

STUDIES IN THE SYNTHESIS OF CAMPTOTHECIN

A THESIS

Presented to

The Faculty of the Division
of Graduate Studies

By

James T. Baxter

In Partial Fulfillment

of the Requirements for the Degree
Master of Science in Chemistry

Georgia Institute of Technology

March, 1976

T-954

STUDIES IN THE SYNTHESIS OF CAMPTOTHECIN

Approved:

L. H. Zalkow

Leon H. Zalkow

D. S. Gaine

Drury S. Gaine

C. L. Liotta

C. L. Liotta

Date approved by Chairman: 2/27/76

ACKNOWLEDGMENTS

I wish to express my gratitude to Dr. Leon H. Zalkow for his counsel and guidance during the course of this work. I would also like to thank Dr. Drury S. Caine and Dr. C. L. Liotta for serving as members of the reading committee.

I also wish to thank Dr. James B. Nabors for his help and ideas during the early part of this work.

Thanks are due the School of Chemistry for financial aid in the form of a Teaching Assistantship.

TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.	ii
GLOSSARY OF ABBREVIATIONS.	v
SUMMARY.	vi
Chapter	
I. INTRODUCTION.	1
II. INSTRUMENTATION AND EQUIPMENT	4
III. EXPERIMENTAL.	5
Preparation of Anhydride <u>2</u>	
Preparation of Furan Acid <u>5</u>	
Preparation of Furan Ester <u>6</u>	
Preparation of Furan Alcohol <u>7a</u>	
Preparation of Furan Acetate <u>7b</u>	
Preparation of Furan Aldehyde <u>8</u>	
Attempted Preparation of Furan Alcohol <u>7a</u> by Reacting the Furan Ester <u>6</u> with LiBH_4 in THF	
Attempted Preparation of Furan Aldehyde <u>8</u> by Reacting the Furan Alcohol <u>7a</u> with σ -Chloranil	
Attempted Preparation of the Furan Analysis <u>8</u> by Reacting the Furan Alcohol <u>7b</u> with CrO_3 in Pyridine	
Attempted Preparation of the Furan Aldehyde <u>8</u> by Reacting the Furan Alcohol <u>7b</u> with DMSO and Acetic Anhydride	
Attempted Preparation of the Furan Methanesulfonate <u>7c</u>	
Attempted Preparation of the Furan Tosylate with p-toluenesulfonyl Chlorine in Pyridine	
Attempted Preparation of the Furan Tosylate <u>7d</u> by Reacting the Furan Alcohol <u>7a</u> with p-Toluenesulfonyl Chloride and Sodium Hydride	
Attempted Cyclization of the Furan Acetate <u>7b</u> by Heating the Furan Acetate <u>7b</u> with Acetic Anhydride and Zinc Chloride	

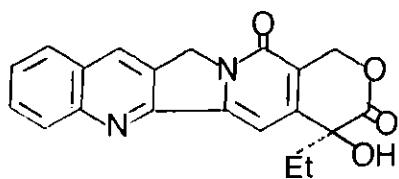
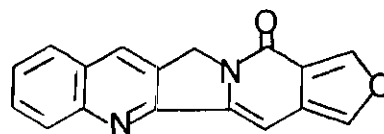
Chapter	Page
Attempted Cyclization of the Furan Acetate <u>7b</u> by Heating the Furan Acetate <u>7b</u> with Acetic Acid Under Nitrogen	
Attempted Cyclization of the Furan Acetate <u>7b</u> by Heating the Furan Acetate <u>7b</u> with Pyridine Under Nitrogen	
Attempted Cyclization of the Furan Acetate <u>7b</u> by Heating the Furan Acetate <u>7b</u> with Acetic Acid and Sodium Acetate	
Attempted Preparation of Cyclized Compound <u>9</u> by Heating the Furan Aldehyde <u>8</u> with Piperidine and Benzene	
Attempted Preparation of Cyclized Compound <u>9</u> by Heating the Furan Aldehyde <u>8</u> with Acetic Anhydride	
IV. DISCUSSION OF RESULTS.	22
V. CONCLUSIONS.	30
VI. RECOMMENDATIONS.	31
BIBLIOGRAPHY.	32

GLOSSARY OF ABBREVIATIONS

Ac	Acetyl ($\text{CH}_3\text{CO}-$)
<u>Anal.</u>	elemental analysis
d	doublet (NMR)
g	gram
hr	hour
IR	infrared spectroscopy
J	coupling constant (NMR)
l	liter
m	multiplet (NMR)
M^+	molecular ion in mass spectrum
m/e	mass to charge ratio
min	minute
NMR	nuclear magnetic resonance spectroscopy
q	quartet (NMR)
s	singlet
t	triplet
TLC	thin layer chromatography
UV	ultraviolet spectroscopy

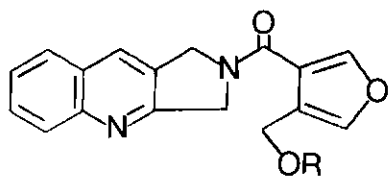
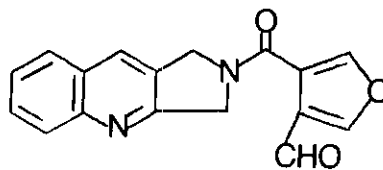
SUMMARY

Our attempt at the synthesis of camptothecin 14, an alkaloid used in the treatment of tumors, was focused on intermediate 9.

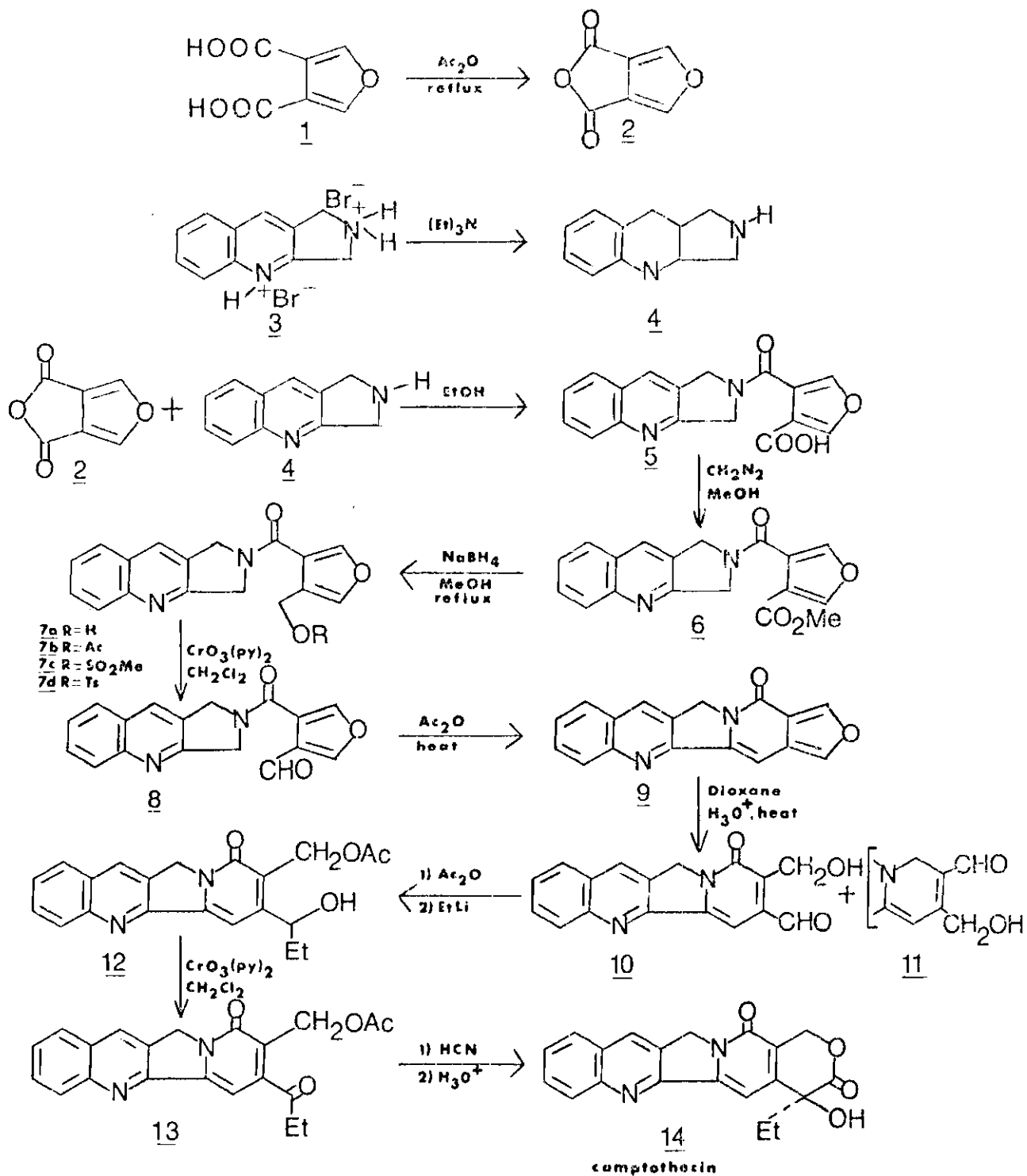
149

Several attempts to prepare intermediate 9 were made by the cyclization of the furan aldehyde 8; however, none were successful.

In the attempted synthesis of intermediate 9 several new compounds were made. These are compounds 7a, 7b, and 8.

7a R = H7b R = Ac8

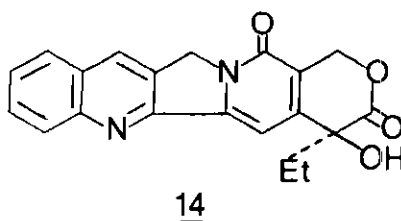
Proposed Sequence of Reactions Leading to Camptothecin



CHAPTER I

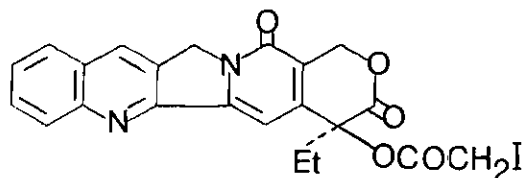
INTRODUCTION

Camptothecin 14, a pentacyclic alkaloid, was isolated from the tree camptotheca acuminata Nyssaceae in 1966.¹⁻⁴ Due to camptothecin's antileukemic and antitumor properties in animals and humans, it has been the subject of considerable research. This research has primarily taken two directions. The first has been the testing of camptothecin on animals and humans, while the second has been the synthesis of camptothecin. The synthesis of camptothecin was important because the only source was the camptotheca acuminata tree, which is native only to mainland China.



M. E. Wall and his coworkers¹⁴ extracted the stem wood of the camptotheca tree with hot hexane-heptane followed by similar extraction with 95% ethanol. The ethanol extract was concentrated and partitioned between chloroform and water. The methanol-insoluble material from the chloroform was chromatographed over silica gel and then

recrystallized from methanol-acetonitrile, which gave camptothecin as pale yellow needles, m.p. 264-267° d; M^+ at m/e of 348.1117 (calcd. for $C_{20}H_{16}N_2O_4$ m/e of 348.1111); $[\alpha]_D^{25} - 31.3^\circ$ ($CHCl_3 - MeOH$ 8:2); $\lambda_{max}^{EtOH} 220$ (ϵ 37,320), 254 (ϵ 29,230), 290 (ϵ 4,980), and 370 (ϵ 19,900) $m\mu$; ν_{max} 3440 (hydroxyl), 1760-1745 (lactone), 1660 (lactam) and 1610, 1585 (aromatic) cm^{-1} ; $\delta(CD_3SCD_3)$: 0.91 (3H, t, $-CH_2CH_3$), 1.90 (2H, complex, $C(OH)CH_2CH_3$), 5.45 (2H, Ar CH_2-O-), and 5.28 (2H, $ArCH_2-N<$).



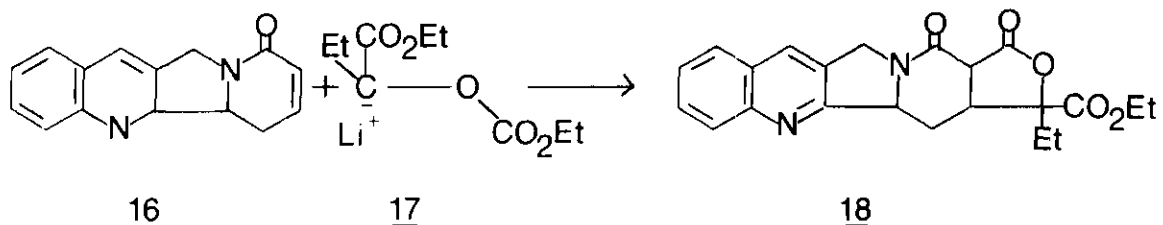
15

X-ray analysis of the iodoacetate 15 confirmed that camptothecin has structure 14.

Camptothecin has been shown to be active against lymphoid leukemia L1210 and Walker 256 (intramuscular) tumors in mice. Camptothecin has also been used in the treatment of human patients for cancer of the colon.⁵⁻⁶ Initial treatments were encouraging; however, due to camptothecin's extreme toxicity it is no longer of prime interest in clinical studies.⁷⁻⁸

The first total synthesis of d,l-camptothecin was reported by Stork and Schultz in 1971.⁹ The key step in the

synthesis was the addition-cyclization of the carbonate of an α -hydroxy ester 17 to an α,β -unsaturated lactam 16.



Compound 18 was then converted into dl-damptothecin by several successive reactions. Since then there has been a number synthesis of camptothecin reported in the literature.¹⁰⁻¹⁶

The purpose of our research was to prepare camptothecin in a yield high enough to lend itself to commercial preparation. The text which follows describes a synthetic approach to camptothecin.

CHAPTER II

INSTRUMENTATION AND EQUIPMENT

Melting points are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus. Infrared spectra were recorded using a Perkin-Elmer 237B spectrophotometer with solids in the form of potassium bromide pellets and liquids as thin films between sodium chloride plates. The band at 1601 cm^{-1} of a polystyrene film was used as a reference point. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian Associates Model A-60D spectrometer using solutions containing tetramethylsilane as an internal standard. Mass spectra were obtained using a Varian Associates Model M-66 mass spectrometer. Microanalyses were performed by Alfred Bernhardt Microanalytisches Laboratorium, Mulheim, West Germany, or by Atlantic Microlab, Inc., Atlanta, Georgia.

CHAPTER III

EXPERIMENTAL

Preparation of Anhydride 2

3,4-Furandicarboxylic acid (5.0g, 3.2×10^{-2} moles), obtained from Aldrich Chemical Co., was dissolved in 25 ml of acetic anhydride and the solution was refluxed for 8 hr. The solution was allowed to cool overnight. During the cooling process a precipitate formed in the bottom of the flask. The supernatant liquid was poured into another flask and the solvent removed on the rotary evaporator until a semisolid remained in the bottom of the flask. The material was then dried in a vacuum oven at 80°C for 24 hours. Periodically the material had to be removed and crushed so that all of the acetic anhydride could be removed.

To the precipitate was added 10 ml of acetic anhydride and the reaction repeated. A white solid (2.9g, 2.1×10^{-2} moles, 66%) was obtained. m.p. 94-96°C; ν_{\max}^{KBr} 3100, 1790, 1740, 1530 cm^{-1} ; M^+ at m/e of 138 (Calcd. for $\text{C}_6\text{H}_2\text{O}_4$, m/e of 138).²⁰

Preparation of Furan Acid 5

The furan acid 5 was prepared by condensing the pyrroloquinoline 4 with the anhydride 2. The pyrroloquinoline

4 was prepared from the pyrroloquinoline dihydrobromide salt 3 in the following manner.¹⁷ The pyrroloquinoline dihydrobromide salt 3 (28g, 8.5×10^{-2} moles) was dissolved in 200 ml of water.¹⁷ The solution was then poured into a separatory funnel.⁶ Ice was added to the separatory funnel in order to keep the solution cool for the anticipated addition of triethylamine. Triethylamine (27 ml) was added to the separatory funnel and the mixture in the separatory funnel was shaken. The solution was extracted 5 times with 100 ml portions of methylene chloride. The combined extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator. The infrared analysis of the free pyrroloquinoline 4 (10.0g, 5.9×10^{-2} moles, 70%) was identical with that of a known sample.²⁰

The pyrroloquinoline 4 was condensed with the anhydride 2 in the following manner: The pyrroloquinoline 4 (10.0g, 5.9×10^{-2} moles) was dissolved in 250 ml of absolute ethanol. The anhydride 2 (8.8g, 6.4×10^{-2} moles), which had been crushed to a fine powder, was added to the pyrroloquinoline solution and the mixture stirred under nitrogen for 1 hour. The reaction mixture was filtered and the precipitate, a white solid 5 (17.4g, 5.5×10^{-2} moles, 96.1%), m.p. 205-220° d; $\nu_{\text{max}}^{\text{KBr}}$ 3100, 1710, 1635 cm^{-1} ; M^+ at m/e of 308 (Calcd. for $\text{C}_{17}\text{H}_{12}\text{O}_4\text{N}_2$, m/e of 308) was obtained.

Preparation of Furan Ester 6

The furan ester 6 was prepared by reacting the furan acid 5 with diazomethane. The diazomethane was prepared in the following manner. Carbitol [2-(2-ethoxyethoxy)-ethanol] (100 ml) and 400 ml of diethyl ether was added to a diazomethane still and cooled to 5°C in an ice water bath. Sodium hydroxide (4.8g) was dissolved in 40 ml of water and added to the contents of the diazomethane still. Dupont EXR-101 (14.0g) was also added to the still. The reaction mixture in the still was warmed with hot water and diazomethane dissolved in diethyl ether was collected in an Erlenmeyer flask which had been cooled to dry ice temperature.

The furan acid 5 (2.0g, 6.5×10^{-3} moles) was suspended in 200 ml of methanol. An ether solution of diazomethane (4.0g, 9.5×10^{-2} moles), generated from Dupont EXR-101, was added to the furan acid 5 suspension and stirred overnight. The solution was filtered and the solvent from the filtrate removed on the rotary evaporator. A brown gum (1.7g, 5.3×10^{-3} moles, 81.5%) was obtained. The brown gum was recrystallized from absolute ethanol. A white solid 6 (0.84g, 2.6×10^{-3} moles, 40%) m.p. 164-166°C; $\nu_{\text{max}}^{\text{KBr}}$ 1715, 1630 cm^{-1} ; M^+ at m/e of 322 (calcd. for $\text{C}_{18}\text{H}_{14}\text{D}_4\text{N}_2$ m/e of 322); anal.: 66.91% C, 8.84% N, 4.45% H (calcd.: 67.07% C, 8.69% N, 4.38% H) was obtained.

Preparation of Furan Alcohol 7a

The furan alcohol 7a was prepared from the furan ester 6 in the following manner. Sodium borohydride (25.9g, 6.8×10^{-1} moles) was placed in a 4 liter beaker. The furan ester 6 (2.2g, 6.8×10^{-3} moles) was dissolved in 300 ml of methanol. The furan ester 6 solution was then poured on to the sodium borohydride. After the initial vigorous reaction the contents were transferred to a round bottom flask and refluxed overnight under nitrogen. After refluxing, an equal volume of water was added to the reaction mixture. Approximately one-half of the methanol was removed on the rotary evaporator. The reaction mixture was then extracted 5 times with 100 ml portions of chloroform. The combined chloroform extracts were washed with water and dried over unhydrous magnesium sulfate. The chloroform was removed on the rotary evaporator leaving a solid material which when recrystallized from absolute ethanol yielded a light brown solid 7a (0.412g, 1.4×10^{-3} moles, 20.5%), m.p. 217° ; ν_{\max}^{KBr} 3400, 1601 cm^{-1} ; mass spectrum showed m/e of 113 (100%), 158 (75%), 159 (48%), 274 (27%), 292 (71%); anal.: 69.43% C, 9.68% N, 4.96% H (anal. calcd. for $\text{C}_{17}\text{H}_{24}\text{O}_3\text{N}_2$: 69.38% C, 9.52% N, 4.80% H).

Preparation of Furan Acetate 7b

The furan acetate 7b was prepared from the furan alcohol 7a in the following manner. The furan alcohol 7a

(0.10g, 3.4×10^{-4} moles) was placed in a 10 ml Erlenmeyer flask with a ground glass stopper. To the furan alcohol 7a was added 6 ml of an acetic anhydride-pyridine (4:1) mixture. The flask was stoppered and the reaction mixture stirred overnight. A cream colored precipitate formed which was collected on a fritted glass funnel. The precipitate was washed with absolute ethanol and dried in a vacuum oven at 55°C for 3 hours. A light pink solid 7b (0.085g, 2.5×10^{-4} moles, 73.5%), m.p. $188-190^{\circ}\text{C}$; $\nu_{\text{max}}^{\text{KBr}}$ 1725, 1620, 1410, 1260 cm^{-1} ; M^+ at m/e of 336 (calcd. for $\text{C}_{19}\text{N}_2\text{O}_4\text{H}_{16}$, m/e of 336) was obtained; anal.: 68.04% C, 8.39% N, 4.94% H (anal. calcd. for $\text{C}_{19}\text{N}_2\text{O}_4\text{H}_{16}$: 67.85% C, 8.33% N, 4.80% H).

Preparation of Furan Aldehyde 8

The furan aldehyde 8 was prepared by oxidizing the furan alcohol 7a with a chromium trioxide complex $[\text{CrO}_3 (\text{C}_5\text{H}_5\text{N})_2]$ in methylene chloride.¹⁸ The $\text{CrO}_3 (\text{py})_2$ complex was prepared in the following manner. Anhydrous pyridine (100 ml) was added to a 300 ml three neck round bottom flask equipped with a magnetic stirrer, thermometer, and drying tube. The pyridine was cooled in an ice-water bath to $15-20^{\circ}\text{C}$. Chromium trioxide (13.3g), which had been stored over phosphorus pentoxide, was added in small portions to the stirred pyridine at a rate so as to keep the temperature below 30°C . After about one-third of the chromium trioxide had been added, the yellow complex began to precipitate. At

the end of the addition (about 1 hour), a slurry of yellow complex in pyridine remained. The temperature of the stirred solution was readjusted to 15°C, and stirred at this temperature until the precipitate reverted to a deep red macrocrystalline form. Petroleum ether (30-60°C) was added to the reaction mixture, the precipitate was allowed to settle, and the solvent mixture was decanted. The residue was washed 3 times with 200 ml portions of petroleum ether, the solvent being removed each time by decantation. The precipitate was collected by suction filtration, dried at room temperature under a vacuum, and stored over phosphorus pentoxide in a desiccator.

The furan aldehyde 8 was prepared by oxidizing the furan alcohol 7a with the $\text{CrO}_3 (\text{py})_2$ complex in the following manner. The furan alcohol 7a (0.75g, 2.5×10^{-3} moles) was dissolved in dry methylene chloride (100 ml). A six fold molar excess of $\text{CrO}_3 (\text{py})_2$ complex was used. This complex was prepared as a 5% solution in dry methylene chloride (4.0g complex and 76 ml of methylene chloride). To the magnetically stirred oxidizing solution at room temperature was added the furan alcohol 7a solution. Immediately upon mixing, a brownish black precipitate formed. The reaction mixture was stirred for 30 minutes then filtered on a fritted glass funnel. The solvent from the filtrate was removed on the rotary evaporator. The brown solid material was chromatographed over silica gel eluting with 2% absolute ethanol in

chloroform. A light tan solid 8 (0.23g, 7.8×10^{-4} moles, 31.4%), m.p. 194-195°C; $\nu_{\text{max}}^{\text{KBr}}$ 2925, 2850, 1673, 1624 cm^{-1} ; mass spectrum showed m/e of 85 (41%), 123 (63%), 159 (100%), 274 (18.5%), 292 (55%); anal.: 19.66% C, 9.65% N, 4.25% H (anal. calcd. for $\text{C}_{17}\text{N}_2\text{H}_{12}\text{O}_3$: 69.86% C, 9.59% N, 4.14% H).

Attempted Preparation of Furan Alcohol 7a by Reacting
the Furan Ester 6 with LiBH_4 in THF

The furan ester 6 (0.40g, 1.24×10^{-3} moles) was dissolved in 30 ml of dry tetrahydrofuran (THF). LiBH_4 (35 ml of 0.08 M) in THF (2.8×10^{-3} moles) was injected into the furan ester 6 solution with a syringe and refluxed overnight. An equal volume of water was added to the reaction mixture and most of the THF removed on the rotary evaporator. The reaction mixture was then extracted four times with 50 ml portions of chloroform. The combined chloroform extracts were then washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator yielding a gummy solid (0.40g) which according to infrared analysis was starting material.

The reaction was repeated using a 1:1 molar ratio of furan ester 6 to LiBH_4 . Again, only starting material could be obtained.

Attempted Preparation of Furan Aldehyde 8 by Reacting
the Furan Alcohol 7a with σ -Chloranil

The furan alcohol 7a (0.0862g, 2.93×10^{-4} moles) was dissolved in 50 ml of chloroform. Ortho-chloranil (0.144g, 5.86×10^{-4} moles) was added to the furan alcohol 7a solution. The reaction flask was wrapped with aluminum foil and the mixture stirred under nitrogen overnight. The solvent was removed on the rotary evaporator. No identifiable product could be obtained. According to infrared analysis the product had only one carbonyl ν_{\max}^{KBr} 1625 cm^{-1} whereas the expected product would have had two carbonyls ν_{\max}^{KBr} 1624 , 1673 cm^{-1} .

Attempted Preparation of the Furan Aldehyde 8 by Reacting
the Furan Alcohol 7b with CrO_3 in Pyridine

The furan alcohol 7b (0.1273g, 4.33×10^{-4} moles) was suspended in 5 ml of pyridine. Chromium trioxide (0.0625g, 6.25×10^{-4} moles) was dissolved in 15 ml of chilled pyridine. The furan alcohol 7b solution was poured into the chromium trioxide solution and the reaction mixture stirred for 45 minutes under nitrogen. The flask was then stoppered and allowed to stand without stirring overnight. An equal volume of chloroform was added and the mixture filtered. The solvent (chloroform and pyridine) from the filtrate was removed on the rotary evaporator. The solid was then

heated in the vacuum oven at 45°C for 1 hour in order to remove the last traces of pyridine. Water (25 ml) was added to the solid and extracted with four ml portions of chloroform. The combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator yielding a brown solid (0.10g). Infrared analysis indicated that some furan aldehyde 8 had been formed; however, the material could not be further purified. The starting material, furan alcohol 7b, had only one carbonyl-- $\nu_{\text{max}}^{\text{KBr}}$ 1601 cm^{-1} . The product had two carbonyls-- $\nu_{\text{max}}^{\text{KBr}}$ 1605, 1675 cm^{-1} .

Attempted Preparation of the Furan Aldehyde 8
by Reacting the Furan Alcohol 7b with DMSO
and Acetic Anhydride

The furan alcohol 7b (1.4g, 4.70×10^{-3} moles) was dissolved in 12 ml of dry dimethylsulfoxide (DMSO). Acetic anhydride (8 ml) was added to the reaction mixture and stirred at room temperature for 20 hours. Absolute ethanol (26 ml) was added to the reaction mixture and stirred for 3 hours. Water (0.5 ml) was added and the reaction mixture cooled with stirring in an ice bath. Next chilled concentrated aqueous ammonia (17.3 ml) was added slowly. Then water (26 ml) was added. A precipitate formed which was filtered. The filtrate was extracted four times with 25 ml portions of chloroform. The combined chloroform extracts

were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator yielding 1.2g of a brown solid. Infrared analysis indicated that oxidation had proceeded only to a slight extent. Mostly, starting material was recovered.

Attempted Preparation of the Furan Methanesulfonate 7c

The furan alcohol 7b (0.0252g, 8.57×10^{-5} moles) and methanesulfonic anhydride (1.4598g, 8.38×10^{-3} moles) were warmed on a steam bath until the furan alcohol 7b was dissolved in the methanesulfonic anhydride. Concentrated sulfuric acid (1 drop) was added to the reaction mixture and heated for 1 hour on the steam bath. The reaction was allowed to cool and 20 ml of water were slowly added. Immediately a precipitate formed. The precipitate was filtered and the filtrate made basic with sodium bicarbonate. This basic solution was extracted four times with 25 ml portions of chloroform. The combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator. A solid was obtained. From infrared analysis it was determined that the desired product had not been obtained.

Attempted Preparation of the Furan Tosylate 7d

by Reacting the Furan Alcohol 7a with
p-toluenesulfonyl chlorine in Pyridine

The furan alcohol 7a (0.40g, 1.36×10^{-3} moles) was

dissolved in 10 ml of dry pyridine. The solution was then cooled to 0°C. p-Toluenesulfonyl chloride (0.518g, 2.72×10^{-3} moles) was dissolved in the cooled reaction mixture. The reaction was then placed in the refrigerator for 24 hours. A dark precipitate formed in the flask. The contents were poured into ice-water. The precipitate was filtered yielding a brown solid (0.20g) which proved to be starting material according to infrared analysis. The filtrate was extracted four times with 25 ml portions of chloroform. The combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator. A light brown solid (0.0344g) was recovered which according to infrared analysis was starting material. The infrared spectra of the product and reactant were identical.

Attempted Preparation of the Furan Tosylate 7d

by Reacting the Furan Alcohol 7a with

p-Toluenesulfonyl Chloride and

Sodium Hydride

The furan alcohol 7a (0.5476g, 1.86×10^{-3} moles) was dissolved in 100 ml of toluene. Sodium hydride (0.0861g of a 57% oil, 10% molar excess) was washed three times with toluene and the washed sodium hydride added to the furan alcohol 7a solution. p-Toluenesulfonyl chloride (0.3900g, 2.05×10^{-3} moles, 10% molar excess) was added to the solution.

The reaction mixture was stirred under nitrogen at room temperature for 24 hours. The reaction mixture was filtered yielding a brown solid (0.2869g). This material proved to be starting material according to infrared analysis. The solvent from the filtrate was removed on the rotary evaporator. This material also proved to be starting material. The reactant and product had identical infrared spectra.

Attempted Cyclization of the Furan Acetate 7b
by Heating the Furan Acetate 7b with Acetic
Anhydride and Zinc Chloride

The furan acetate 7b (0.0541g , 1.61×10^{-4} moles) was placed in a 10 ml round bottom flask. To the furan acetate 7b was added fused ZnCl_2 (0.0245g , 1.79×10^{-4} moles) and 5 ml of acetic anhydride. The reaction mixture was refluxed overnight. After refluxing, the reaction mixture turned black. The solvent, acetic anhydride, was removed on the rotary evaporator. Water was added to the black solid and the mixture extracted five times with 25 ml portions of chloroform. The combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent, chloroform, was removed on the rotary evaporator yielding a black solid (0.0400g). This material was chromatographed on a preparative TLC plate (fluorescent silica gel, 20 cm x 20 cm, 2.0 mm in thickness) using

absolute ethanoethylacetate (1:4) as a solvent. No identifiable product could be obtained. Two bands on the TLC plate were not starting material. This was determined by the fact that these bands had different R_f values and different infrared spectra from starting material. These products were present in such small amounts that they could not be further purified or identified.

Attempted Cyclization of the Furan Acetate 7b by

Heating the Furan Acetate 7b with Acetic

Acid Under Nitrogen

The furan acetate 7b (0.02g , 5.95×10^{-5} moles) was refluxed in 5 ml of acetic acid under nitrogen overnight. The solvent was removed on the rotary evaporator yielding a brown solid (0.020g). This material was dried in a vacuum oven at 45°C for 1 hour. According to infrared analysis this material proved to be starting material. Both starting material and product had identical infrared spectra.

$\nu_{\text{max}}^{\text{KBr}}$ 1620, 1725 cm^{-1} .

Attempted Cyclization of the Furan Acetate 7b

by Heating the Furan Acetate 7b with

Pyridine Under Nitrogen

The furan acetate 7b (0.0297g , 8.3×10^{-5} moles) was refluxed overnight in 10 ml of pyridine under nitrogen. The solvent was removed on the rotary evaporator yielding a

brown solid (0.0265g). This material was dried in a vacuum oven at 45°C for 1 hour. According to infrared analysis this material proved to be starting material. Both starting material and product had identical infrared spectra.

ν_{\max}^{KBr} 1620, 1725 cm^{-1} .

Attempted Cyclization of the Furan Acetate 7b by

Heating the Furan Acetate 7b with Acetic

Acid and Sodium Acetate

The furan acetate 7b (0.0265g, 7.88×10^{-5} moles) was refluxed in 15 ml of acetic acid containing sodium acetate (0.030g, 3.66×10^{-4}) under nitrogen. The solvent was removed on the rotary evaporator yielding a brown solid. Water (25 ml) was added and the material extracted five times with 25 ml portions of chloroform. The combined chloroform extracts were washed with water and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator yielding a brown solid (0.20g). According to infrared analysis this material proved to be starting material. Both starting material and product had identical infrared spectra. ν_{\max}^{KBr} 1620, 1725 cm^{-1} .

Attempted Preparation of Cyclized Compound 9 by Heating the

Furan Aldehyde 8 with Piperidine and Benzene¹⁹

The furan aldehyde 8 (0.0642g, 2.20×10^{-4} moles) was dissolved in a minimum amount of chloroform. Benzene (20 ml)

was added and the mixture refluxed in a 25 ml round bottom flask with a Dean-Stark phase separating head attached. Approximately 4 ml of solvent was removed from the trap. This was done in order to remove chloroform and any water that might be present. This procedure had to be used to dissolve the furan aldehyde 8 in benzene. The reaction mixture was cooled well below the boiling point and piperidine (2.5×10^{-2} ml, 2.94×10^{-4} moles) was added. The mixture was then refluxed for 3 hours. The solvent was removed on the rotary evaporator leaving a yellow solid (0.0600g) which was dried in the vacuum oven at 70°C for 2 hours. This material was chromatographed on silica gel. According to infrared and ultraviolet analysis the expected product was not obtained.

Attempted Preparation of Cyclized Compound 9 by Heating
the Furan Aldehyde 8 with Acetic Anhydride

The furan aldehyde 8 (0.2238g, 7.66×10^{-4} moles) was refluxed for 8 hours in acetic anhydride. The acetic anhydride was removed on the rotary evaporator yielding a black gum (0.2230g) which was dried for 3 hours in a vacuum oven at 70°C. This material was then chromatographed over silica gel; however, no identifiable product could be obtained. No product was obtained that had only one carbonyl as would be expected in the cyclized product. The detection of carbonyls was done by infrared analysis.

I. Attempted Preparation of Cyclized Compound 9 by Heating the Furan Aldehyde 8 with Acetic Anhydride and Benzene

The furan aldehyde 8 (0.163g, 5.58×10^{-4} moles) was placed in a 25 ml round bottom flask. Anhydrous benzene (17 ml) and acetic anhydride (0.06 ml, 5.58×10^{-4} moles) were added to the flask and the contents refluxed for 83 hours. Water (10 ml) was added to the flask and the solution stirred for 1 hour. The reaction mixture was neutralized with sodium bicarbonate. The benzene layer was separated in a separatory funnel, washed with water, and dried over anhydrous magnesium sulfate. The solvent was removed on the rotary evaporator yielding a yellow solid (0.0552g). This material was subjected to column chromatography using silica gel then preparative thin layer chromatography using silica gel. Infrared analysis indicated new products had formed; however, no identification could be made.

II. Attempted Preparation of Cyclized Compound 9 by Heating the Furan Aldehyde 8 with Acetic Anhydride and Benzene

The furan aldehyde 8 (0.141g, 4.83×10^{-4} moles) was placed in a 25 ml round bottom flask. Anhydrous benzene (15 ml) and acetic anhydride (0.0493 ml, 4.83×10^{-4} moles) were added to the flask and the contents refluxed for 24 hours. The solvent was removed on the rotary evaporator yielding a solid (0.141g) which was dried in the vacuum oven at 50°C for 5 hours. The material proved to be starting

material according to infrared analysis. The starting material and product had identical infrared spectra.

CHAPTER IV

DISCUSSION OF RESULTS

As stated in the Introduction, the purpose of this research was to prepare camptothecin in a yield high enough to lend itself to commercial preparation. Two factors caused this goal to be unobtainable. First, the furan aldehyde 8 could not be prepared in good yield, and second, the furan aldehyde 8 could not be cyclized. Several new compounds were prepared.

As shown in Figure 1 the proposed synthesis of camptothecin started with the preparation of anhydride 2. 3,4-Furandicarboxylic acid was dissolved in acetic anhydride and refluxed for eight hours. The precipitate which formed when the solution cooled overnight was dried in the vacuum oven at 80°C for 24 hours. Removing acetic anhydride from the furan anhydride 2 proved to be very difficult. The precipitate had to be crushed to a fine powder so that all of the acetic anhydride could be removed. The furan anhydride was obtained in 63% yield and melted between 94°C and 96°C. The infrared spectrum showed bands at 3100 cm^{-1} (aromatic), 1740 cm^{-1} (anhydride), and (1530 cm^{-1}) aromatic. Mass spectrum analysis gave M^+ at m/e 138 (calcd. for $\text{C}_6\text{H}_2\text{O}_4$, m/e of 138).

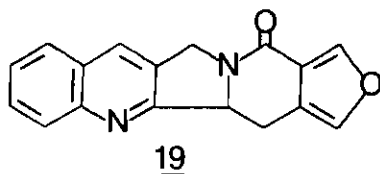
The furan anhydride 2 was then reacted with the pyrroloquinoline 4 at room temperature for one hour in ethanol. The product obtained was the furan acid 5 which had a melting range of 205-220°d. Infrared analysis showed bands at 3100 cm^{-1} (aromatic) and 1635 cm^{-1} (amide). Mass spectral analysis gave M^+ at m/e of 308 (calcd. for $C_{17}H_{12}O_4N_2$, m/e of 308).

The furan ester 6 was prepared in the following manner. An ether solution of diazomethane, generated from Dupont EXR-101, was added to the furan acid 5, suspended in methanol, and stirred overnight. The solution was filtered and the solvent was removed on the rotary evaporator yielding a brown gum. The brown gum was recrystallized from absolute ethanol yielding a white solid (m.p. 164-166°C) which proved to be the furan ester 6. The infrared spectrum showed bands at 1715 cm^{-1} (ester) and 1630 cm^{-1} (amide). Mass spectral analysis gave M^+ at m/e of 322 (calcd. for $C_{18}H_{14}O_4N_2$, m/e of 322). Combustion analysis gave 66.91% C, 8.84% N, 4.45% H (calcd.: 67.07% C, 8.69% N, 4.38% H).

The furan alcohol 7a was prepared by reacting the furan ester 6 with sodium borohydride in methanol. It was found that a 100 fold molar excess of sodium borohydride was necessary in order to consistently get a 20% yield of furan alcohol. Lithium borohydride was also tried as a reducing agent; however, according to infrared analysis only starting material was found in the reaction mixture. The furan ester

6 was dissolved in methanol and was poured on to the sodium borohydride. After the initial vigorous reaction subsided, the reaction mixture was refluxed overnight. After refluxing, an equal volume of water was added to the reaction mixture. Approximately one-half of the methanol was removed on the rotary evaporator and the resulting solution extracted with chloroform. Removing the chloroform by the use of a rotary evaporator left a brown solid, which when recrystallized from absolute ethanol, yielded a light brown solid (m.p. 217°C), which proved to be the furan alcohol 7a. Infrared analysis showed bands at 3400 cm^{-1} (alcohol) and 1601 cm^{-1} (amide). Mass spectral analysis gave M^+ at m/e of 292 (calcd. for $\text{C}_{17}\text{H}_{14}\text{O}_3\text{N}_2$, m/e of 292). Combustion analysis gave 69.43% C, 9.68% N, 4.96% H (calcd.: 69.38% C, 9.52% N, 4.80% H).

The preparations of several derivatives of the furan alcohol 7a were attempted. Only one of the three derivatives (the Furan acetate 7b, the furan mesylate 7c, and the furan tosylate 7d) was prepared, the furan acetate 7b. The purpose of the derivatives was to prepare a compound with a leaving group from which compound 19 could be synthesized. The furan acetate 7b was prepared by stirring the furan



alcohol 7a in an acetic anhydride-pyridine (4:1) mixture. The light pink solid 7b had a melting point of 188-190°C. The infrared spectrum showed bands at 1725 cm^{-1} (acetate), 1625 cm^{-1} (amide), and 1410 cm^{-1} (acetate). Mass spectral analysis gave M^+ at m/e of 336 (calcd for $\text{C}_{19}\text{N}_2\text{O}_4\text{H}_{16}$, m/e of 336). Combustion analysis gave 68.04% C, 8.39% N, 4.94% H (calcd.: 67.85% C, 8.33% N, 4.80% H). The attempted preparation of the furan methanesulfonate 7c was carried out in the following manner. The furan alcohol 7a was dissolved in methanesulfonic anhydride on a steam bath. One drop of sulfuric acid was added and the mixture heated one hour. The product obtained was not the furan methanesulfonate 7c according to infrared analysis. Two different methods were used in the attempted preparation of the furan tosylate 7d. In one method p-toluenesulfonyl chloride in pyridine was used and in the other method p-toluenesulfonyl chloride and sodium hydride was used. In each case the infrared spectra of the reactant and product was identical.

Since the only derivative of the furan alcohol 7a which could be prepared was the furan acetate 7b it was decided to try and cyclize the furan acetate 7b to compound 15. Four methods were used in the attempted preparation of compound 15 by the cyclization of the furan acetate 7b. In method I the furan acetate 7b and a catalytic amount of fused zinc (II) chloride were refluxed overnight in acetic anhydride. The reaction mixture turned black. After workup

the material was chromatographed on a preparative TLC plate. No identifiable products could be obtained. Three other methods were used in the attempted cyclization of the furan acetate. In method II the furan acetate 7b was refluxed overnight in acetic acid under nitrogen. In method III the furan acetate 7b was refluxed overnight in pyridine under nitrogen. Finally, in method IV the furan acetate 7b and a catalytic amount of sodium acetate were refluxed overnight in acetic acid. In each case both starting material and product had identical infrared spectra.

Since compound 15 could not be prepared it was decided to prepare the furan aldehyde 8 from the furan alcohol 7a. Attempts would then be made to cyclize the furan aldehyde 8 in order to prepare compound 9. Four methods were used in the preparation of the furan aldehyde 8; however, only one proved successful in producing the furan aldehyde 8 in satisfactory amounts. In method I the furan alcohol 7a and a two fold molar excess of ortho-chloranil were stirred in chloroform under nitrogen overnight. The reaction flask had been wrapped in aluminum foil. According to infrared analysis the expected product had not been obtained. Method II involved the use of chromium trioxide and pyridine, chromium trioxide was dissolved in chilled pyridine. The furan alcohol 7b, which had been suspended in pyridine, was poured into the chromium trioxide solution and the reaction mixture stirred for 45 minutes under nitrogen.

The flask was then stoppered and the reaction mixture was allowed to stand overnight. After workup it was determined by infrared analysis that a small amount of furan aldehyde 8 had been prepared; however, another preparative method which would give the expected product in higher yield was needed. The product had two carbonyls-- $\nu_{\text{max}}^{\text{KBr}}$ 1605, 1675 cm^{-1} . Method III used dimethylsulfoxide (DMSO) in acetic anhydride as the oxidant. The furan alcohol 7b was dissolved in dry DMSO. Acetic anhydride was added and the reaction mixture stirred at room temperature for 20 hours. After workup infrared analysis indicated that oxidation had proceeded to only a slight extent. Mostly starting material was recovered. Method IV was the method which proved to be the most successful in the preparation of the furan aldehyde 8. This method used a chromium trioxide complex $[\text{CrO}_3 (\text{C}_5\text{H}_5\text{N})_2]$ in methylene chloride as the oxidizing agent. The $\text{CrO}_3(\text{py})_2$ complex was prepared in the following manner. Anhydrous pyridine was cooled in a ice-water bath to 15-20°. Chromium trioxide which had been stored over phosphorus pentoxide was added over a period of one hour at a rate so as to keep the temperature below 30°C. A yellow precipitate had formed which when stirred an additional hour at 15°C reverted to a deep red macrocrystalline form. The precipitate $[\text{CrO}_3(\text{py})_2]$ was washed with petroleum ether and collected by suction filtration. The oxidation of the furan alcohol 7b by the $\text{CrO}_3(\text{py})_2$ complex was carried out in the following manner.

A six fold molar excess of the $\text{CrO}_3(\text{py})_2$ complex was used. This complex was prepared as a 5% solution in dry methylene chloride. Immediately upon mixing the furan alcohol 7b and $\text{CrO}_3(\text{py})_2$ complex solutions a brownish black precipitate formed. This mixture was stirred for 30 minutes then filtered. The solvent from the filtrate was removed on the rotary evaporator yielding a brown solid. When this material was chromatographed on silica gel a light tan solid was separated which proved to be the fural aldehyde 8 (31.4%), m.p. 194-195°; $\nu_{\text{max}}^{\text{KBr}}$ 2925, 2850, 1673, 1624 cm^{-1} ; mass spectrum showed m/e of 85 (41%), 123 (63%), 159 (100%), 274 (18.5%), 292 (55%); anal.: 69.66% C, 9.65% N, 4.25% H (anal. Calcd. for $\text{C}_{17}\text{N}_2\text{H}_{12}\text{O}_3$: 69.86% C, 9.59% N, 4.14% H).

Four methods were used in order to try to synthesize compound 9 from the furan aldehyde 8. Method I involved the attempted cyclization of the furan aldehyde 8 by refluxing it with a catalytic amount of piperidine in benzene for three hours. A yellow solid was produced which was chromatographed on silica gel. According to infrared and ultraviolet analysis the expected product was not obtained. In method II the furan aldehyde 8 was refluxed for 8 hours in acetic anhydride. A black gum formed which when chromatographed over silica gel yielded no identifiable product. No product was obtained which had only one carbonyl. The detection of carbonyls was done by infrared analysis. Method III in which the furan aldehyde 8 is heated with a

CHAPTER V

CONCLUSIONS

Several new compounds have been prepared during the course of this research. A number of attempts were made to cyclize the furan aldehyde 8 to intermediate 9. Since the furan aldehyde 8 could only be obtained in poor yield the proposed synthesis, had it worked, would not have been of commercial value.

CHAPTER VI

RECOMMENDATIONS

Since camptothecin no longer holds the promise that it once did in clinical testing, a low cost synthesis would be of limited value.

Further attempts to cyclize the furan aldehyde 8 into intermediate 9 might be worthwhile in order to test the biological activity of intermediate 9.

BIBLIOGRAPHY

1. M. E. Wall, M. C. Wani, C. E. Cook, K. H. Palmer, A. T. McPhail and G. A. Sim, J. Amer. Chem. Soc. 88, 3888 (1966).
2. M. E. Wall, K. H. Palmer, M. C. Wani, C. E. Cook, G. A. Sim, and A. T. McPhail, Abstracts, 4th International Symposium on Natural Products, IUPAC, Stockholm, June, 1966, p. 103.
3. Chem. Eng. News, 44 (28), 19 (1966).
4. Chem. Eng. News, 44 (51), 65 (1966).
5. J. A. Gottlieb, A. M. Quarino, J. B. Call, V. T. Oliverio and J. B. Block, Cancer Chemother. Rep. 54, 461 (1970).
6. Jane E. Collins and James F. Kieley, HEW News, National Institutes of Health, National Cancer Institute, April 9, 1970.
7. U. Schaeppi, D. A. Cooney, and R. D. Davis, U. S. Clearinghouse Fed. Sci. Tech. Inform., PB Rep 1967, PB-180549, 13 pp.
8. U. Schaeppi, H. Rosenkrantz, M. M. Mason, P. S. Schein, and R. D. Davis, U. S. Clearinghouse Fed. Sci. Tech. Inform., PB Rep 1968, PB-179993, 158 pp.
9. G. Stork and A. Schultz, J. Amer. Chem. Soc. 93, 4074 (1971).
10. R. Volkmann, S. Danishefsky, J. Egglev and D. M. Solomn, J. Amer. Chem. Soc. 93, 5576 (1971).
11. A. S. Kende, T. J. Bentley, R. W. Draper, J. K. Jenkins, M. Joyeux and I. Kubo, Tetrahedron Letters 1307-1310 (1973).
12. M. C. Wani, H. F. Campbell, G. A. Brine, J. A. Kepler, Monroe E. Wall and S. G. Levine, J. Amer. Chem. Soc. 94, 3631 (1972).
13. Tsutomu Sugawara, Tatsuo Toyoda, and Kazuyuk Sasakura, Tetrahedron Letters 5109-5112 (1972).

14. E. Winterfeldt, T. Korth, Dennis Pike and M. Boch, Angew. Chem. Internat. Ed. 11, 289 (1972).
15. Cyril Tang and Henry Rapoport, J. Amer. Chem. Soc. 94, 8615 (1972).
16. A. I. Meyers, Robert L. Nolen, Eric W. Collington, Thomas A. Norwid, and Roger C. Strickland, J. Org. Chem. 38, 1974-1982 (1973).
17. J. B. Nabors, Ph.D. Thesis, Georgia Institute of Technology, (1969).
18. J. C. Collins, W. W. Hess, and F. J. Frank, Tetrahedron Letters, 3363 (1968).
19. Organic Synthesis, 41, 38 (1961).
20. D. A. Murphy, M. S. Thesis, Georgia Institute of Technology, (1970).