD ring system was constructed through a five-step pathway involving acetic anhydride induced rearrangement of pyridone N-oxide to 2(1H)-pyridone. Picolinic acid N-oxide was esterified to its methyl ester and on heating in acetic anhydride gave 6-carbomethyl-2(1H)-pyridone (130a). Treatment of 130a with methyl acrylate led to 4-carbomethoxy-3-oxopyrrolidino[2,1-f]-2-(1H)-pyridone (132a), which was decarboxylated to give 3-oxopyrrolidino-[2,1-f]-2(1H)-pyridone (128). Finally, Friedlander condensation of 128

with o-aminobenzaldehyde led to 1-oxo-indolizino-11H-[1,2-b]quinoline (79).

In an attempt to construct the E ring, 3,4-dimethylpyridine was selectively oxidized to 4-methylnicotinic acid. After esterification and reduction to 3-hydroxymethyl-4-methylpyridine (158), it was esterified to its carbonate ester 159a. Attempts to effect intramolecular ethoxycarbonylation of 159a under various conditions were not successful.

In the third approach, the intermediate 2,3-dihydro-2-pyruvoyl-1H-pyrrolo[3,4-b]quinoline (181) was prepared and condensed with malononitrile to give a small amount of intermediate [1-[1,3-dihydro-2H-pyrrolo[3,4-b]quinolin-2-yl)carbonyl]ethylidene]-malononitrile (182). In the last approach, intermediate 3-furfurylidene-1,3-dihydro-β-oxo-2H-pyrrolo[3,4-b]quinoline-2-propionaldehyde dimethyl acetal (195) was prepared through a four-step pathway, but initial attempts to cleave the furan nucleus were not fruitful.

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