AN ABSTRACT OF THE THESIS OF

Guy Alan Schiehser for the degree of Doctor of Philosophy in Chemistry

presented on November 2, 1979

Title: I. THE ISOLATION AND STRUCTURE OF LEPTOSPHAERIN: A METABOLITE OF THE MARINE ASCOMYCETE, LEPTOSPHAERIA ORAEMARIS (LINDER) II. A SYNTHESIS OF THE MARINE TOXIN, 9-ISOCYANOPUPUKEANANE

Abstract approved: Redacted for Privacy

Dr. James D. White

The ethyl acetate extract of the culture filtrate of the marine Ascomycete, Leptosphaeria oraemaris (Linder) yielded a novel metabolite designated by the common name leptosphaerin. An x-ray crystallographic structure analysis was disproved by the synthesis of 3-acetoxy-5-(1,2-dihydroxyethyl)-3-pyrrolin-2-one 1, starting with N,N-dibenzylhydroxylamine 47 and D-mannitol 44. Oxidation of 47 followed by acid catalyzed hydrolysis gave N-benzylhydroxylamine 49. D-mannitol 44 was converted to a bis acetonide and the resulting vicinal diol was cleaved with lead tetraacetate to afford 2,3-O-isopropylidene-D-glyceraldehyde 46. Condensation of 46 and 49 gave N-benzyl-N-(2,3-O-isopropylideneglyceraldimine)-N-oxide 50 which, upon thermolysis in the presence of methyl acrylate yielded 2-benzyl-3-(1,2-O-isopropylidene dihydroxyethyl)-5-carbomethoxyisoxazolidine 75. Catalytic hydrogenolysis followed by oxidation and acetylation produced 3-acetoxy-5-(1,2-O-isopropylidenedihydroxyethyl)-3-pyrroline-2-one 81. Removal of the acetonide protecting group generated 1, which proved to be nonidentical with the natural product.

The structure of the metabolite was established by spectral analysis

and literature comparisons to be 2-acetamido-2,3-dideoxy-erythro-hex-2-enono-1,4-lactone 86.

II

A formal synthesis of the marine toxin, 9-isocyanopupukeanane 1 is described starting with 4-methylanisole 23. Birch reduction of 23 afforded 1-methoxy-4-methylcyclohexa-1,4-diene 24 which was sequentially cyclopropanated with methylene iodide-zinc-silver couple and ethyldiazoacetate to give 4-carboxyethyl-1-methoxy-5-methyltricyclo[5.1.0.$0^{3,5}$]octane 26. Acid catalyzed hydrolysis and rearrangement of 26 followed by ketalization and elimination of methanol yielded methyl 1,5-dimethyl-4-methoxy-2,4-cyclohexadienyl acetate 30. Conversion of the acetic ester residue of 30 to a vinyl ketone was effected by a conventional sequence of reduction, oxidation, addition of vinyl magnesium bromide and oxidation to produce (1,5-dimethyl-4-methoxy-2,4-cyclohexadienyl)-but-3-en-2-one 36. The generation of the [4.3.1.$0^{3,7}$]tricyclodecane skeleton was accomplished by an intramolecular Diels-Alder reaction of 36 which gave 1,5-dimethyl-9-methoxy-5-oxotricyclo[4.3.1.$0^{3,7}$]dec-8-ene 47. Treatment of 47 with isopropenyllithium afforded 1,3-dimethyl-5-isopropenyl-9-methoxytricyclo[4.3.1-$0^{3,7}$]dec-8-en-5-ol 52. Thermolysis of 52 in dimethyl sulfoxide led to the elimination of the tertiary alcohol and was attended by the hydrolysis of the vinyl ether to yield 1,3-dimethyl-5-isopropenyl-9-oxatricyclo[4.3.-1.$0^{3,7}$]dec-4-ene 54. Upon catalytic hydrogenation with platinum, diene 54 produced 1,3-dimethyl-5-isopropyl-9-oxotricyclo[4.3.1.$0^{3,7}$]decane 5 which constitutes a formal synthesis of the toxin by virtue of its previous conversion to 9-isocyanopupukeanane.

THE ISOLATION AND STRUCTURE OF LEPTOSPHAERIN: A METABOLITE OF THE MARINE ASCOMYCETE, LEPTOSPHAERIA ORAEMARIS (LINDER)

and

A SYNTHESIS OF THE MARINE TOXIN, 9-ISOCYANOPUPUKEANANE

by

Guy Alan Schiehser

A THESIS

submitted to

Oregon State University

in partial fulfillment of

the requirements for the

degree of

Doctor of Philosophy

Commencement June 1980

APPROVED:

Redacted for Privacy

Professor of Chemistry, in charge of major

Redacted for Privacy

Chairman of Department of Chemistry

Redacted for Privacy

Dean of Graduate School

Date thesis is presented November 2nd, 1979

our souls touch

our hearts feel

our bodies meet

and we are

one

complete

allness

Susan Polis Schutz

To

Nancy,

Dawn Marie,

and Michael Alan

and

To

my parents

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Professor James D. White for his continual support and encourangement. It has been largely through his efforts that my graduate experience has been one of immense personal growth and discovery.

I also wish to thank Dr. Philip Catalfomo for his patient guidance, tolerance and critical perceptions during my initial graduate work.

I gratefully acknowledge the generous support of the Nicholas L. Tartar Foundation.

TABLE OF CONTENTS

PART I

THE ISOLATION AND STRUCTURE OF LEPTOSPHAERIN

A. INTRODUCTION

Interest in marine natural products has its origins in antiquity, yet only in the last several decades has a serious effort been made to evaluate and exploit the ocean's chemical resources.[1] Representing a logical extension of terrestrial natural products chemistry, the investigation of marine organisms has derived much of its impetus from the search for new sources of physiologically active compounds.[2] As the field has evolved, this preoccupation with biomedicinals has yielded somewhat reluctantly to more academic pursuits such as chemotaxonomy and chemical ecology.[3]

The organisms studied to date, while they only constitute a small percentage of the estimated marine species, span the taxonomic spectrum and have provided an impressive array of structurally novel and functionally unique metabolites.[4,5]

It is curious, in view of these intense research efforts that one group of organisms, the marine fungi, have been the subject of only a few chemical studies and little is known of their secondary metabolic capabilities or their chemical role in the marine environment.[6] This is even more remarkable when one considers the abundance of metabolic data available on their terrestrial counterparts.[7] In contrast to the majority of marine organisms, the marine fungi possess the significant property of ready adaptability to artificial liquid culture. As a consequence, many

of the difficulties associated with acquisition of specimens for natural products work, such as seasonal and geographical fluctuations in availability and metabolic patterns can be eliminated. A more important consequence of this adaptability is the potential for precise control of growth conditions necessary to laboratory investigations and which have been exploited commercially to optimize the production of economically important metabolites, most notably the penicillins and ergot alkaloids, from terrestrial fungi.

The first reported chemical studies of marine fungi appeared in the literature in 1957 with Vishniac's[8] isolation of cholesterol in Myxomycete Labyrinthula minuta var. atlantica. Schafer and Lane[9] subsequently identified twelve amino acids in the acid hydrolysate of Lulworthia floridana.

An investigation by Kirk and Catalfomo[10] of fourteen isolates representing ten species of higher filamentous marine fungi led to the identification of choline in two isolates and established the presence of ergosterol in six isolates. The isolation of sterols from marine fungi was also reported by Teshima and Kanazawa,[11] who identified ergosterol and campesterol in five species of marine yeasts.

In 1972, Catalfomo et.al.[12] isolated and identified choline sulfate (ester) in Zalerion maritimum (Linder) on the basis of infrared and nuclear magnetic resonance spectra. The metabolite was also identified in eighteen additional isolates, representing ten species of marine Ascomycetes by comparative thin layer chromatography.

The hydrolysis of the triglyceride fractions from Corollospora maritima Werdermann and Zalerion maritimum (Linder) Anistasiou, described by Block et.al.[13] in 1973, yielded as major components, oleic, palmitic

and linoleic acids while minor amounts of stearic, palmitoleic, caproic, myristic, behenic and linolenic acids were also detected.

In a recent communication, Peters et.al.[14] reported the determination of free amino acids in eight Ascomycetes and two Fungi Imperfecti utilizing a complementary system of two-dimensional thin layer and gas-liquid chromatography.

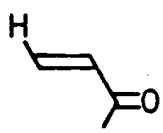
In drawing attention to the potential importance of marine fungi as a source of nutrients and vitamins or their precursors in the detritus food webs of coastal and estuarine environments, Kirk[15] has cited evidence suggesting specific nutrutional relationships between marine fungi and such marine organisms as nematodes and marine woodborres. Noting the occurrence of free hydroxyproline, a major constituent of collagen in marine and terrestrial animals, Peters[14] has suggested that marine fungi may constitute an important source of amino acids in detritus feeding animals.

A suggestion by Catalfomo[12] that the presence of choline sulfate in higher marine fungi and red algae, with its conspicuous absence in the lower fungi, lent support to the hypothesis of a Floridean ancestry of Ascomycetes, was recently cited by Kohlmeyer[16] in a re-evaluation of the evidence for the phylogenetic origin of Ascomycetes.

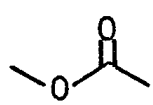
The purpose of the present investigation was to further delineate the metabolic capabilities of marine fungi by the isolation and structural elucidation of previously unreported metabolites[10,12,14] of the marine Ascomycete Leptosphaeria oraemaris (Linder).

B. STRUCTURE ELUCIDATION

An isolate of the marine Ascomycete Leptosphaeria oraemaris (Linder) was placed into artificial liquid culture and the concentrated culture filtrates were partitioned with hexane and ethyl acetate. Chromatographic examination of the ethyl acetate extract yielded 25 mg of a white crystalline solid melting between 189 and 190°C and designated as leptosphaerin. The empirical formula was determined by high resolution mass spectrometry to be $C_8H_{11}NO_5$. Subsequent spectral analysis permitted the identificiation of several of the structural features present in the molecule. The ultraviolet absorption at 246 nm, together with the infrared absorption at 1670 and 1640 cm^{-1}, suggested an α,β-unsaturated carbonyl system in which the proton of the β-carbon appeared as a doublet at δ 7.51 in the nmr spectrum.

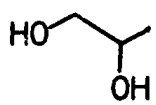


A peak in the infrared spectrum at 1745 cm^{-1} was considered indicative of an ester functionality.



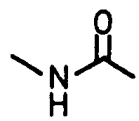
Two exchangeable proton resonances, one a doublet at δ 4.25 and the other in the complex centered at δ 3.80 pointed to a diol in which at least one of the alcohols was secondary. In order to determine the precise nature of the exchangeable protons, advantage was taken of the ability of dimethyl sulfoxide to cause downfield shifts and permit the observation to spin-spin coupling in hydroxylic protons.[7] The spectrum recorded in d_6-dimethyl sulfoxide revealed a primary hydroxyl as a triplet (δ 4.80 J=5Hz, removed by D_2O exchange) while the secondary hydroxyl was obscured in a two proton multiplet at δ 5.14. Cooling the sample to 10°C shifted the resonance downfield (δ 5.26, J=5.5Hz), where it appeared as a doublet. The corresponding methylene ($-CH_2OH$) and methine (-CHOH) protons were observed as two proton (δ 3.44) and one proton (δ 3.71) multiplets, respectively.

Acetylation of leptosphaerin led to the disappearance of the two high field exchangeable resonances and the complementary appearance of two three-protons singlets at δ 2.08 and δ 2.05. In addition, the characteristic 8-line pattern (δ 4.15, 1H, J=4,13Hz and δ 4.46, 1H, J=5,13Hz), representing the AB portion of the ABX ($-CH(OAc)-CH_2OAc$) system, established the group as a terminal 1,2-diol.

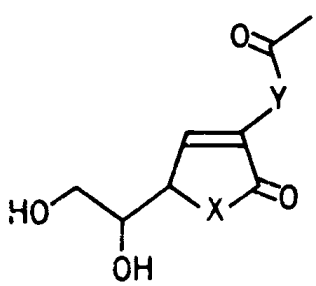


The presence of the amide group found its principal evidence in the broad, exchangeable resonance at δ 8.88 (d_6-acetone). This signal exhibited an anticipated solvent shift in dimethyl sulfoxide, where it appeared as a broadened singlet at δ 9.99. A more pronounced effect was

noted for the diacetate, where the N-H proton fell at δ 7.66 in $CDCl_3$ and δ 10.12 in d_6-dimethyl sulfoxide. Although multiple absorptions (3435, 3355, and 3290 cm^{-1}) in the infrared spectrum of the diol prevented assignment of the N-H stretching band, the acetylated product showed only a single peak above 3000 cm^{-1} (3360 cm^{-1}).



Subjecting the diol to a double resonance experiment provided further clarification of the structure. Irradiation of the one proton doublet of doublets (δ 5.12, J=1.75, 4.75Hz) collapsed the doublet at δ 7.51 to a singlet while the multiplet centered at δ 3.78 showed apparent decoupling although the complex remained unresolved. Taking into consideration the singlet (3H) at δ 2.14 and the mass spectral base peak at m/e 141.037 representing the loss of the elements of acetic acid, two plausible structures, 1 and 2, were proposed.



1 X = NH, Y = O

2 X = O, Y = NH

At this juncture, lacking sufficient quantities of material for additional chemical transformation, we prepared, to constant melting point, crystals of leptosphaerin which were submitted for an x-ray structure determination by Dr. Jon Clardy at Iowa State University. The computer simulated drawing (Figure 1) showed the compound to be an α,β-unsaturated-γ-lactam with an acetoxy group at C-3 and an ethanediol substituent at C-5. Although the absolute stereochemistry was not determined, the relative orientation at C-5,6 was as depicted in the drawing.

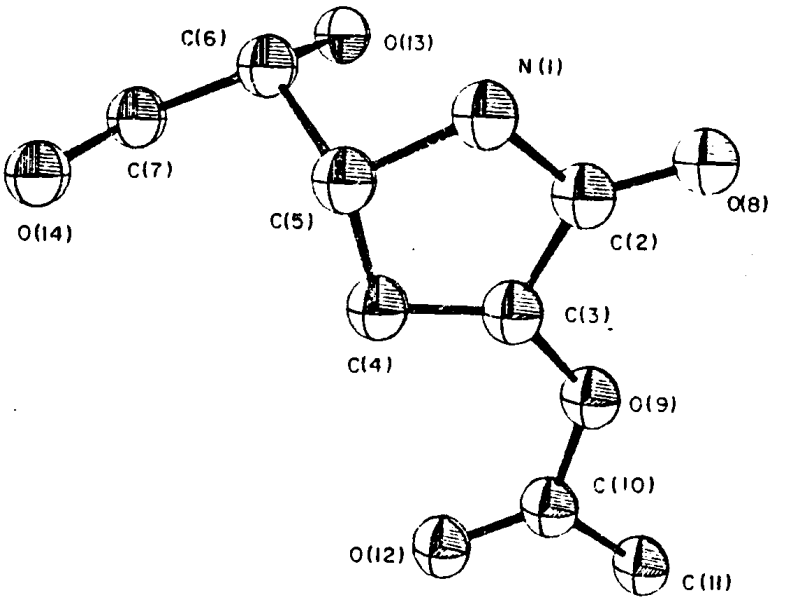


Figure 1

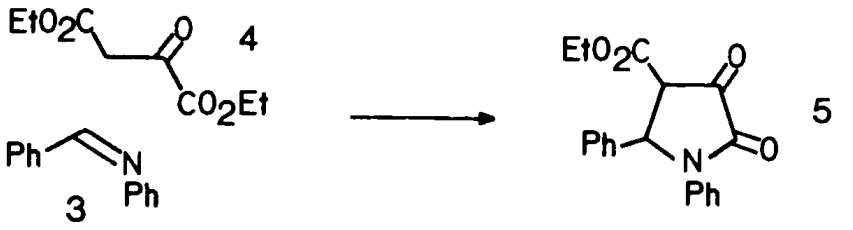
With a structure in hand, our interest was directed toward the development of a synthetic approach to this compound. It was hoped that a synthesis would not only provide sufficient quantities of material for biological testing but would also allow the determination of the abso-

lute stereochemistry of the product. In order to accomplish the latter, a route with absolute stereochemical control of at least one of the two asymmetric centers in the molecule is required. Since the center at C-5 is potentially epimerizable, it was decided that the center at C-6 should be introduced stereospecifically. For synthetic convenience, we assumed the center at C-6 to be S. This was based primarily upon the marked resemblance of the six carbon skeleton of leptosphaerin to an amino sugar and its presumed biogenesis from D-glucose.

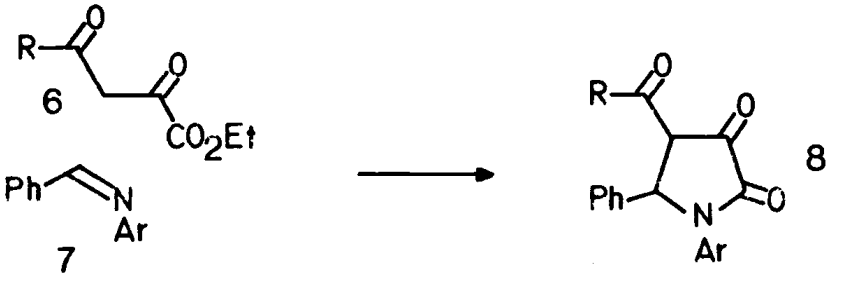
C. SYNTHESIS OF THE PUTATIVE LEPTOSPHAERIN

Although no 3-acetoxy-3-pyrrolin-2-ones have been reported to occur naturally, synthetic approaches to this system and their progenitors, the 2,3-dioxopyrrolidines and the 3-hydroxy-γ-lactams, have been described by a number of investigators.

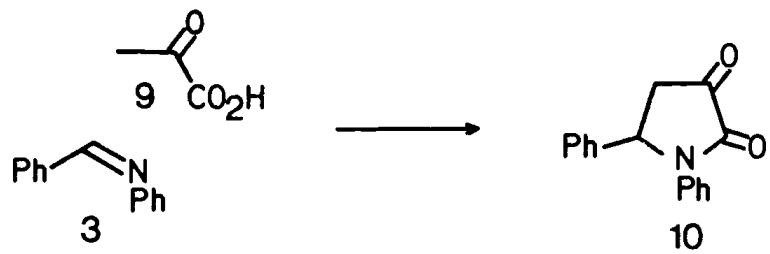
In the first known example, Schiff and Bertini[18] prepared the 3-oxo-2-pyrrolidone 5 by condensation of benzalaniline 3 and ethyl oxalacetate



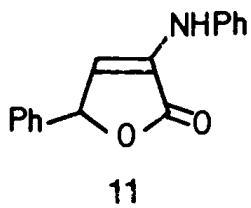
4. In 1898, Schiff and Gigli[19] prepared a series of 4-acyl-pyrrolidin-2, 3-diones 8 by pyrolysis of substituted oxaloesters 6 and benzaldehyde



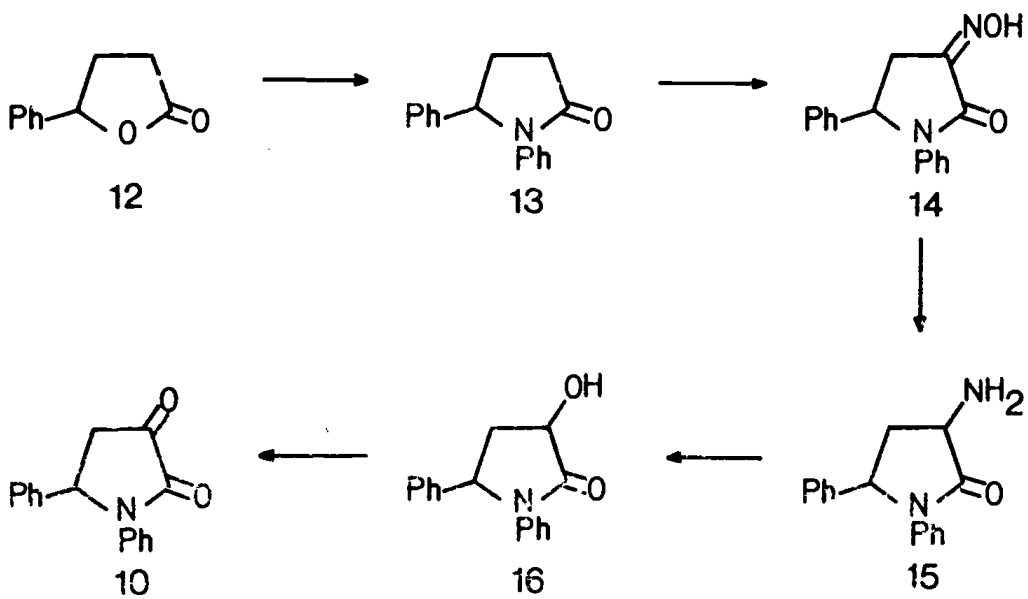
imines 7. The authors also reported the reaction of pyruvic acid 9 with benzalaniline 3 to give a crystalline product identified as 1,5-diphenyl-2,3-pyrrolidinedione 10. The result was to go unchallenged for over half



a century when two independent studies [20,21] established the correct structure as lactone 11. Meyers and Vaughan[22] prepared authentic 1,5-diphenyl-

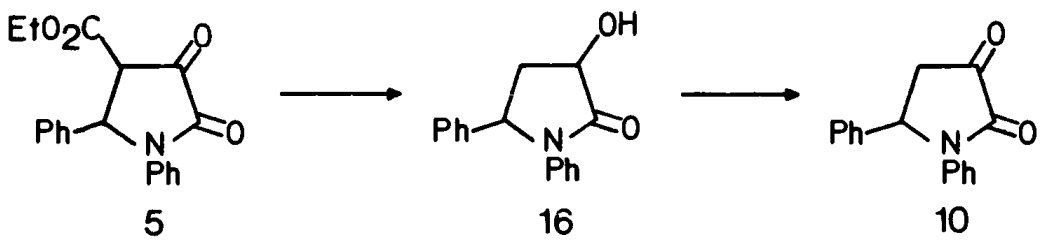


3-oxo-2-pyrrolidone 10 by the route outlined in Scheme I.



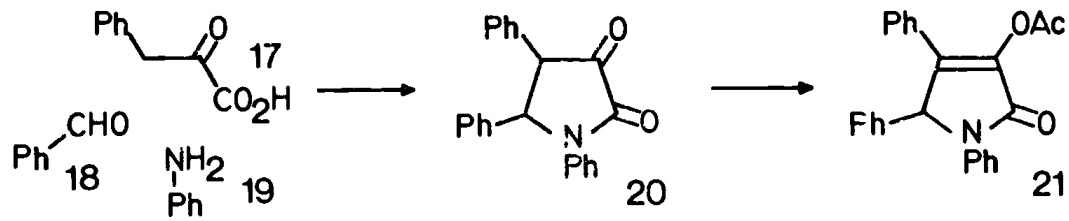
Scheme 1

Support for the anilinolactone 11 was also provided by the unambiguous synthesis of dione 10 by Wasserman and Koch[23] (Scheme II).

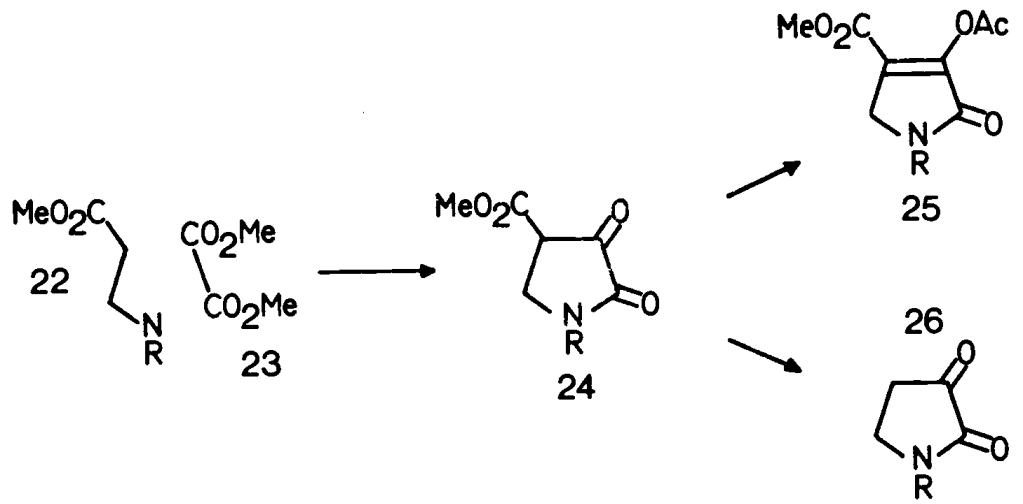


Scheme II

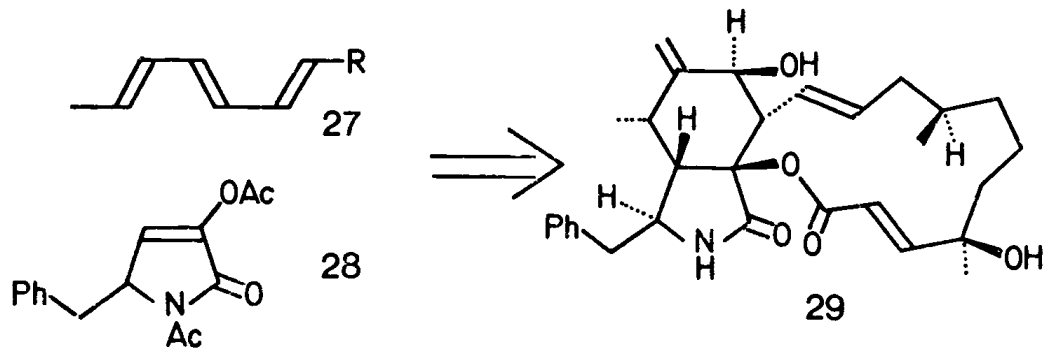
Borsche[24] prepared a series of 1,4,5-triaryl-3-oxo-2-pyrrolidones by condensing arylamines and aryl aldehydes with 3-arylpyruvic acids. Treatment of 20 with sodium acetate and acetic anhydride produced the



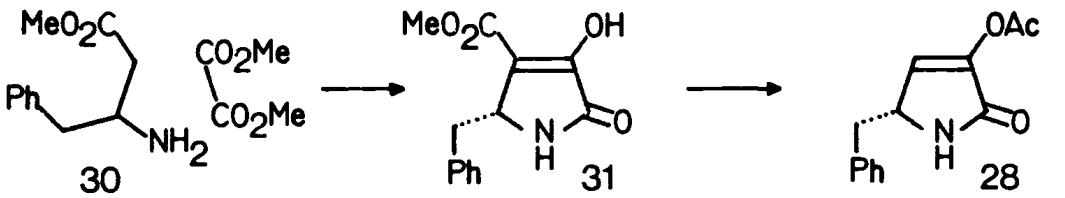
enol acetate 21. Southwick[25] generated a number of 4-carboalkoxy-3-acetoxypyrrolidones 25 (R=H, methyl, isopropyl) by the base-catalyzed addition of β-aminopropionates to dimethyl oxalate followed by treatment of the



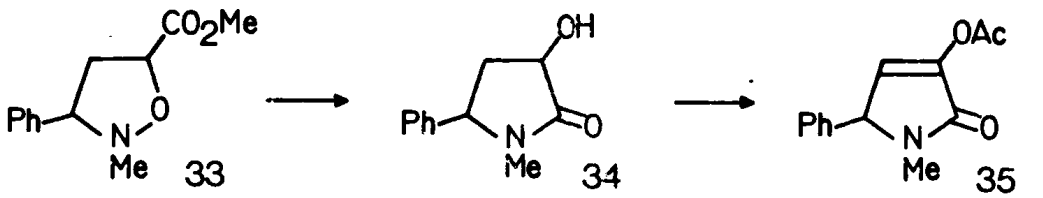
derived α-oxolactams 24[26] with ketene. Acid-catalyzed hydrolysis and decarboxylation of intermediates of type 24 (R=n-butyl, isobutyl, t-butyl, cyclohexyl, phenyl and β-phenethyl) led to the corresponding 3-oxo-2-pyrrolidones 26. A recent application of this approach appeared in Stork's[27] synthesis of cytochalasin B 29 which featured a Diels-Alder reaction of triene 27 with acetoxypyrrolone 28. The preparation of the dienophile 28



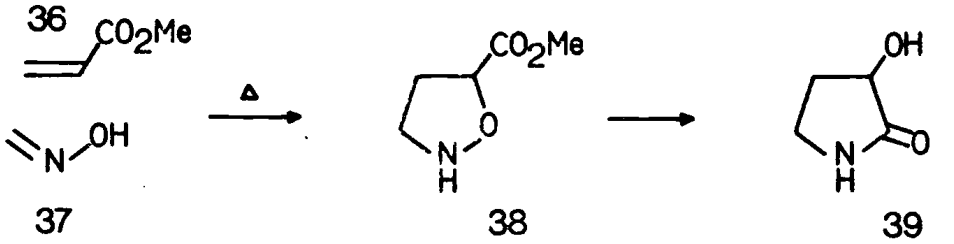
was achieved by condensation of aminoester 30 with diethyl oxalate to give 3-oxolactam 32. Subsequent diacetylation, hydrolysis and decarboxylation, and finally reacetylation gave enol acetate 28.



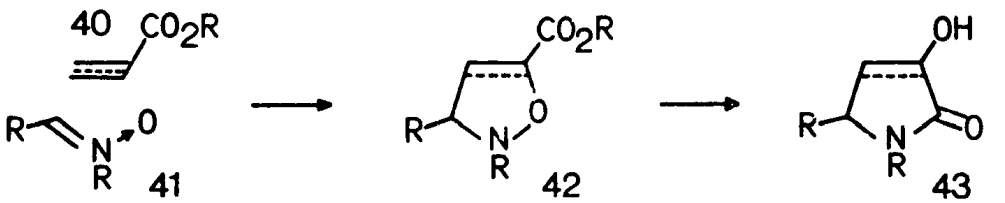
Dagne and Castagnoli[28], in their synthesis and structural assignment of hydroxyconitine, prepared the enol acetate 35 by hydrogenolysis of isoxazolidine 33 followed by oxidation and acetylation of the intermediate



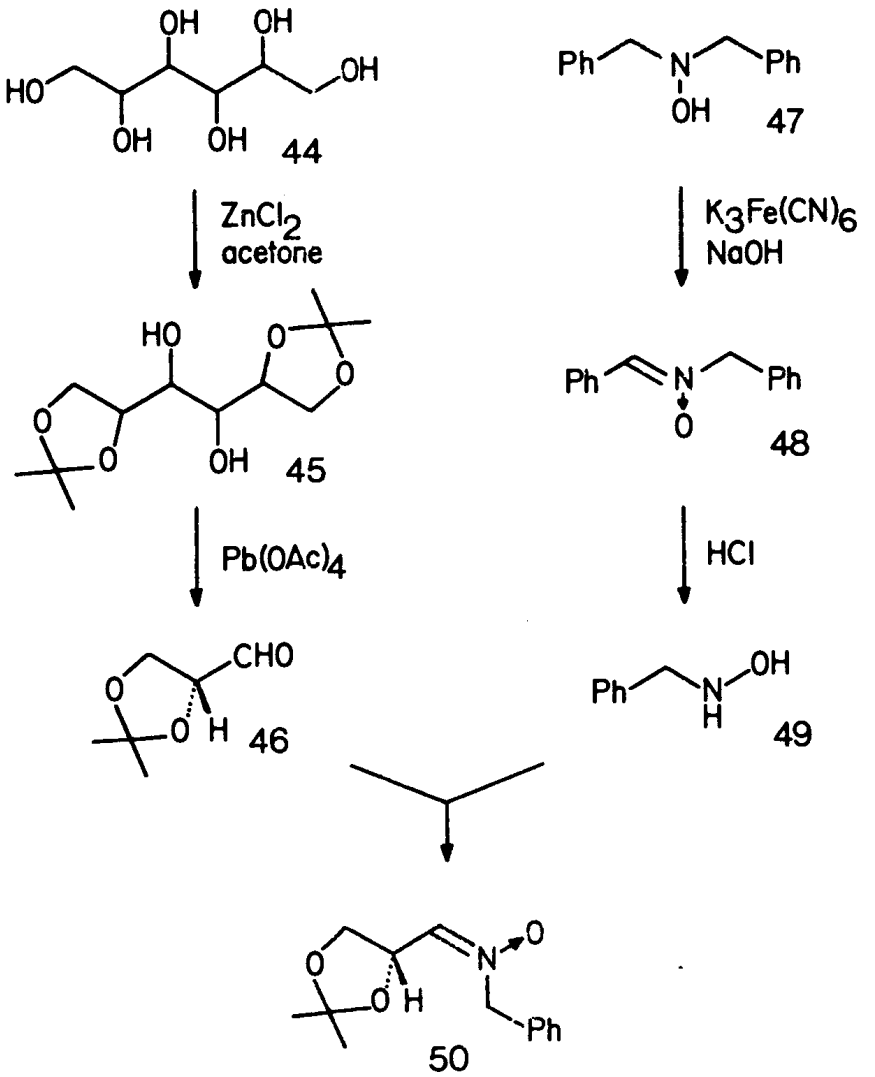
hydroxylactam 34. In a unique 1,3-dipolar cycloaddition, Morita[29] prepared the N-unsubstituted isoxazolidine 38 from methyl acrylate and formaldoxime. Palladium on carbon cleavage and lactamization gave the hydroxylactam 39.



Our approach to the lactam nucleus of leptosphaerin was based upon Huisgen's reported hydrogenolysis of 5-carboalkoxyisoxazolidines which undergo facile in situ ring closure to give 3-hydroxy lactams.[30] It was



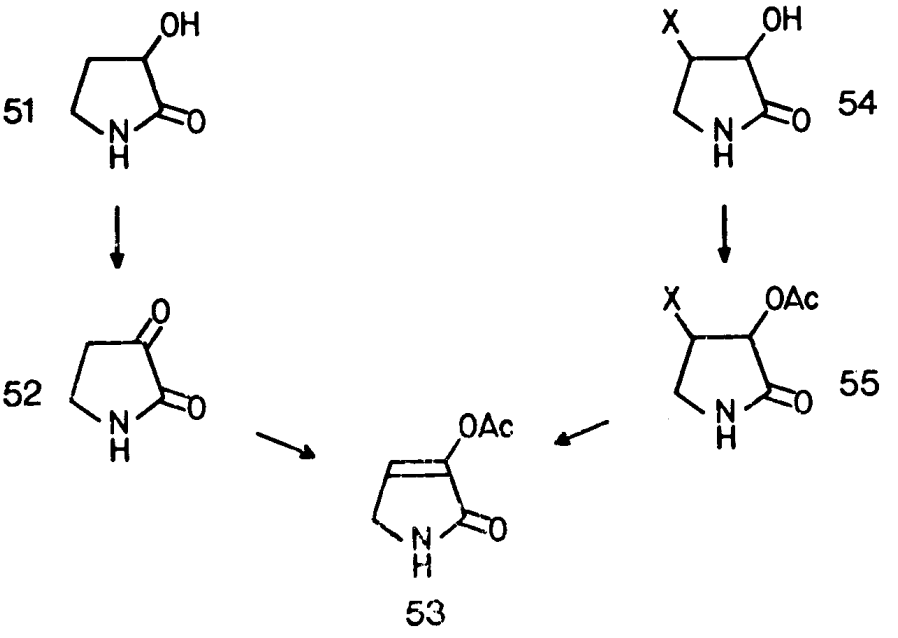
reasoned that the requisite isoxazolidine could be generated by means of a 1,3-dipolar cycloaddition of a suitably functionalized nitrone to an acrylic acid derivative. The nitrone component, ideally, should incorporate the diol side chain with the appropriate absolute configuration required at C-6 in leptosphaerin as well as a readily removable protecting group. To satisfy these criteria, the nitrone 50, which was used through the course of this investigation, was prepared as illustrated on the following page.



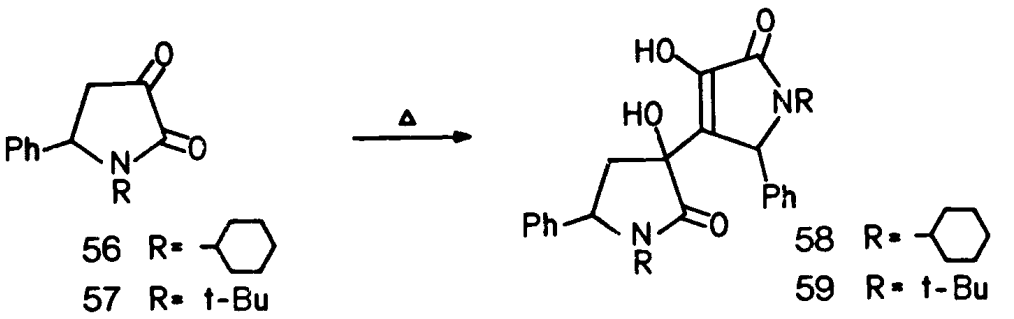
Bisacetonization of D-mannitol 44 followed by cleavage of the derived glycol 45 with lead tetraacetate gave 2,3-O-isopropylidene-D-glyceraldehyde 46.[31] N-Benzylhydroxylamine 49 has been prepared by a number of methods,[32,33,34] of which we found the following to be most expeditious.[35,36] N,N-Dibenzylhydroxylamine 47, prepared from hydroxylamine and benzyl chloride,[36] was oxidized with potassium ferricyanide and sodium hydroxide.[35] The resulting nitrone 48 was hydrolyzed with concentrated hydrochloric acid to give N-benzylhydroxylamine 49. The condensation of 49 and the aldehyde 46 proceeded spontaneously and in excellent yield at

room temperature in chloroform to afford nitrone 50. This substance, as a result of its derivation from a D-sugar, possesses the configuration required for an enantioselective synthesis of 6R-1.

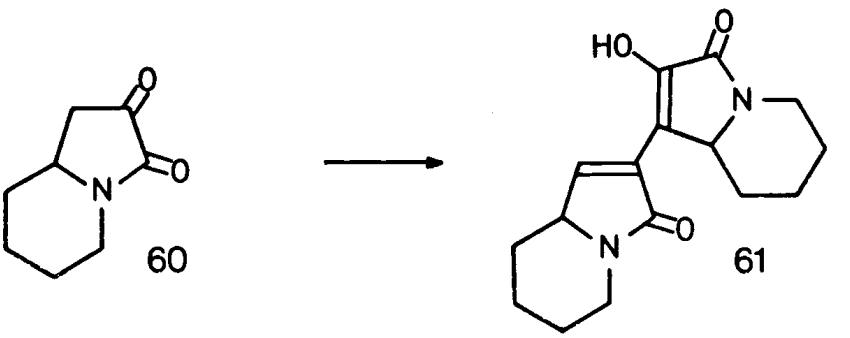
Attention was now turned to the construction of isoxazolidine 42 through a 1,3-dipolar cycloaddition of nitrone 50 to an appropriate dipolarophile. Selection of the dipolarphile was dictated by the requirement for functionality in the isoxazolidine, and consequently the derived γ-lactam 43, from which the enol acetate of leptosphaerin would be readily accessible. Two logical targets for this purpose are the 3-hydroxy-γ-lactam 51 and a 4-substituted derivative 54. In the former case, oxidation should lead to 52 which upon acetylation would afford 53. The alternative route would entail acetylation of 54, followed by elimination, to yield enol acetate 53. Of the intermediates in these two pathways, the 3-oxolactam 52 intially caused most concern predicated upon literature descriptions of instability for systems of this type. Southwick[25] noted the



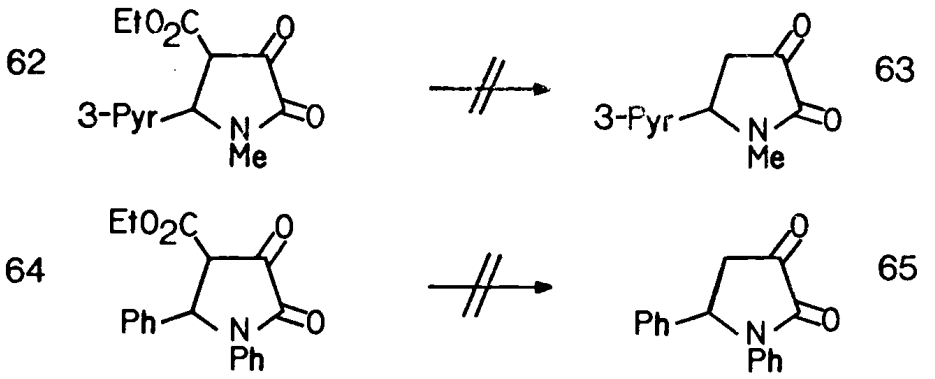
ease with which some derivatives of the 1-substituted-2,3-dioxopyrrolidines undergo self-condensation. The 1-cyclohexyl analogue 56 reacted to give the aldolization product 58 in ethanol containing pyridine. The N-substituted derivatives 56 and 57 underwent a similar transformation when heated in refluxing toluene. Rapoport[37] has also characterized a dehydrated dimer 61 formed when α-oxolactam 60 was subjected to silica gel



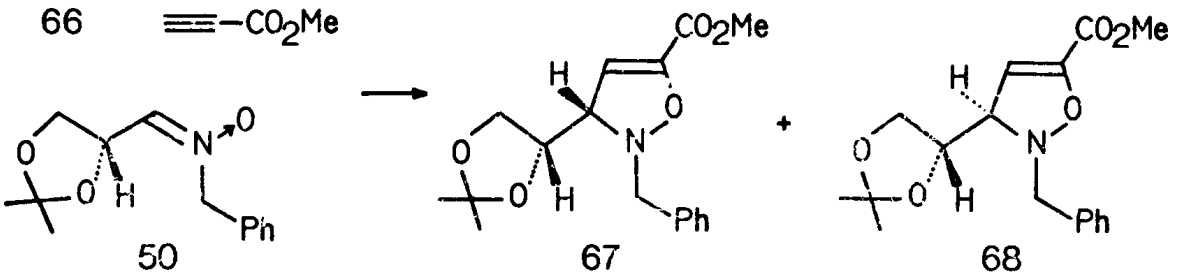
chromatography or upon standing at 0°C for several days. Dagne and Castagnoli[28] and Waterman and Koch[23] have ascribed their inability to



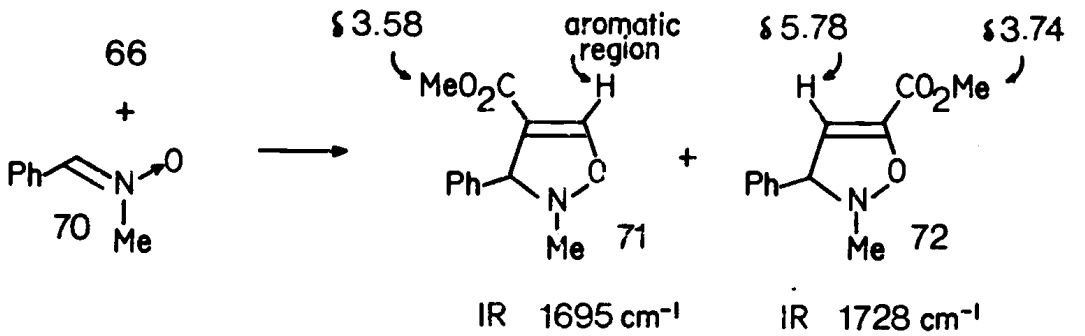
obtain diones 63 and 65 respectively, to self-condensation.



Our initial studies utilized methyl propiolate 66 as dipolarophile for cycloaddition to the previously described nitrone 50. It was hoped that the resulting 5-carboalkoxyisoxazolines 67 and 68 could by hydrogenolyzed directly to the 3-oxolactam 69, whose potential instability might be minimized by a judicious choice of reaction conditions. The desired cycloaddition was effected in refluxing toluene to give two products which were separated chromatographically. The more polar product displayed

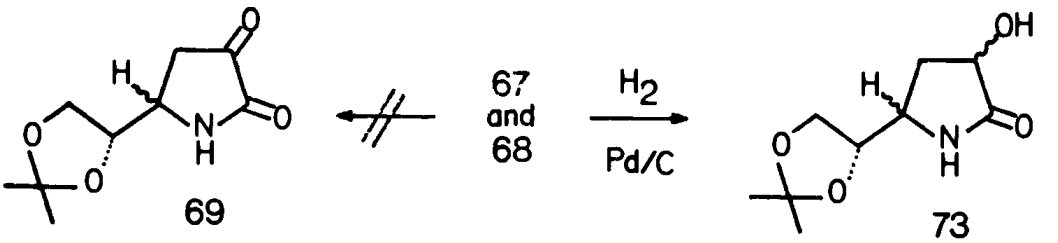


a one-proton (J=3Hz) at δ 5.70, whereas the more mobile component exhibited a one-proton doublet (J=2Hz) at δ 5.92. Both compounds showed a three proton singlet at δ 3.80 and possessed infrared carbonyl bands at 1735 cm^{-1}. The structures of the two products were tentatively assigned as the C-3 epimeric isoxazolidines 67 and 68. Support for this assignment was found in Huisgen's[38] reported cycloaddition of N-methyl-C-phenyl nitrone 70 to methyl propiolate 66 which produced a mixture of both possible regioisomers, 71 and 72 in a ratio of 52:48. The two adducts were

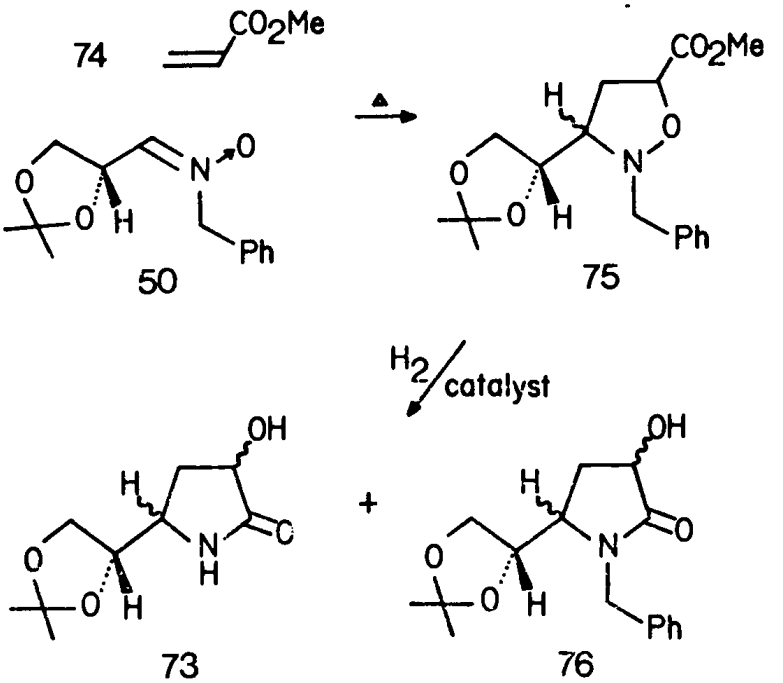


differentiated by the chemical shifts of the vinylic and methyl proton resonances in the NMR spectrum as well as by the infrared carbonyl bands of the methyl esters. Spectral comparisons of 67 and 68 to 71 and 72 firmly established the identity of the former cycloadducts as epimeric 5-carboalkoxyisoxazolines.

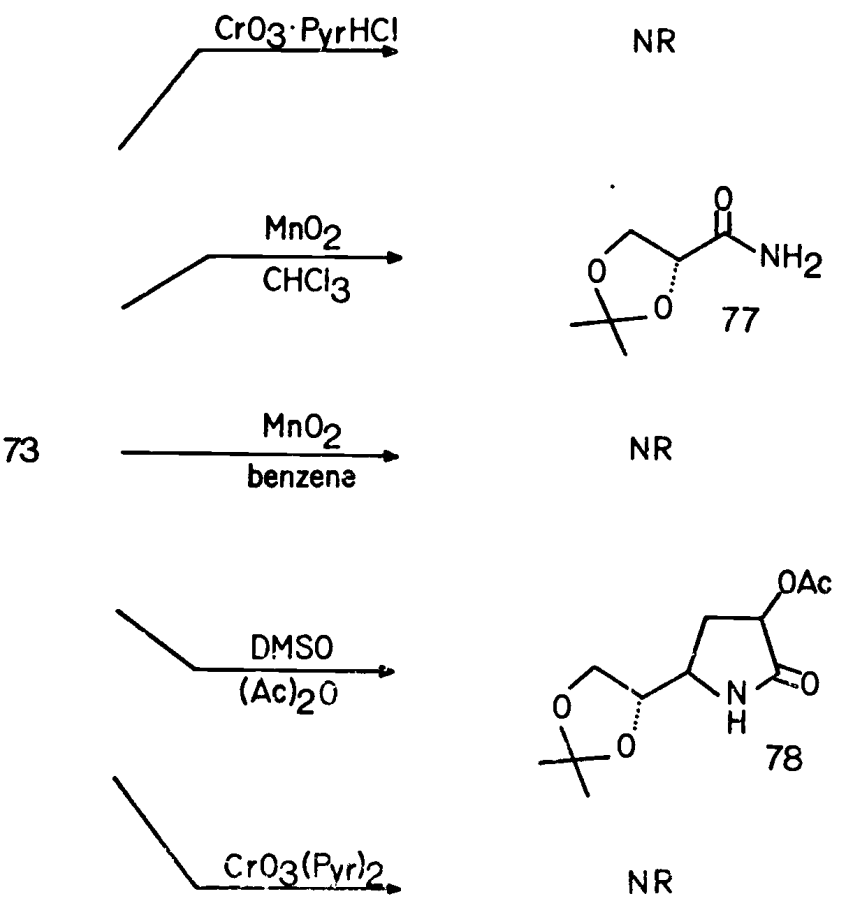
Catalytic hydrogenolysis of the mixture of epimers 67 and 68 with palladium on carbon failed to yield the oxolactam 69 but did give the reduced hydroxylactam 73. The same product as an epimeric mixture was



obtained more efficiently when isoxazolidine 75, prepared by the cycloaddition of nitrone 50 and methyl acrylate 74 in refluxing toluene, was hydrogenolyzed with palladium on carbon, platinum oxide or rhodium on carbon (incomplete hydrogenolysis of 75 resulted in the isolation of appreciable quantities of N-benzylhydroxylactam 76 in addition to 73).

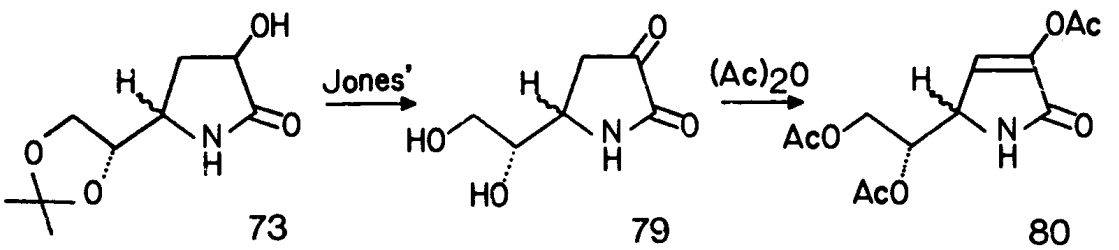


With ample quantities of hydroxylactam 73 available, a series of oxidations were investigated with the intention of generating 3-oxolactam 69. Previous workers had noted the apparent resistance of 3-hydroxypyrrolidines to a variety of oxidants[39,40] including cupric acetate, bismuth oxide, N-bromosuccinimide and Collin's reagent.[23] Our initial oxidation attempts utilizing pyridinium chlorochromate,[41] manganese dioxide in benzene, and chromium trioxide-dipyridine complex[42] showed a similar lack of reactivity, with starting alcohol being recovered in all cases. Manganese dioxide in chloroform gave as sole product 2,3-O-isopropylidene-D-glyceramide 77. Oxidation with dimethylsulfoxide and acetic anhydride yielded the monoacetate 78. However, when hydroxylactam 73 was treated with

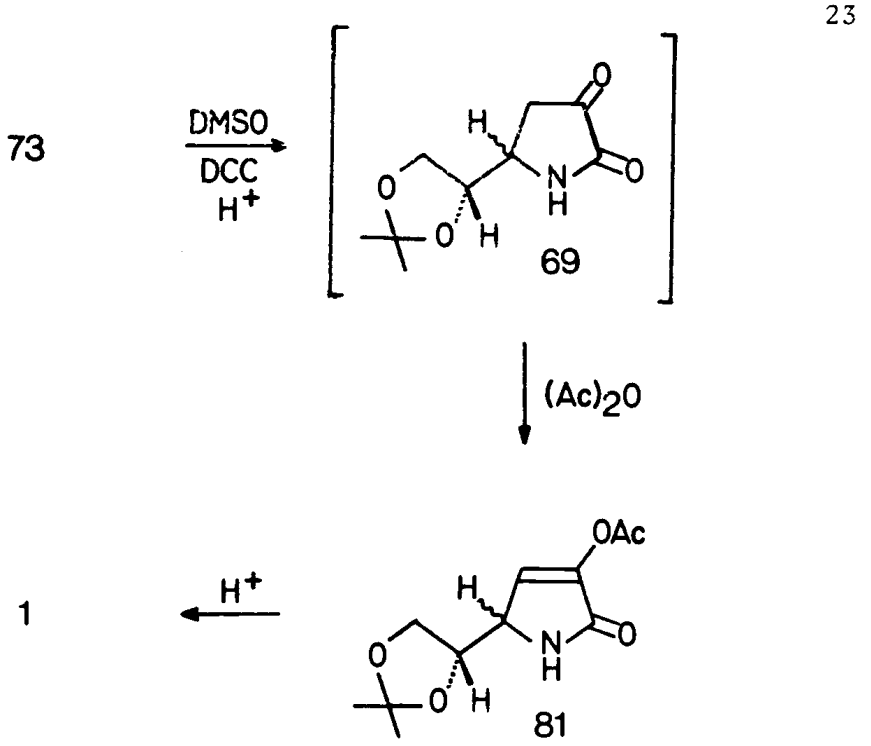


Jones' reagent,[43] it was rapidly converted into a material which gave a positive ferric chloride test. This intermediate was acetylated, without isolation, to give a product whose spectral characteristics were consistent with a pair of diastereomeric triacetates 80.

Unfortunately, spectral comparisons of 80 with acetylated leptosphaerin from the natural source failed to show a correspondence.



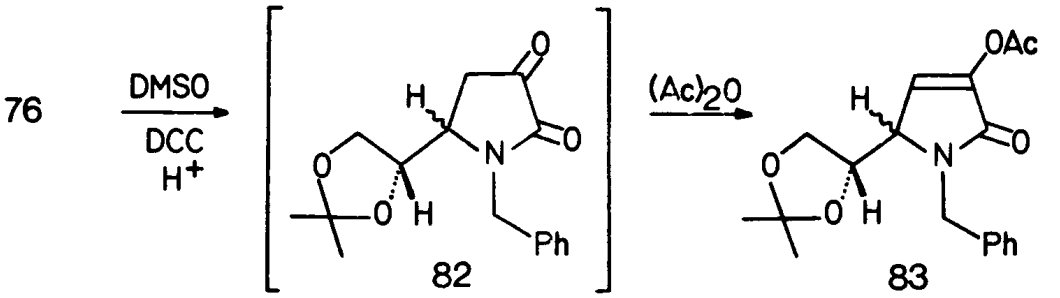
The inadvertent loss of the acetonide group, which presumably occurred under the acidic conditions prevailing during workup of the Jones' oxidation, could be prevented by subjecting alcohol 73 to a Moffatt oxidation.[44] As in the previous reaction, a ferric chloride-positive material was observed, which was acetylated without isolation to give a crystalline acetonide 81. The infrared band at 1770 cm^{-1}, the three proton singlet at δ 2.29 and the prominent loss of ketene (M^+-42) in the mass spectrum of 81 provided support for the presence of enol acetate. The absorptions at 1720 and 1625 cm^{-1} were within accepted limits for an α,β-unsaturated-γ-lactam. In addition, the vinyl hydrogen at C-4 and the methine proton at C-5 both exhibited NH-coupling as demonstrated by decoupling and D_2O exchange experiments.



With the pyrrolidone 81 secured, hydrolysis of the acetonide was attempted in the expectation that the product would be either identical or epimeric (at C-5) with natural leptosphaerin. Treatment of 81 with oxalic acid in aqueous ethanol[45] afforded a crude diol whose spectral characteristics were consistent with enol acetate 1. However, when direct spectral comparison was made with the natural material no correspondence was observed. Particularly striking was the presence of NH coupling to the protons at C-4 and C-5 in the synthetic material, a feature which was not observed in the natural product. The infrared band at 1770 cm^{-1} and the three proton singlet at δ 2.30 in the NMR spectrum of synthetic 1 also reflected a significant structural difference from the natural material in which the corresponding absorptions appeared at 1735 cm^{-1} and δ 2.14. Perhaps the most disturbing property of the synthetic compound was its marked instability to silica gel chromatography, especially in view of

the demonstrated stability of leptosphaerin.

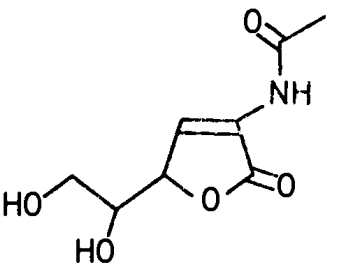
Cognizant of the documented lability of certain 1-unsubstituted α-oxolactams, the previously described sequence was applied to the N-benzyl-hydroxylactam 76 obtained by incomplete hydrogenolysis of isoxazolidine



75. The derived enol acetate 83 exhibited spectral characteristics which were in excellent agreement with 81 and again dissimilar to those of leptosphaerin.

It was now clear from the lack of correspondence in physical and chemical properties between synthetic 1 and natural leptosphaerin that the structure of the latter was misassigned, in spite of an x-ray analysis apparently confirming 1. At this juncture, it became essential to critically reevaluate the chemical evidence leading to the incorrect structural designation.

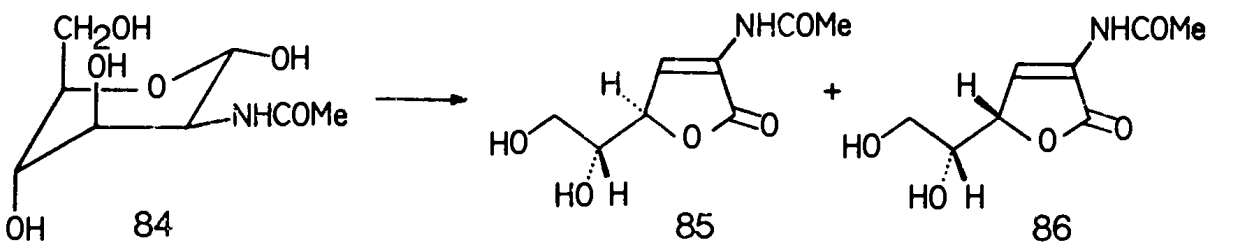
One possibility which had been suggested earlier was lactone 2. The low field shift of the protons at C-4 and C-5 as well as the lack of NH-coupling in leptosphaerin relative to lactam 1 was readily accomodated by the alternative formulation 2. Similarly, the absence of a prominent loss of ketene, characteristic of enol acetates, in the mass spectrum of



2

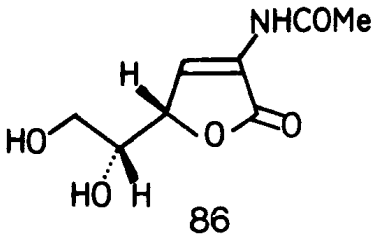
the natural product is consistent with the enol acetamide group. The infrared bands of leptosphaerin at 1745, 1670, and 1640 cm^{-1} could be reasonably assigned to the lactone carbonyl, amide carbonyl and the conjugated olefin, respectively.

Careful scrutiny of the carbohydrate literature, in fact, revealed that 2 had been prepared previously. In a study of the bromine oxidation products of N-acetylmannosamine 84, Previc and Fletscher[46] reported the formation, after base induced dehydration, of the epimeric lactones 85 and 86. Comparison of the spectral properties of leptosphaerin with



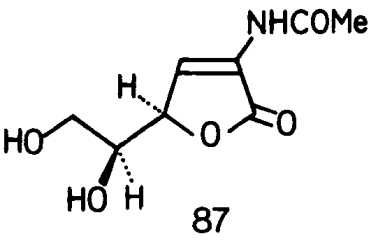
those reported for the erythro epimer 86 showed an excellent correlation. This relative stereochemical assignment was identical to that established by the original x-ray analysis.

The absolute configuration of leptosphaerin was thus reduced to two possible enantiomeric lactones 86 and 87. In view of the natural product's presumed biogenetic origin in D-glucose, through the intermediacy of N-



86

D 5S,6R

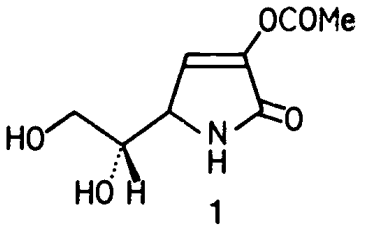


87

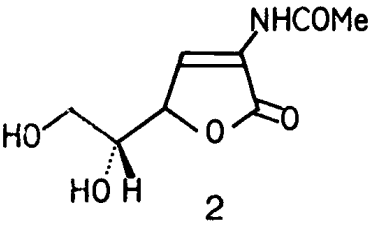
L 5R,6S

acetylglucosamine of N-acetylmannosamine 84, the D-lactone 86 appears to be the more probable absolute stereochemical formulation.

Finally, a reevaluation by Professor Clardy of the x-ray data secured from leptosphaerin revealed that 1 as well as 2 were compatible with the diffraction data. Refinement values for 1 and 2 of 0.078 and 0.072, respectively are well within the error limits normally associated with an unequivocal structure assignment. Apparently, leptosphaerin possesses an unusual coincidence of structural dimensions whereby the lactone oxygen and amide nitrogen cannot be distinguished even at a reasonably advanced



1



2

level of refinement of the x-ray data. Further refinement, which might have permitted a distinction between the two structures by location of the hydrogens, was prohibited by experimental difficulties associated with the data collection process.

In conclusion, it now appears certain that leptosphaerin possesses structure 2 rather than the lactam formulation 1. A definitive proof must await an unambiguous synthesis of lactone 86.

D. EXPERIMENTAL

Isolate R-697, Leptosphaeria oraemaris (Linder), was supplied and authenticated by Dr. Paul W. Kirk, Old Dominion University, Norfolk, Virginia. The stock culture was maintained on slants of medium 5M (Table I).[10] Inoculation flasks were prepared by transferring segments from the stock cultures to 125 ml Erlenmeyer flasks containing 30 ml of medium 6M (Table I).[10] All media were autoclaved for 20 minutes at 120°C prior to inoculations. The flasks were subsequently placed on a gyrotory shaker and incubated in the dark for 7 days at 25°C. The contents of each inoculum

Table I. Culture Media

Medium 5M[10]

Glucose*	10 g
Yeast Extract*	1 g
Agar*	20 g
Rila Marine Mix**	40 g
Distilled Water	to 1 liter

Medium 6M[10]

Glucose*	10 g
Ammonium Succinate	1 g
Yeast Extract*	1 g
Rila Marine Mix**	40 g
Distilled Water	to 1 liter

*Difco Laboratories, Detroit, Michigan.
**Rila Products, P.O. Box 114, Teaneck, N.J.

flask was homogenized in a sterile semi-micro Waring blender and quantitatively transferred to an equal number of 500 ml Erlenmeyer flasks con-

taining 150 ml of medium 6M. Following a 7 day incubation period at 25°C on a rotary shaker, the cultures were harvested by suction filtration to separate the medium from the mycelium. The mycelium was washed with distilled water and dried in a forced air dryer for 48 hours at 50°C. The culture filtrate was concentrated to dryness on a rotoevaporator and stored under refrigeration.

The concentrated media was extracted six times with 150 ml volumes of hexane. The insoluble residue was dissolved in 1 liter of water and was exhaustively extracted with ethyl acetate.

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Boiling points are uncorrected. Ultraviolet (UV) and infrared (IR) spectra were recorded on Beckman DB-GT and Perkin-Elmer 727 spectrophotometers, respectively. Proton nuclear magnetic resonance (NMR) spectra were obtained on a Varian Associates EM-360, EM-360A, or HA-100 spectrometer. Chemical shifts are given in parts per million (δ) downfield from the internal standard tetramethylsilane. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. Coupling constants (J) are measured in Hertz. Electron impact mass spectra were recorded on a Varian MAT CH-7 (low resolution) and CEC-103B (high resolution) spectrometers using an ionization voltage of 70 ev.

Exact mass determinations were made on a CEC-103B spectrometer. Combustion analysis was perfomed by Micro-Tech Laboratories, Skokie, Illinois. Preparative and analytical thin layer chromatography plates of silica gel GF-254 were supplied by Analtech. Column chromatography was conducted with ICN silica gel Woelm (Activity II) or Merck neutral alumina (Activity)

II).

Anhydrous tetrahydrofuran (THF) was prepared by distillation from lithium aluminum hydride and was stored over sodium wire. Dimethyl sulfoxide (DMSO) was dried by distillation from calcium hydride at reduced pressure and was stored over Molecular Sieves. Other solvents were purified using standard methods.

Leptosphaerin

The ethyl acetate extract of the culture filtrate was subjected to column chromatography on silica gel, using ethyl acetate followed by methanol, as eluting solvent. Evaporation of the initial fractions yielded 25 mg of crude but crystalline material. Preparative thin layer chromatography (ethyl acetate) gave a UV active band (R_f 0.35, PF-254) which was eluted with acetone. Recrystallization from ethyl acetate yielded fine needles, m.p. 189.5-190.5°C; UV (MeOH) 246 nm; IR (KBr) 3435, 3355, 3290, 1745, 1670, 1640, 1540, 1340, 1260, 1140, 1075, 1035, 870, and 780 cm^{-1}; NMR (acetone-d_6) δ 8.88 (1H, broad s, removed by D_2O exchange), 7.51 (1H, d, J=1.75Hz), 5.12 (1H, dd, J=1.75, 4.75Hz), 4.25 (1H, d, J=5.3Hz, removed by D_2O exchange), 3.60-3.97 (4H, m, 1H removed by D_2O exchange), 2.14 (3H, s); (DMSO-d_6) δ 10.12 (1H, broad s, removed by D_2O exchange), 7.45 (1H, d, J=1.7Hz), 5.14 (2H, m, 1H removed by D_2O exchange), 4.11 (1H, t, removed by D_2O exchange), 3.72 (1H, m), 3.48 (2H, m), 2.08 (3H, s); mass spectrum m/e 201.057 (M^+, 2% calcd for $C_8H_{11}NO_5$: 201.064), 141.037 (M^+-60, 100% calcd for $C_6H_7NO_3$: 141.043), 123 (72%), 99 (52%), 98 (31%), 70 (40%) and 43 (78%).

Acetylation of Leptosphaerin

A solution of 5 mg of leptosphaerin in 0.2 ml of acetic anhydride containing a catalytic amount of pyridine was maintained at reflux for 1 h and then was allowed to stand at room temperature for 24 h. The reaction mixture was diluted with water and extracted with chloroform. The combined organic extracts were washed with 1N hydrochloric acid, 1N sodium bicarbonate and water, dried over magnesium sulfate and the solvent removed in vacuo. The crude acetylated product was subjected to preparative thin layer chromatography (silica gel; ethyl ether) and the UV active band (R_f 0.69) was eluted to give a crystalline solid: m.p. 145-146°C; IR (KBr) 3340, 3145, 1755, 1735, 1695, 1675, 1620, 1535, 1385, 1320, 1255, 1110, 1025, 965, 880, 835, 780, and 680 cm^{-1}; NMR (DMSO-d_6) δ 10.12 (1H, bs), 7.45 (1H, d, J=2Hz), 5.34 (2H, m), 4.33 (1H, dd, J=3.7,12.4Hz), 4.08 (1H, dd, J=4.3,12.4Hz), 2.17 (3H, s), 2.08 (3H, s), 2.05 (3H, s).

N,N-Dibenzylhydroxylamine (47)

According to the method of Jones and Sneed,[36] a solution of 28 g (0.4 mol) of hydroxylamine hydrochloride, 100 g (0.8 mol) of benzyl chloride and 120 g (1.0 mol) of sodium carbonate monohydrate in 400 ml of 70% ethanol was refluxed for 2 h. The supernatant was removed by decantation and was placed under refrigeration until crystallization was complete. The remaining solids were diluted with water to precipitate the residual N,N-dibenzylhydroxylamine. The solutions were filtered and the crude products removed of solvent in a drying pistol to give 59.4 g (70%) of 47 as white needles: m.p. 120-121°C [lit.[36] mp 125°C]; IR (KBr) 3200 cm^{-1};

NMR ($CDCl_3$) δ 7.28 (10H, s), 6.85 (1H, broad s, removed by D_2O exchange), 3.66 (4H, s); mass spectrum m/e 213.

N-Benzylidene aniline-N-oxide (48)

According to the method of Behrend and Leuchs,[35] saturated solutions of 59 g (179 mmol) of potassium ferricyanide and 11.1 g (197 mmol) of potassium hydroxide were added to 14.8 g (69.5 mmol) of 47 in 30 ml of ethyl ether and shaken in a separatory funnel for 20 min. Continuous liquid-liquid extraction of the reaction mixture with ethyl ether followed by removal of solvent in vacuo gave 12.9 g (87%) of crude 48: mp 80-81°C [lit.[36] mp 81-82°C]; IR (KBr) 1580, 940 cm^{-1}; NMR ($CDCl_3$) δ 8.21 (2H, m), 7.43 (10H, m), 5.07 (2H, s); mass spectrum m/e 211.102 (M^+, calcd for $C_{14}H_{13}NO$: 211.100).

N-Benzylhydroxylamine (49)

A solution of 12.9 g (60 mmol) of nitrone 48 in 30 ml of concentrated hydrochloric acid was steam distilled until all of the benzaldehyde had been removed. The solution was cooled to 0°C and neutralized with saturated sodium carbonate monohydrate and extracted with pentane using a continuous liquid-liquid extractor. Removal of the solvent gave 5.91 g (79%) of 49: An analytical sample was prepared by sublimation to give a white solid: mp 56-57°C; IR (KBr) 3270, and 3140 cm^{-1}; NMR ($CDCl_3$) δ 7.30 (5H, s), 6.08 (2H, broad s), 3.95 (2H, s); mass spectrum m/e 123.

1,2,5,6-Di-O-isopropylidene-D-mannitol (44)

The procedure of Baer and Fischer[31] was followed. To a solution of 60 g (0.44 mol) of fused zinc chloride in 300 ml of acetone was added 20 g (0.11 mol) of D-mannitol. The mixture was stirred for 14 h at room temperature and was poured into a solution of 70 g of potassium carbonate in 20% aqueous ethyl ether. The resulting mixture was shaken for 20 min and the acetone-ether solution decanted. The inorganic salts were washed twice with 100 ml of acetone:ethyl ether (1:1) and the combined extracts were reduced to dryness in vacuo. The crude product was extracted with hot petroleum ether and the resulting solutions were placed under refrigeration until crystallization was complete. Filtration of the fine white needles gave 13.6 g (56%) of 44: mp 117-118°C [lit.[31] 122°C]; mass spectrum m/e 247 (M^+-15).

2,3-O-Isopropylidene-D-glyceraldehyde (46)

To a solution of 4.88 g (22 mmol) of diacetonide 44 in 100 ml of dry benzene was added 9.0 (20 mmol) of lead tetraacetate. The mixture was stirred for 3 h at room temperature, filtered through sintered glass and the filtrate removed of solvent in vacuo below 25°C. Three 15 ml quantities of carbon tetrachloride were added and the solutions evaporated in vacuo to remove residual acetic acid. Distillation under reduced pressure yielded 2.98 g (52%) of 46 as a pale yellow oil: bp 48-51°C (15 torr) [lit.[31] bp 35-42°C (8-11 torr)]; IR (film) 1730, 1380, and 1370 cm^{-1}; NMR ($CDCl_3$) δ 9.65 (1H, d, J=1.7Hz), 4.50-4.03 (3H, m), 1.44 (3H, s), 1.39 (3H, s).

N-Benzyl-N-(2,3-O-isopropylideneglyceraldimine-N-oxide) (50)

A solution of 497 mg (4.0 mmol) of sublimed N-benzylhydroxylamine 49 and 525 mg (4.0 mmol) of 2,3-O-isopropylidene-D-glyceraldehyde 46 in 15 ml of chloroform was stirred at room temperature for 15 min. Removal of the solvent under reduced pressure gave 950 mg (94%) of crude nitrone 50: mp 79-81°C; IR (KBr) 1600 cm^{-1}; NMR ($CDCl_3$) δ 7.38 (5H, s), 6.87 (1H, d, J=5Hz), 3.70 (1H, dd, J=7.5,9Hz), 1.39 (3H, s), 1.36 (3H, s); Anal. calcd for $C_{13}H_{17}NO_3$: C, 66.36; H, 7.38; N, 5.95. Found: C, 66.04, H, 7.15; N, 5.91.

2-Benzyl-3(1,2-O-isopropylidene dihydroxyethyl)-5-carbomethoxy-4-isoxazoline (67, 68)

A solution of 235 mg (1.0 mmol) of nitrone 50 and 90 mg (1.0 mmol) of methyl propiolate in 5 ml of toluene was maintained at reflux temperature for 1 h. Removal of the solvent gave 73% of crude 10 as a mixture of two epimers. Preparative layer chromatography [benzene:ethyl acetate (9:1)] yielded 67 and 68 at Rf 0.52 and 0.48 respectively: Compound 67: IR (film) 1735 and 1640 cm^{-1}; NMR ($CDCl_3$) δ 7.32 (5H, s), 5.92 (1H, d, J=2Hz), 4.28 (1H, d, J=12Hz), 3.97 (5H, m), 3.80 (3H, s), 1.32 (3H, s), 1.26 (3H, s); mass spectrum m/e 319.143 (calcd for $C_{17}H_{21}NO_5$: 319.142). Compound 68: IR (film) 1735 and 1640 cm^{-1}; NMR ($CDCl_3$) δ 7.33 (5H, s), 5.70 (1H, d, J=2.5Hz), 4.35 (1H, d, J=13Hz), 4.21 (1H, m), 3.98 (2H, m), 3.96 (1H, d, J=13Hz), 3.81 (3H, s), 3.72 (1H, m), 1.28 (3H, s), and 1.26 (3H, s); mass spectrum m/e 319.140 (calcd for $C_{17}H_{21}NO_5$: 319.142).

2-Benzyl-3(1,2-Di-O-isopropylidene ethanediol)-5-carbomethoxyisoxazolidine (75)

A mixture of 47 mg (0.2 mmol) of nitrone 50 and 4 ml of methyl acrylate 74 was maintained at reflux for 1 h. The solution was rotoevaporated to a light syrup which was subjected to preparative layer chromatography utilizing benzene:ethyl ether (1:2) as eluent. The band at Rf 0.75 was eluted to give 62 mg (97%) of 75: IR (film) 1750, 1370, and 1360 cm^{-1}; NMR ($CDCl_3$) δ 7.33 (5H, m), 4.87-4.47 (1H, m), 4.34-3.45 (6H, m), 3.77 (3H, s), 3.39-2.99 (1H, m), 2.85-2.26 (2H, m), 1.34 (3H, s), 1.32 (3H, s); mass spectrum m/e 321.156 (M^+, calcd for $C_{17}H_{21}NO_5$: 321.158).

3-Hydroxy-5(1,2-O-isopropylidene ethanediol)pyrrolidin-2-one (73)

A solution of 1.5 g (4.8 mmol) of isoxazolidine 75 in 15 ml of 100% ethanol was hydrogenolyzed utilizing 300 mg of Pd/C (10%), under 1 atm of H_2. After 18 h the solution was filtered through Celite and removed of solvent under reduced pressure to give a pale green syrup. Recrystallization of the crude residue from acetone gave 477 mg (51%) of hydroxy lactam 73: IR (KBr) 3420, 3225, and 1710 cm^{-1}; NMR ($CDCl_3$) δ 6.78 (broad s, 1H), 4.46-3.65 (mc, 6H), 2.74-2.00 (m, 1H), 1.87-1.53 (m, 1H), 1.43 (s, 3H), 1.33 (s, 3H); mass spectrum m/e 201.102 (M^+, calcd for $C_9H_{15}NO_4$: 201.100).

3-Acetoxy-5-(1,2-O-isopropylidene dihydroxyethyl)-3-pyrrolin-2-one (81)

To a solution of 40 mg (0.2 mmol) of hydroxylactam 73 in 3 ml of benzene:dimethylsulfoxide (9:1) was added 120 mg (0.6 mmol) dicyclohexylcarbodiimide and 1 drop of polyphosphoric acid. The mixture was stirred at room temperature for 18 h, diluted with ethyl acetate and treated with 54 mg (0.6 mmol) of oxalic acid. The precipitate was removed by filtration and the filtrate was evaporated to dryness. The crude product [Rf 0.80; ethyl acetate:methanol (9:1)] was dissolved in 3 ml of acetic anhydride containing a catalytic amount of pyridine and was stirred at room temperature for 12 h. The reaction mixture was poured into ice cold aqueous sodium bicarbonate and was extracted with ethyl ether. The combined ethereal extracts were dried and removed of solvent to give 28 mg (58%) of crude enol acetate 81 as a 3:1 mixture of diastereomers. Column chromatography [ethyl acetate:methanol (9:1)] on silica gel yielded the major isomer as a colorless solid: mp 152-156°C; IR 1770, 1720, and 1625 cm^{-1}; NMR ($CDCl_3$) δ 7.64 (1H, broad s, removed by D_2O exchange), 6.89 and 6.76 (1H, t, J=2Hz), 4.35 (1H, ddd, J=2,2,6Hz), 4.16 (1H, q, J=6Hz), 4.00 (1H, dd, J=6,8Hz), 3.71 (1H, dd, J=5,8Hz), 2.29 (3H, s), 1.46 (3H, s), and 1.34 (3H, s); mass spectrum m/e 241.097 (calcd for $C_{11}H_{15}NO_5$: 241.095).

3-Acetoxy-5(1,2-dihydroxyethyl)-3-pyrrolin-2-one (1)

To a solution of 10 mg (0.05 mmol) of acetonide 81 in 1 ml of 80% aqueous ethanol was added 10 mg of oxalic acid. The reaction mixture was maintained at 60°C for 5 h. After removal of the solvent the crude residue was subjected to preparative layer chromatography. Removal of the band at Rf 0.25 [ethyl acetate:methanol (9:1)] gave the unstable diol 1: IR 1770, 1720, and 1625 cm^{-1}; NMR (d_6-acetone) δ 7.56 (1H, broad s, removed by D_2O exchange), 6.96 and 6.85 (1H, t, J=2Hz), 4.30 (2H, m, 1H removed by D_2O exchange), 3.75-3.30 (4H, m), 2.22 (3H, s); mass spectrum m/e 201.

1-Benzyl-3-acetoxy-5(1,2-O-isopropylidene dihydroxyethyl)-3-pyrrolin-2-one (83)

N-benzyl hydroxylactam 76 (658 mg, 2.26 mmol) was dissolved in 30 ml of anhydrous benzene:dimethylsulfoxide (9:1) and 1.4 g (6.78 mmol) of dicyclohexylcarbodiimide and 99 mg (1.13 mmol) of polyphosphoric acid were added. The mixture was stirred at room temperature for 3 h, diluted with ethyl acetate, filtered and the solvent removed in vacuo.

The crude dione 82 was acetylated with acetic anhydride containing a catalytic amount of pyridine for 1 h at room temperature. Following removal of solvent the product was chromatographed preparatively to give enol acetate 83 as a 3:1 mixture of two diastereomers: IR 1780, 1710, 1660, and 1640 cm^{-1}; NMR ($CDCl_3$) δ 7.27 (5H, broad s), 6.76 and 6.64 (1H, d, J=2Hz), 5.24 and 5.05 (1H, d, J=15Hz), 4.34 (1H, d, J=15Hz), 4.36 (1H, m), 4.13 (1H, broad t, J=4Hz), 3.97 (1H, dd, J=7,9Hz), 3.66 (1H, dd, J=6, 9Hz), 2.29 (3H, s), 1.43 (3H, s), and 1.30 (3H, s); mass spectrum m/e 331.

E. BIBLIOGRAPHY

(1) R.J. Seltzer, Chem. Eng. News, Dec. 16, 1974, p. 20.

(2) B.W. Halstead, "Poisonous and Venomous Marine Animals," Vol. I and II, U.S. Government Printing Office (1965).

(3) E. Sondheimer and J.B. Simeone, Eds., "Chemical Ecology", Academic Press, New York, N.Y. (1970).

(4) D.J. Faulkner, Tetrahedron, 33, 1421 (1977).

(5) P.J. Scheuer, "Chemistry of Marine Natural Products", Academic Press, New York (1973).

(6) T.W. Johnson, Jr. and F.K. Sparrow, Jr., "Fungi in Oceans and Estuaries", J. Crame, Weinheim (1961).

(7) N.W. Miller, "The Pfizer Handbook of Microbial Metabolites", McGraw-Hill, New York, N.Y. (1961).

(8) H.S. Vishniac, Acta Biochim. Biophys., 26, 430 (1957).

(9) R.D. Schafer and C.E. Lane, Bull. Mar. Sci., 7, 289 (1957).

(10) R.W. Kirk, Jr. and P. Catalfomo, Phytochemistry, 9, 595 (1970).

(11) S. Teshima and A. Kanazawa, Bull. Jap. Soc. Sci. Fish., 37, 68 (1971).

(12) P. Catalfomo, J.H. Block, G.H. Constantine, Jr., and P.W. Kirk, Jr., Mar. Chem., 1, 157 (1972-73).

(13) J.H. Block, P. Catalfomo, G.H. Constantine, Jr., and P.W. Kirk, Jr., Mycologia, 65, 488 (1973).

(14) J.E. Peters, P. Catalfomo, G.H. Constantine, Jr., and P.W. Kirk, Jr., J. Pharm. Sci., 64, 176 (1975).

(15) P.W. Kirk, Jr., P. Catalfomo, J.H. Block, and G.H. Constantine, Jr., Food-Drugs from the Sea Proceedings, Marine Technology Society, Washington, D.C., 1972, p. 223.

(16) J. Kohlmeyer, Bioscience, 25, 86 (1975).

(17) O.L. Chapman and R.W. King, J. Am. Chem. Soc., 86, 1256 (1964).

(18) R. Schiff and C. Bertini, Ber., 30, 601 (1897).

(19) R. Schiff and L. Gigli, Ber., 31, 1306 (1898).

(20) H.H. Wasserman and R.C. Koch, Chem. Ind. (London), 428 (1957).

(21) W.L. Meyer and W.R. Vaughan, J. Org. Chem., 22, 98 (1957).

(22) W.L. Meyer and W.R. Vaughan, J. Org. Chem., 22, 1554 (1957)

(23) H.H. Wasserman and R.C. Koch, J. Org. Chem., 27, 35 (1962).

(24) W. Borsche, Ber., 42, 4072 (1909).

(25) P.L. Southwick, E.P. Preuic, J. Casanova, Jr., and E.H. Carlson, J. Am. Chem. Soc., 78, 1087 (1956).

(26) P.L. Southwick and R.T. Crouch, J. Am. Chem. Soc., 75, 3413 (1953).

(27) G. Stork, Y. Nakahara, and W.J. Greenlee, J. Am. Chem. Soc., 100, 7775 (1978).

(28) E. Dagne and N. Castagnoli, Jr., J. Med. Chem., 15, 356 (1972).

(29) M. Ochiai, M. Obayashi and K. Morita, Tetrahedron, 23, 2641 (1967).

(30) R. Huisgen, H. Hauck, R. Grashey and H. Seidl, Chem. Ber., 101, 2568 (1968).

(31) E. Baer and H.O.L. Fischer, J. Biol. Chem., 128, 463 (1936).

(32) R. Behrend and C. Neubauer, Justus Liebigs Ann. Chem., 298, 200 (1879).

(33) H. Fever, B.F. Vincent, and R.S. Bartlett, J. Org. Chem., 30, 2877 (1965).

(34) G. Minunni and G. Corselli, Chem. Zentrall., 1892, 904.

(35) R. Behrend and K. Leuchs, Justus Liebigs Ann. Chem., 257, 203 (1890).

(36) L.W. Jones and M.C. Sneed, J. Am. Chem. Soc., 39, 674 (1917).

(37) D.R. Bender, L.F. Bjeldanes, D.R. Knapp, and H. Rapoport, J. Org. Chem., 40, 1264 (1975).

(38) H. Seidl, R. Huisgen and R. Knorr, Chem. Ber., 102, 904 (1969).

(39) J.E. McKeon, Ph.D. dissertation, Yale University, 1960.

(40) J. Meinwald and O.L. Chapman, J. Am. Chem. Soc., 80, 633 (1950).

(41) E.J. Corey and J.W. Suggs, Tetrahedron Lett., 2647 (1975).

(42) J.C. Collins, W.W. Hess, and F.J. Frank, Tetrahedron Lett., 3363 (1968).

(43) K. Bowden, I.M. Heilbron, E.R.H. Jones, and B.C.L. Weedon, J. Chem. Soc., 39 (1946).

(44) K.E. Pfitzner and J.G. Moffatt, J. Am. Chem. Soc., 87, 5661, 5670 (1965).

(45) F. Imperato, J. Org. Chem., 41, 3478 (1976).

(46) N. Pravdic and H.G. Fletcher, Jr., Carbohydr. Res., 19, 339 (1971).

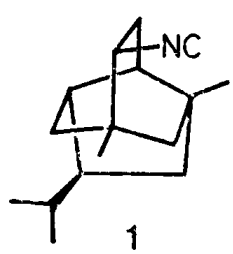
PART II

A SYNTHESIS OF THE MARINE TOXIN, 9-ISOCYANOPUPUKEANANE

A. INTRODUCTION

In reviewing the defense mechanisms observed in molluscs of the class Opisthobranchi, Thompson, in 1960,[1] noted that while some species produce acidic secretions when aroused and others employ cnidophores, a number of species, in which neither protective system was present, were seldom victims of predation by fish. This observation led to the postulation of a third defense mechanism by Johannes, who reported the the nudibranch Phyllidia varicosa (Lamarck, 1801) secretes a strong smelling, thermally stable and volatile substance which was lethal to fish and crustaceans.[2] In view of the fact that this mucus excretion is characteristic of most Phyllidia species,[3] and considering the widespread occurrence of skin glands among the opisthobranchs, whose structure and function appears to be defensive,[1] the secretion of toxic substances may eventually prove to be a protective device of general distribution in the class.

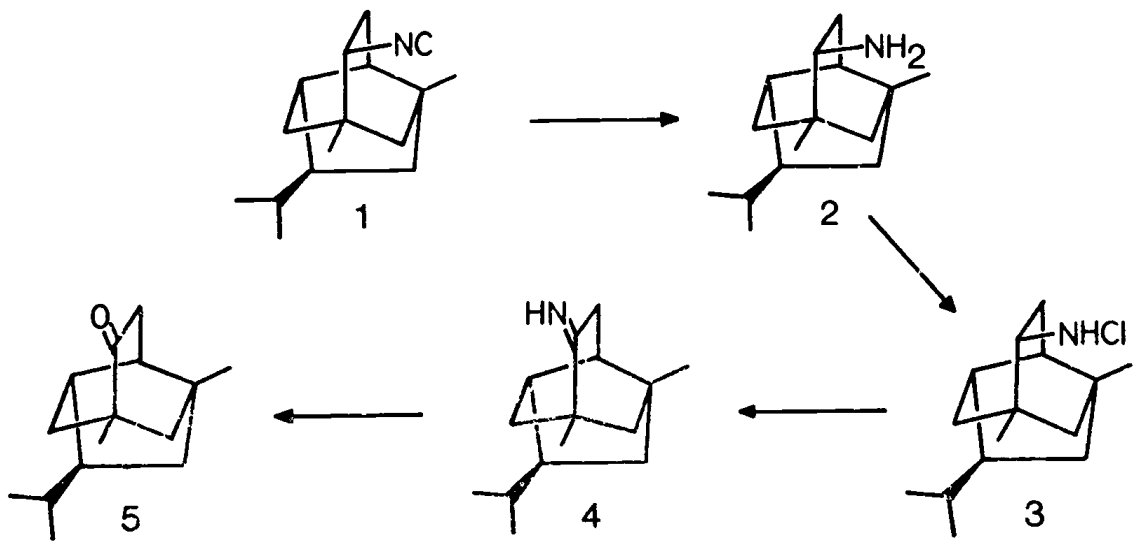
Based on the previous reports, Burreson and Scheuer, et.al., in 1975,[4] isolated and identified the toxic principle, 9-isocyanopupukeanane



1

1, from mucus extracts of Phyllidia varicosa. The toxin was also shown to be of dietary origin[4] when the nudibranch was discovered feeding on a species of sponge belonging to the genus Hymeniacidon. It was the site of this observation, off Pupukea on the north shore of Oahu, which lent its name to the metabolite. Biologically, the isocyanide not only protects the nudibranch from predation but also serves as the kairomone[5] of the browser-prey relationship. The natural product is of chemical interest by virtue of the unique [4.3.1.$0^{3,7}$]tricyclodecane skeleton which represents a biogenesis through a previously unreported isoprenoid rearrangement. In addition, the isocyano group is of relatively rare occurrence, having been observed only in the marine sponges,[6-13] Penicillium notatum[14] and Trichoderma sp.[15,16]

In the course of their structural work, Burreson and Scheuer[4] reported the degradation of the isocyanide 1 through the amine 2 and, without isolation of the intermediate chloroamine 3 and imine 4, to pupukeanone 5.



Our approach to the synthesis of 9-isocyanopupukeanane was based upon a proposed intramolecular Diels-Alder reaction, shown schematically in Figure 1, of a suitably functionalized dihydro derivative of meta-xylene to which is appended a butenyl side chain. It was anticipated that the

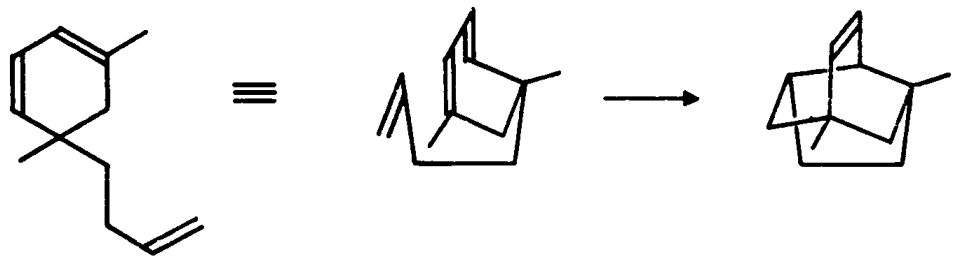


Figure 1

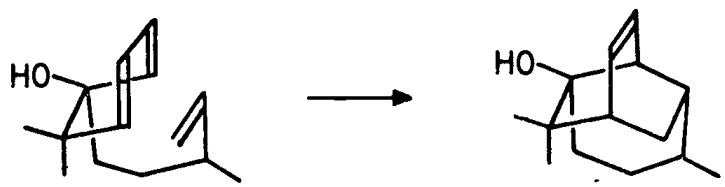
regiochemistry of the addition would be controlled by the steric constraints inherent in the transition state and might be assisted by polarization effects to give exclusively the requisite [4.3.1.$0^{3,7}$]tricyclodecane skeleton (as opposed to a twistane framework).

The intramolecular Diels-Alder reaction, although a relatively recent extension of its intermolecular progenitor, is a well documented and exceptionally powerful synthetic tool. Since the original systematic study of House and Cronin in 1963,[17] the reaction has seen broad application in the generation of multi-ringed systems.[18,19]

The use of this approach in the synthesis of a [4.3.1.$0^{3,7}$]tricyclodecane skeleton was, until very recently,[20] unprecedented in the literature. However, a number of related applications have been reported. Thermolysis of triene 6 was shown by Krantz[21] to give, in addition to other products the [3.3.1.$0^{4,7}$]tricyclononane 7. Ohloff's synthesis of patchoulol[22] in-

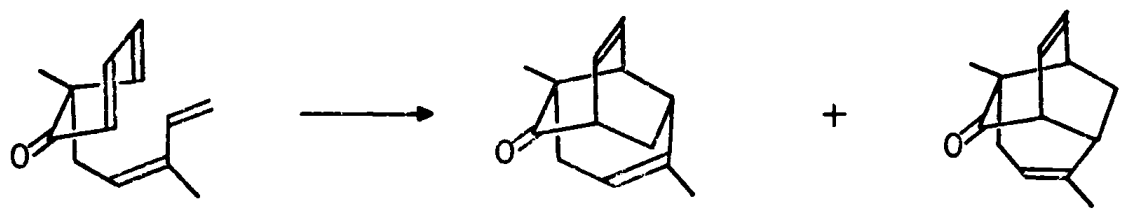
6 7

cluded an intramolecular addition in which the cyclohexadiene 8, containing an appended pentenyl chain yielded the [5.3.1.0]tricycloundecene 9. The



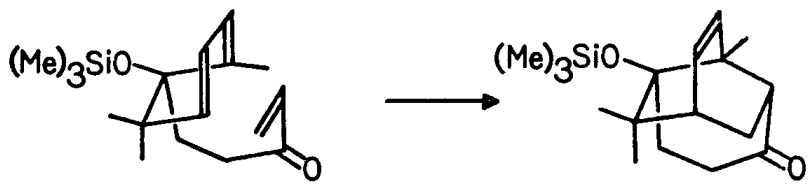
8 9

synthesis of seychellene by Frater[23] features a similar cycloaddition, in which the pentadienyl chain of 10 again forms a three-carbon bridge across



10 11 3:1 12

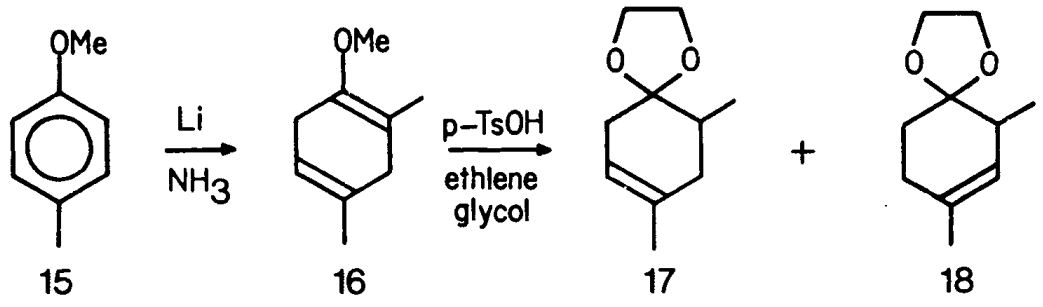
the [2.2.2] system. This cyclization however, failed to exhibit strict regioselectivity and both possible modes of addition were observed (11 and 12). In contrast, Oppolzer's recent synthesis of norpatchoulenol[24] employed the closure of trienone 13 to generate the [5.3.1.$0^{3,8}$] system 14 as the sole product.



13 14

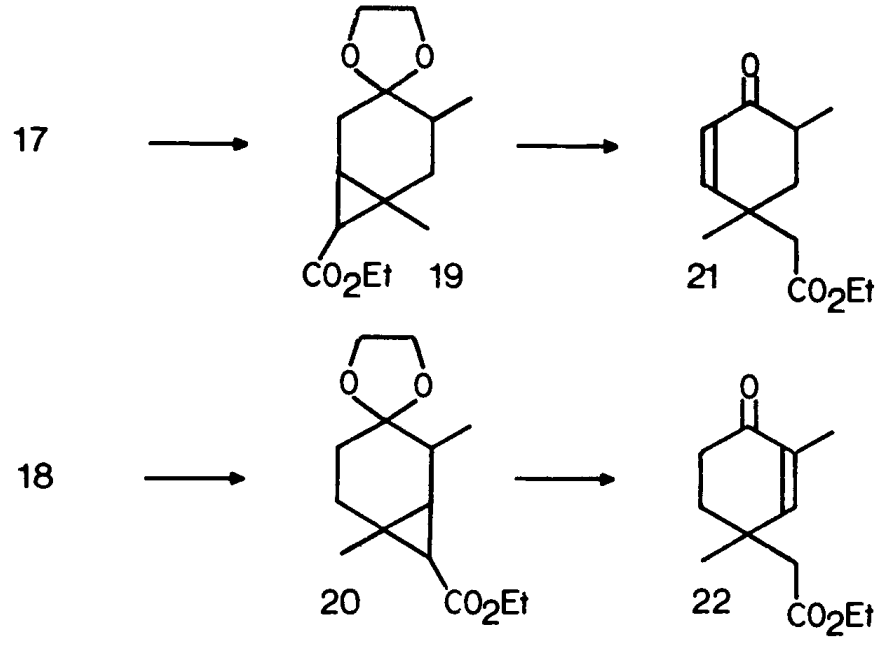
B. SYNTHESIS OF 9-PUPUKEANONE

Encouraged by these precedents, an approach to 9-isocyanopupukeanane was initiated by the Birch reduction of 2,4-dimethylanisole 15 which gave the 1,4-cyclohexadiene 16 in 69% yield. Applying the methodology developed by Stipanovic and Turner,[25] and diene 16 was treated with ethylene

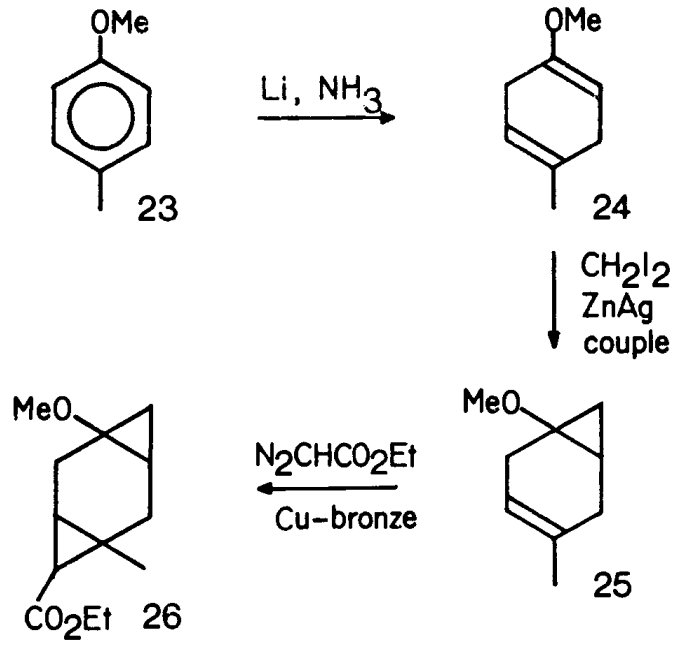


15 16 17 18

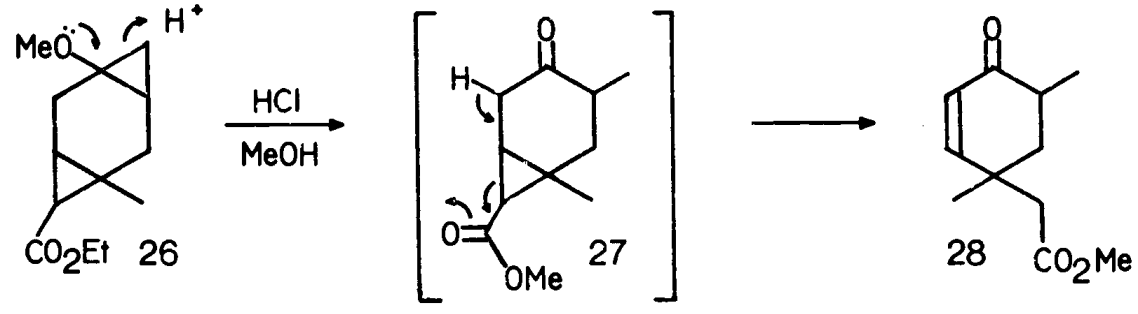
glycol and p-toluenesulfonic acid to give what was eventually shown to be a mixture of isomeric olefin ketals 17 and 18. Exposure of the mixture to ethyl diazoacetate in the presence of copper-bronze gave the corresponding cyclopropyl esters 19 and 20. Acid catalyzed transketalization with acetone, followed by a sodium acetate induced rearrangement produced enone esters 21 and 22 in 73% yield but unfortunately in a 1:1 isomeric ratio. Since the components of this mixture defied routine separation and since it appeared unlikely that the facile double bond isomerization could be suppressed in the ketalization of 16, an alternative route to the enone 21 was clearly required.



After sagacious consideration it became apparent that advantage might be taken of the inherent difference in reactivity between the vinyl ether and the trisubstituted double bond in the Birch reduction product 24 prepared from 4-methylanisole 23.[26] Selective cyclopropanation of the diene 24, utilizing the modified Simmons-Smith reaction developed by Conia,[27] afforded the cyclopropyl ether 25 in 64% yield, thus introducing a masked α -methyl ketone.[28] Ethyl diazoacetate addition to olefin 25, catalyzed by copper-bronze, proceeded at 140°C to give the biscyclopropyl ester 26 as a mixture of cis-trans and endo-exo isomers. Subsequent treat-

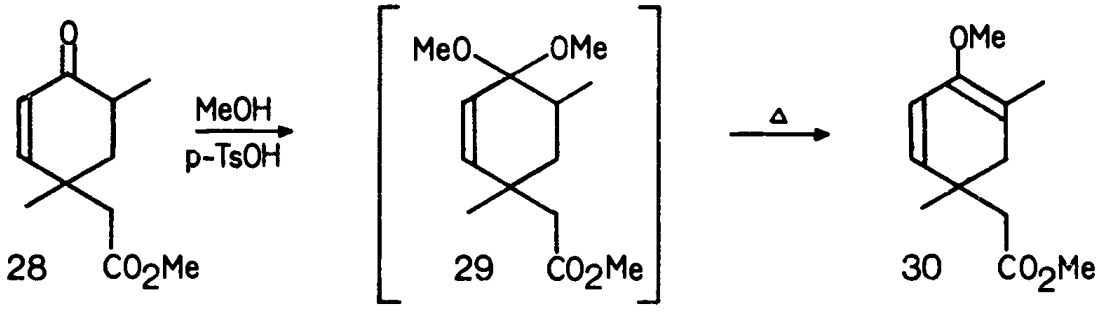


ment of the diasteromeric mixture with aqueous methanolic hydrochloric acid gave enone 28 as a 1:1 mixture of cis and trans isomers. The reaction



apparently proceeds through the intermediacy of ketone 27, formed by an initial fragmentation and hydrolysis of the cyclopropyl methyl ether.

This is followed by a further fragmentation of the cyclopropyl ester in a direction controlled by the acidity of the ketone α-methylene protons. Completion of the required diene unit for the Diels-Alder reaction was accomplished by ketalization of enone 28 and, without isolation, thermal elimination of methanol to give dienyl ester 30 (79% from 28).



It remained at this point to convert the acetic ester residue of 30 to a form which would constitute the dienophilic portion of the Diels-Alder precursor. The method initially attempted for this transformation was that reported by Floyd,[29] which involves the preparation of vinyl ketones by the addition of two equivalents of vinyllithium 31[30] to a carbo-

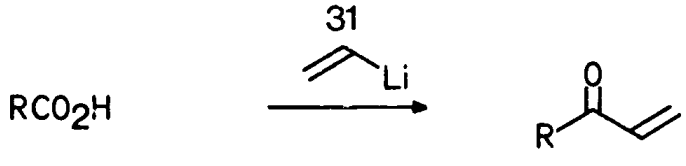
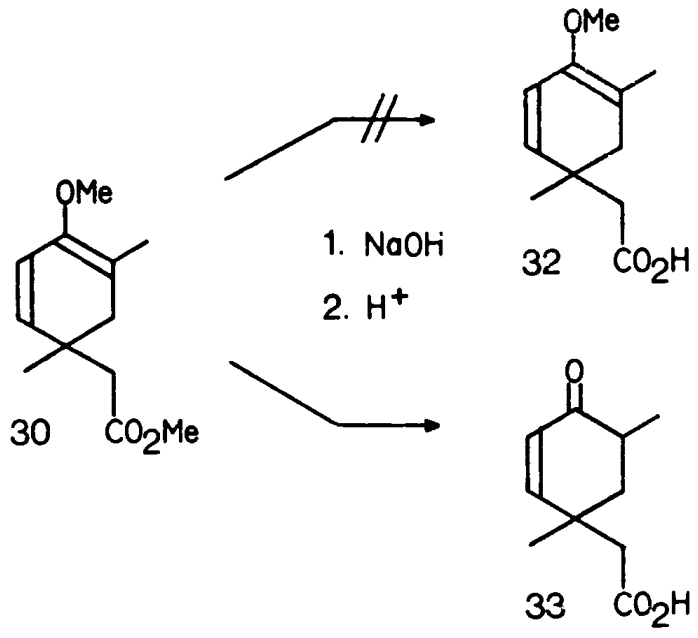
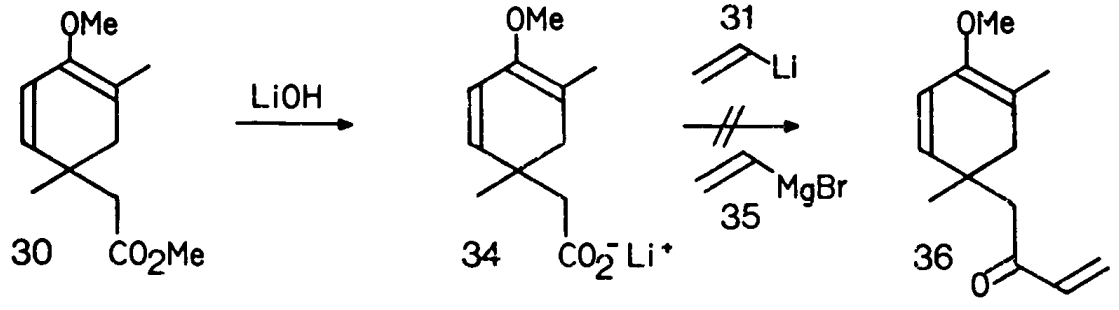


Figure 2

xylic acid (Figure 2). Our attempts to secure the free carboxylic acid 32 by basic hydrolysis of 30 followed by acidification led to hydrolysis

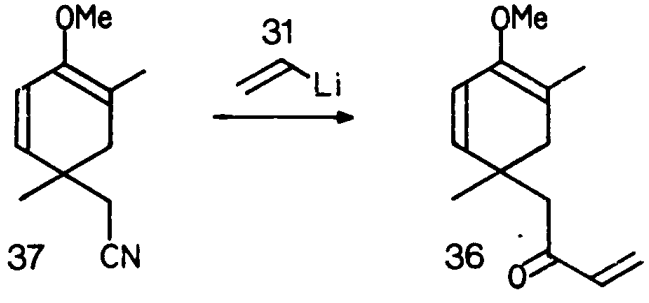


of the vinyl ether and formation of enone acid 33 instead. Since the addition of one equivalent of vinyllithium to a lithium carboxylate would constitute an equivalent version of the vinylation reaction, the lithium



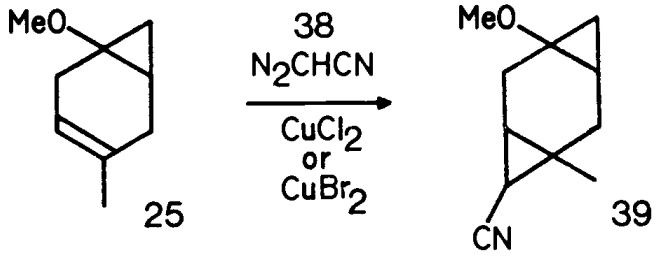
salt 34 was prepared by direct saponification of the methyl ester 30 using lithium hydroxide. However, despite considerable efforts, which included variations in solvent, temperature and reaction time, no identifiable products were observed when 34 was exposed to vinyllithium. Similar attempts to effect the conversion to 34 to 36 utilizing vinyl magnesium bromide 35 were equally futile.

Since the addition of lithium reagents to nitriles often affords acceptable yields of the corresponding ketones,[31] our attention turned to the preparation of dienyl nitrile 37. In the search for convenient access

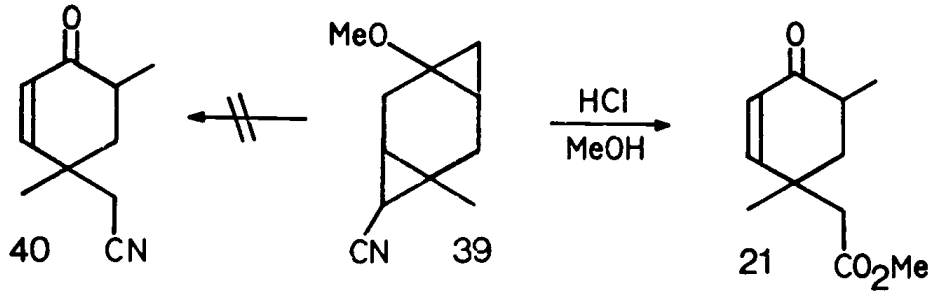


to this compound, we became aware of the reagent, diazoacetonitrile, which was originally prepared by Curtius in 1898.[32] Surprisingly, the sole example of its use as a cyclopropanating agent is the report of Harper and Sleep, in 1955,[33] in their synthesis of the chrysanthemic acids. Both the rapid decomposition of the reagent at temperatures above 50°C and the progressive deactivation of the catalyst at lower temperatures due to the deposition of insoluble polymeric material appears to have prevented the routine use of the reagent in the generation of cyano-substituted cyclo-

propanes. Nonetheless, olefin 25 was converted smoothly at ambient temperature to biscyclopropyl nitrile 39 by treatment with diazoacetonitrile 38 in the presence of cupric chloride or cupric bromide.



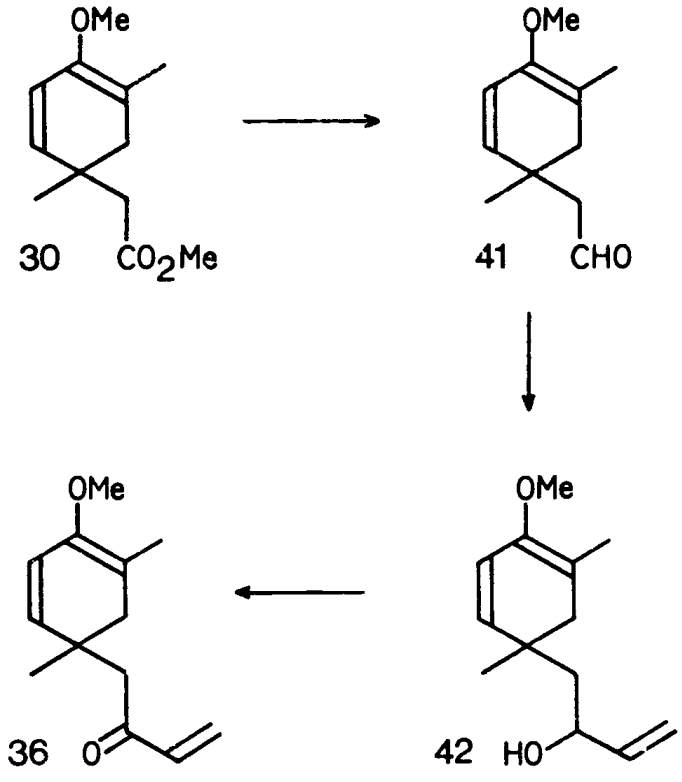
Although gradual deactivation of the catalyst was encountered, the difficulty was conveniently resolved by the periodic addition of fresh catalyst. Unfortunately, attempts to convert tricyclooctane 39 to ketonitrile 40 resulted in methanolysis of the cyano group and led to the



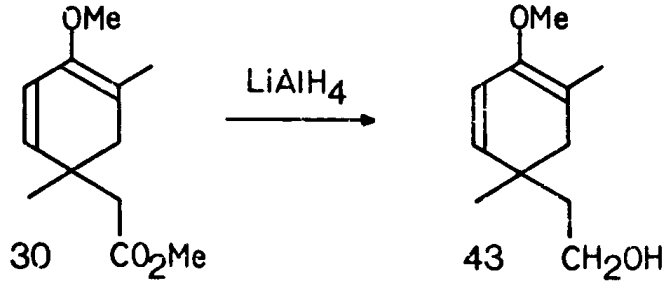
same enone methyl ester 21, previously obtained from 26.

At this point, it was decided that a more conventional route from

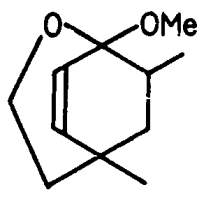
30 to vinyl ketone 36 should be investigated. Attempts to reduce methyl



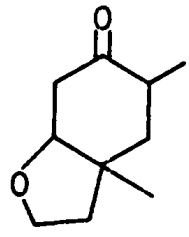
ester 30 directly to the aldehyde 41 with diisobutyl aluminum hydride (DIBAL)[34] or sodium bismethoxyethoxyaluminum hydride (Vitride)[35] led only to the overreduced product 43.



Upon standing or on silica gel chromatography, primary alcohol 43 gave a number of products of which two were tentatively assigned structures 44 and 45. The former product is the internal ketal of dienyl alcohol



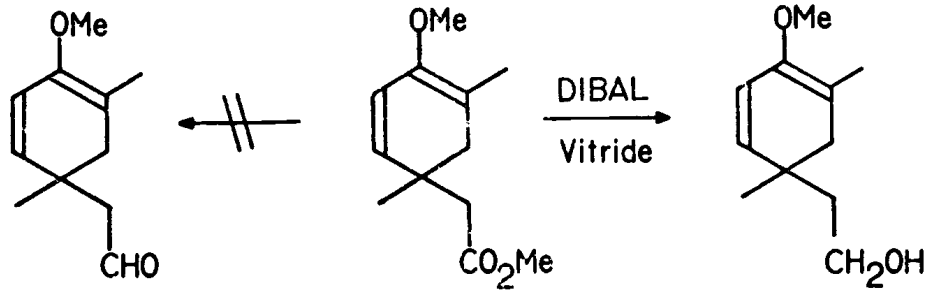
44



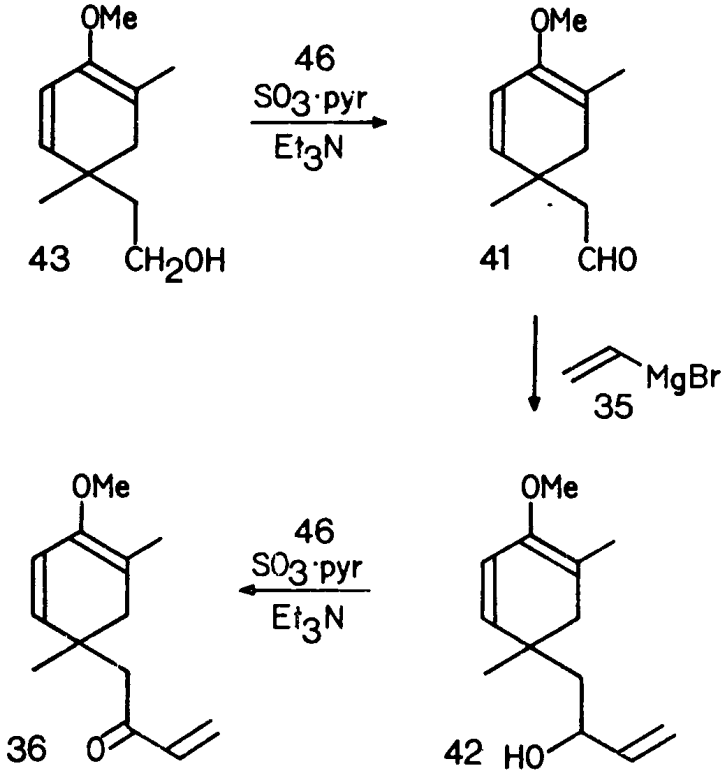
45

43, while the latter presumably arises by hydrolysis of the vinyl ether followed by a Michael addition of the hydroxyl group to the resulting enone.

With direct access to aldehyde 41 ruled out, dienyl ester 30 was reduced with lithium alumnium hydride to give primary alcohol 43 cleanly in 86% yield. The sensitivity of the product was again evident as a number of oxidation methods stubbornly refused to yield the desired aldehyde. Typical of these results was the use of Collin's reagent[36] which, although

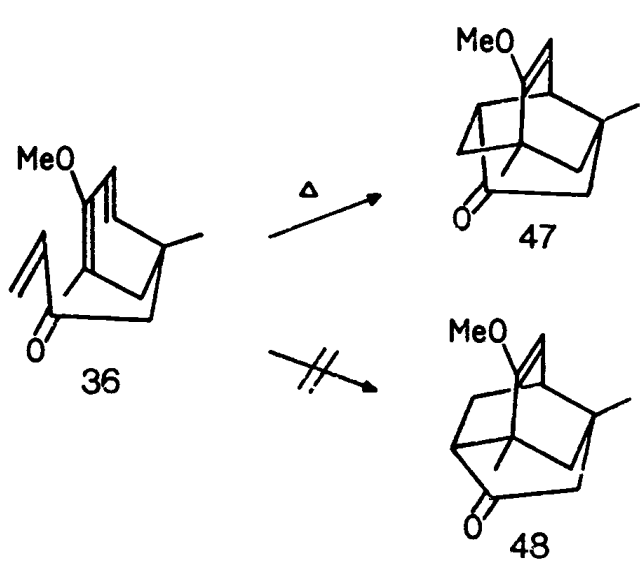


giving an aldehyde product, apparently led to the oxidation of the cyclohexadienyl system as well. The Moffatt oxidation[38] and silver-carbonate on Celite[39] gave no identifiable products, although the lack of vinyl resonances in the NMR spectra of crude material suggested that an acid catalyzed ring closure of the type noted previously had occurred. The conversion to 41 was ultimately effected with sulfur trioxide-pyridine complex 46 in dimethylsulfoxide and triethylamine[40] and gave the chromatographically stable (alumina) aldehyde 41. Addition of vinyl magnesium bromide proved to be routine, affording a diastereomixture of unstable allylic alcohols 42.

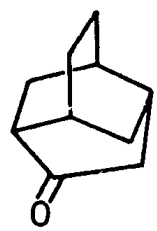


Transformation of 42 to the elusive vinyl ketone 36 was realized by subjecting the isomeric alcohols to oxidation with sulfur trioxide-pyridine under the conditions used for the preparation of 41. The product exhibited the distinctive ABX pattern of a vinyl ketone in the region of the NMR spectrum from δ 6.2 and 5.1, as well as the AB vinyl resonances due to the cyclohexadiene (δ 5.72).

With the precursor 36 in hand, the stage was now set for the crucial intramolecular Diels-Alder reaction. It should be noted that the methoxy and ketone groups in the diene and dienophile units of this structure are oriented so as to guide the cycloaddition in the direction leading to formation of the pupukeanane skeleton 47 rather than the alternative twistane 48. This electronic effect is undoubtedly reinforced by the reduced steric strain in the transition state associated with the generation of the natural system 47 as compared to skeleton 48.

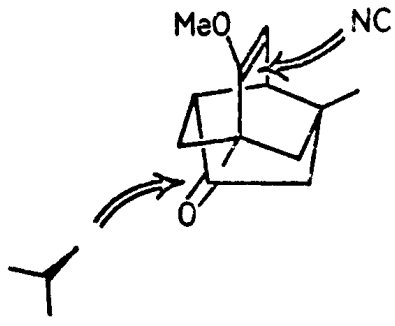


As anticipated, thermolysis of the vinyl ketone 36 in benzene at 100°C proceeded smoothly to give the tricyclodecane 47 as the sole product in 87% yield. The transformation was characterized spectrally by the loss of all the vinyl proton absorptions in the NMR spectrum between δ 6.2 and δ 5.1 with the concomitant appearance of a single vinyl doublet (J=7Hz) at δ 4.47. While the methyl ether resonance showed only a small upfield shift to δ 3.18, the disappearance of the vinyl methyl singlet (δ 1.73) in the precursor was accompanied by the rise of a second saturated methyl signal in 47. Confirmation of the exclusive formation of regioisomer 47 was provided by the infrared spectrum, which exhibited a single carbonyl absorption at 1740 cm^{-1}. The twistane 48 would be expected, by analogy to ketone 49,[41] to show a carbonyl band in the region of 1718 cm^{-1}.

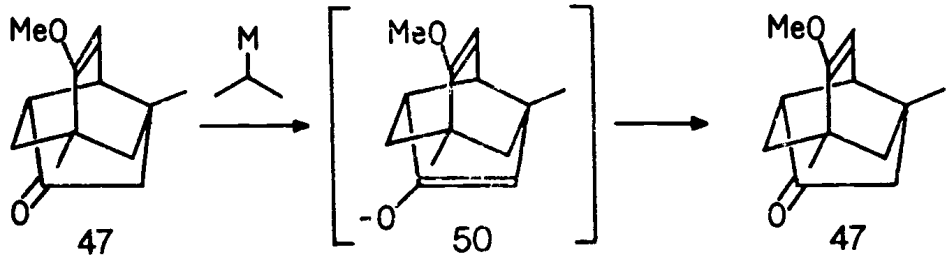


49

With the pupukeanane skeleton successfully assembled, completion of a route to the toxin required introduction of the endo isopropyl group at C-5 and the isocyano group at C-9.

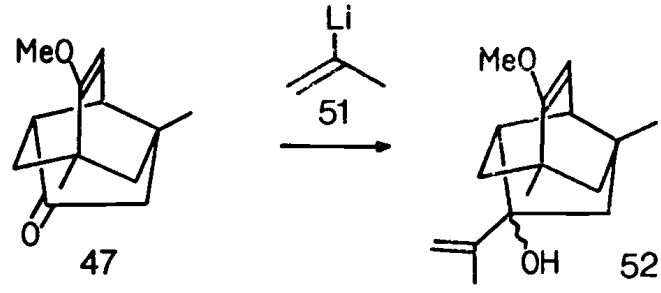


The first of these operations proved to be a nontrivial exercise. Although failure of the Wittig reaction, using the ylid derived from isopropyl triphenylphosphonium bromide,[42] was not surprising in view of the high steric demand of the reagent and the relatively hindered ketone of 47, the isolation of only starting material upon treatment of 47 with isopropyl magnesium bromide[43] or isopropyllithium[44] was quite unexpected. This result, supported by reports on related systems,[45,46] suggested that conversion of 47 to its enolate 50 is facile and thus effectively precludes addition of a highly basic isopropyl residue to the carbonyl carbon. Successful introduction of the isopropyl substituent was finally

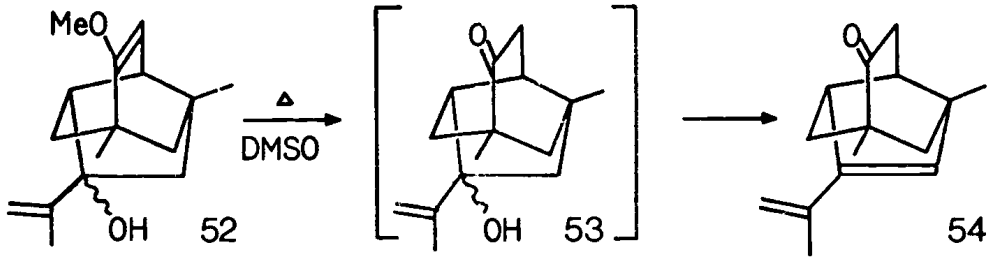


M = Li, MgBr

achieved indirectly by treatment of 47 with isopropenyllithium 51,[47] which yielded tertiary alcohol 52.



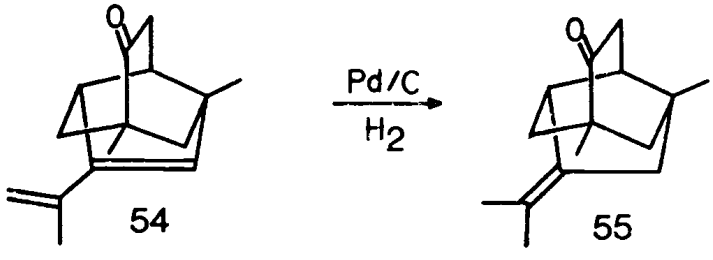
A judicious selection from among the plethora of dehydration methods was rewarded when, upon gradual heating to 120°-130°C in dimethyl sulfoxide,[48] the vinyl ether 52 underwent an initial conversion to ketone 53 and then, upon further heating at 150°-160°C, the tertiary alcohol was eliminated to give diene 54. This pair of transformations took place in overall yield of 94%.



It now remained to hydrogenate the diene 54, with the expectation that pupukeanone 5, the degradation product of the natural isocyanide obtained by Scheuer, would result. It was presumed that following hydrogenation of the isopropenyl double bond, addition of hydrogen to the endocyclic olefin would occur from the exo face of this system to produce the desired endo configuration of the isopropyl substituent.

At this point, we became aware of a just completed synthesis of 9-isocyanopupukeanane which proceeded through the intermediacy of the same dienyl ketone 54.[49] Attempts to establish an identity of the two synthetic dienes, based upon spectral data made available to us by Professor Yamamoto, proved to be inconclusive and it thus became crucial to convert 54 to pupukeanone 5 to obtain a definitive correlation.

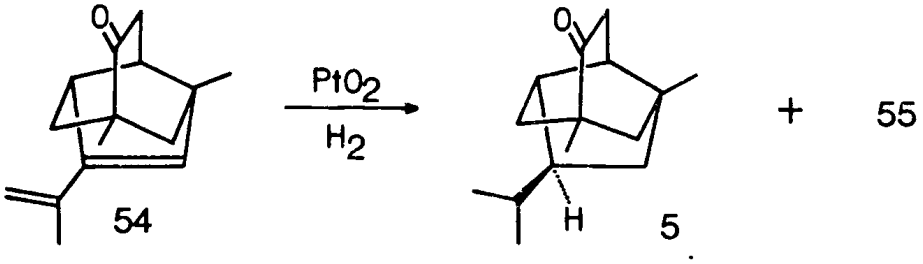
Reduction of diene 54, utilizing palladium on carbon as catalyst, furnished a single product which was tentatively assigned as isopropylidene 55 based upon a mass spectral molecular ion at m/e 218 and the absence of vinyl proton resonances in the NMR spectrum.



54 55

Additional support for the proposed structure was found in the proton spectrum which revealed the presence of vinylic methyl groups and a multiplet at δ 2.60 corresponding to the allylic bridgehead hydrogen at C-6. The formation of this product presumably arises by either a direct 1,4-addition of hydrogen to the diene 54, or by an initial hydrogenation of the terminal isopropenyl double bond followed by subsequent isomerization of the endocyclic olefin to the tetrasubstituted exo-position.

Platinum catalyzed hydrogenation of diene 54 afforded a mixture of underreduced olefin 55 and pupukeanone 5. The latter product, obtained



in pure form by subjecting the mixture of 55 and 5 to hydrogenation utilizing iridium black as catalyst, exhibited a two proton doublet (J=2Hz) at δ 2.34 and saturated methyl singlets at δ 1.04 and δ 0.91 in the NMR spectrum and was spectroscopically identical to a sample obtained by degradation of the natural product and furnished by Professor Yamamoto.

With the preparation of pupukeanone 5, whose conversion to the natural isocyanide has been described independently by two groups,[20,5] a formal total synthesis of 9-isocyanopupukeanane has been realized.

C. EXPERIMENTAL

1-Methoxy-2,4-dimethylcyclohexa-1,4-diene (16)

To 750 ml of liquid ammonia was added sequentially 130 ml of anhydrous t-butanol, 130 ml of anhydrous tetrahydrofuran and 40.7 g (0.3 mol) of 2,4-dimethylanisole. Lithium metal (4.2 g, 0.6 mol) was added and the reaction was maintained until the blue color had discharged. An additional 4.2 g (0.6 mol) of lithium was introduced and the reaction was continued for 2 h. The mixture was quenched with ammonium chloride and the liquid ammonia was allowed to evaporate overnight at room temperature. The crude residue was diluted with water and extracted with ethyl ether. The combined ethereal extracts were washed with water and brine, were dried over magnesium sulfate and the solvent evaporated in vacuo. Distillation afforded 28.6 g (69%) of 16: bp 33-35°C (150 microns); NMR (CCl_4) δ 5.32 (1H, m), 3.46 (3H, s), 2.64 (4H, m), 1.66 (3H, bs), 1.58 (3H, bs).

1,1-Ethylenedioxy-4,6-dimethylcyclohex-3-ene (17) and 1,1-Ethylenedioxy-2,4-dimethylcyclohex-3-ene (18)

In a Dean-Stark apparatus, a mixture of 300 mg (2.2 mmol) of diene 16, 1 ml of ethylene glycol and a catalytic amount of p-toluenesulfonic acid in 20 ml of benzene was maintained at reflux for 3 h. The reaction mixture was made alkaline with sodium bicarbonate, extracted with ethyl ether and washed with water and brine. The ethereal extracts were dried over magnesium sulfate and the solvent was removed in vacuo to give 273 mg

(84%) of a mixture of ketals 17 and 18: NMR (CCl_4) δ 5.31 (1H, m), 3.97 (3H, s), 2.18 (4H, m), 2.00 (1H, m), 1.69 (3H, bs), 0.94 (3H, d, J=6Hz); mass spectrum m/e 168.114 (M^+, calcd for $C_{16}H_{10}O_2$: 168.115).

7-Carboethoxy-4,4-ethylenedioxy-1-methylbicyclo[4.1.0]heptane (19) and 7-Carboethoxy-3,3-ethylenedioxy-6-methylbicyclo[4.1.0]heptane (20)

A mixture of 130 mg (0.77 mmol) of ketals 17 and 18 and 10 mg of copper-bronze in 0.4 ml of diglyme was heated under argon to 140°C. Ethyl diazoacetate[51] (0.22 ml, 2.1 mmol) was added dropwise over 4 h. The reaction mixture was cooled, filtered through Celite and the solvent removed in vacuo. Distillation yielded 95 mg (48%) of a mixture of cyclopropyl esters 19 and 20: NMR ($CDCl_3$) δ 4.16 (2H, q, J=8Hz), 3.96 (4H, m), 2.30-1.50 (5H, m), 1.28 (3H, t, J=8Hz), 1.22 (3H, s), 1.12-0.76 (5H, m); mass spectrum m/e 254.

1-Methoxy-4-methylcyclohexa-1,4-diene (24)

To 750 ml of liquid ammonia was added sequentially 150 ml of anhydrous t-butanol, 150 ml of anhydrous tetrahydrofuran and 48.8 g (51 ml, 0.4 mol) of 4-methylanisole. Lithium metal (11.5 g, 1.6 mol) was added over 40 min and the reaction was maintained for an additional 2.5 h. Excess lithium was quenched with ammonium chloride and the ammonia was allowed to evaporate at room temperature overnight. The crude residue was diluted with water and extracted with ethyl ether. The combined ethereal extracts were washed thoroughly with water and brine and were dried over magnesium sulfate. Distillation yielded 31.6 g (64%) of 24 as a colorless

oil: bp 64-66°C (10 mm) [lit.[52] 74°C (17 mm)]; NMR (CCl_4) δ 5.28 (1H, m), 4.46 (1H, m), 3.46 (3H, s), 2.63 (4H, bs), 1.67 (3H, s).

1-Methoxy-4-methylbicyclo[4.1.0]hept-3-ene (25)

To a solution of 100 mg of purified silver acetate in 50 ml of glacial acetic acid heated to 100°C was added 8.5 g (0.13 mol) of zinc dust. The mixture was stirred vigorously for 1 min, and the acetic acid was removed by decantation. The residue was washed with anhydrous ethyl ether until all traces of acetic acid had been removed. The couple was suspended in 75 ml of anhydrous ether and 17.4 g (65 mmol, 5.25 ml) of diiodomethane was added at such a rate as to maintain a moderate reflux. The suspension was stirred for 1 h, 6.2 g (50 mmol) of diene 24 was added and the reaction was maintained at reflux for 2 h. An additional 8.5 g of zinc-silver couple and 5.25 ml of diiodomethane in 75 ml of ethyl ether was introduced and the reaction was continued under reflux for 12 h. Workup entailed slow dilution with saturated aqueous sodium bicarbonate and extraction with ethyl ether. The combined ethereal extracts were washed with sodium bicarbonate and brine and dried over magnesium sulfate. The solvent was removed in vacuo and the residue was distilled to give 4.4 g (64%) of cyclopropyl ether 25: bp 75-78°C (10 mm); NMR (CCl_4) δ 5.16 (1H, m), 3.19 (3H, s), 2.49 (2H, m), 2.31 (1H, m), 2.09 and 1.92 (1H, m), 1.57 (3H, broad s), 1.18 (1H, m), 0.70 (1H, dd, J=5,10Hz), 0.31 (1H, dd, J=5,6Hz); mass spectrum m/e 138.106 (M^+, calcd for $C_9H_{14}O$: 138.104).

4-Carboxyethyl-1-methoxy-5-methyltricyclo[5.1.0.$0^{3,5}$]octane (26)

A mixture of 9.59 g (69 mmol) of olefin 25 and 250 mg of copper-bronze was heated to 140°C under a nitrogen atmosphere. A solution (1M) of ethyl diazoacetate[52] in methylene chloride was added dropwise over 7.5 h until tlc showed the absence of starting material (100 ml). The crude reaction mixture was filtered through Celite, evaporated under reduced pressure and distilled to give 8.86 g (56.9%) of tricyclic ester 26: bp 86-110°C (250 microns); NMR (CCl_4) δ 4.04 (2H, q, J=7Hz), 3.16 and 3.11 (3H, s), 2.34-1.78 (3H, m), 1.68-0.82 (4H, m), 1.22 (3H, t, J=7Hz), 1.07 and 1.04 (3H, s), 0.57 (.5H, dd, J=5,10Hz), 0.26 (.5H, dd, J=5,6Hz), 0.05 (.5H, m); mass spectrum m/e 224.143 (M^+, calcd for $C_{13}H_{20}O_3$: 224.141).

Methyl 1,5-dimethyl-4-oxo-2-cyclohexenyl acetate (28)

In a 100 ml round bottom flask, 8.86 g (39.6 mmol) of 26 was treated with 7% aqueous methanolic hydrochloric acid and was maintained at reflux under a nitrogen atmosphere for 24 h. The reaction mixture was concentrated, diluted with water, and extracted with ethyl ether. The organic extracts were washed with water and brine, and dried over magnesium sulfate. Evaporation of the solvent in vacuo gave 7.19 g (92.7%) of enone ester 28 as a mixture of two diastereomers: IR (film) 1735 and 1675 cm^{-1}; NMR (CCl_4) δ 6.72 and 6.60 (1H, dd, J=2,10Hz), 5.80 and 5.78 (1H, d, J=10Hz), 3.64 (3H, s), 2.42 (2H, m), 2.33 (1H, s), 1.71 (2H, t, J=13Hz), 1.29 and 1.22 (3H, s), 1.08 (3H, d, J=6Hz); mass spectrum m/e 196.112 (M^+, calcd for $C_{11}H_{16}O_3$: 196.110).

Methyl 1,5-dimethyl-4-methoxy-2,4-cyclohexadienyl acetate (30)

To a solution of 4.2 g (21.4 mmol) of enone 28 in 30 ml of anhydrous methanol was added 3 ml of trimethylorthoformate and a catalytic amount of p-toluenesulfonic acid. The mixture was maintained at reflux for 22 h, following which the methanol and trimethylorthoformate were removed by distillation at atmospheric pressure. The bath temperature was raised to 160-170°C for 15 min, the reaction was allowed to cool, and the product was distilled under reduced pressure to give 3.54 g (79%) of dienyl ether 30 as a pale yellow oil: bp 78-82°C (250 microns); IR (film) 1735 and 1675 cm^{-1}; NMR (CCl_4) δ 5.81 (1H, d, J=10Hz), 5.56 (1H, d, J=10Hz), 3.61 (3H, s), 3.49 (3H, s), 2.26 (2H, s), 2.13 (2H, m), 1.68 (3H, s), 1.11 (3H, s); mass spectrum m/e 210.128 (M^+, calcd for $C_{12}H_{18}O_3$: 210.126).

Lithium 1,5-dimethyl-4-methoxy-2,4-cyclohexadienyl acetate (33)

A solution of 2.1 g (10 mmol) of dienyl ester 30 and 420 mg (10 mmol) of lithium hydroxide monohydrate in 10% aqueous methanol was maintained at reflux for 7 h. The product was concentrated and washed with anhydrous benzene. Drying at 60°C for 36 h on a vacuum pump gave 1.9 g (94%) of lithium carboxylate 33 as a white amorphous solid: mp 98-102°C; NMR (d_6-acetone) δ 5.90 (1H, d, J=10Hz), 5.72 (1H, d, J=10Hz), 3.47 (3H, s), 2.25 (2H, m), 2.06 (2H, m), 1.65 (3H, s), 1.11 (3H, s).

Vinyllithium (34)[30]

To 4.5 g (20 mmol, 3.64 ml) of tetravinyltin[53] under an argon atmosphere was added 18.2 ml (40 mmol) of 2.2M n-butyllithium in n-hexane. The mixture was stirred for 60 min during which the colorless vinyllithium precipitated. The reaction mixture was filtered through sintered glass and washed with 2x5 ml of anhydrous n-hexane. The vinyllithium was dissolved in 45 ml of anhydrous tetrahydrofuran and the resulting solution was transferred by canula to a double sealed dark bottle. The concentration was determined, by NMR, to be 0.44M; NMR[54] (THF) δ 7.41 (1H, dd, J=19.5, 23Hz), 6.79 (1H, dd, J=8,19.5Hz), 6.05 (1H, dd, J=8,23Hz).

Diazoacetonitrile (38)[55]

Caution. Explosive. A solution of 9.3 g (0.1 mol) of aminoacetonitrile hydrochloride in 25 ml of water and 60 ml of methylene chloride was cooled to -5°C under a nitrogen atmosphere. An ice-cold solution of 8.3 g (0.12 mol) of sodium nitrite in 25 ml of water was added, the temperature was lowered to -9°C and 9.5 g of 5% by weight sulfuric acid was added dropwise to maintain the reaction temperature below 1°C. The reaction was terminated after 15 min when heat was no longer evolved. The mixture was transferred to a separatory funnel and the methylene chloride layer was added to 100 ml of cold 5% sodium bicarbonate solution. The aqueous layer was extracted once with 10 ml of methylene chloride. The sodium bicarbonate and combined methylene chloride solutions were shaken in a separatory funnel until no trace of acid remained. The organic layer was separated and dried over anhydrous sodium sulfate. The concentration

of the resulting solution of 38 was approximately 0.5M.

1-Methoxy-4-cyano-5-methyltricyclo[5.1.0.$0^{3,5}$]octane (39)

To a solution of 276 mg (2 mmol) of olefin 25 in 1 ml of methylene chloride was added 50 mg of cupric chloride. A methylene chloride solution of diazoacetonitrile was added portionwise until tlc monitoring revealed the absence of starting material. The solution was filtered through silica gel, evaporated, and subjected to column chromatography (benzene:ethyl acetate, 9:1) to give 180 mg (51%) of nitrile 39 as a mixture of two major isomers: NMR (CCl_4) δ 3.20 and 3.15 (3H, s), 2.6-1.7 (4H, m), 1.50-0.75 (5H, m), 1.25 and 1.08 (3H, s); mass spectrum m/e 177.

1,5-Dimethyl-5-(2-hydroxyethyl)-2-methoxycyclohex-1,3-diene (43)

To a solution of 840 mg (4 mmol) of dienyl ester 30 in 10 ml of anhydrous tetrahydrofuran cooled to -78°C was added 91 mg (2.4 mmol) of lithium aluminum hydride. The reaction mixture was allowed to come to room temperature and after 45 min was quenched with water. The mixture was extracted from aqueous saturated sodium bicarbonate with ethyl ether and the combined ethereal extracts were dried over magnesium sulfate. Removal of the solvent in vacuo gave 626 mg (86%) of crude alcohol 43: NMR (CCl_4) δ 5.76 (1H, d, J=10Hz), 5.45 (1H, d, J=10Hz), 3.47 (3H, s), 3.80-3.10 (3H, m), 2.10 (2H, m), 2.80 (2H, m), 1.64 (3H, s), 1.00 (3H, s); mass spectrum m/e 182.

Sulfur trioxide-pyridine (46)

The reagent was prepared according to the procedure of Baumgarten.[56] To a stirred solution of 20 ml (38.4 g, .48 mol) of sulfur trioxide in 80 ml of carbon tetrachloride cooled to 0°C was added dropwise 38 g (0.48 mol) of pyridine over 1 h. Following the addition, the reaction mixture was stirred for an additional 15 min. The reaction mixture was filtered and the white precipitate was washed with ice cold water. The solid was dried in a drying pistol (phosphorus pentoxide, vacuum pump) for 25 h to give 41.5 g (54%) of complex 46: mp 173°C [lit.[57] mp 175°C].

1,5-Dimethyl-4-methoxy-2,4-cyclohexadienylacetaldehyde (41)

To a solution of 450 mg (2.47 mmol) of alcohol 43 in 5 ml of anhydrous triethylamine and 5 ml of anhydrous dimethyl sulfoxide was added a solution of 1.9 g (11.9 mmol) of sulfur trioxide-pyridine complex[40] in 7 ml of anhydrous dimethyl sulfoxide. The reaction mixture was stirred at room temperature for 2.25 h and then partitioned between water and ethyl ether. The combined ethereal extracts were washed with water and brine and dried over magnesium sulfate. The solvent was removed to give 323 mg of crude product (72%). Column chromatography on alumina (benzene) gave a sample of pure aldehyde 41: IR (film) 1700 cm^{-1}; NMR (CCl_4) δ 9.63 (1H, t, J=3H), 5.87 (1H, d, J=10Hz), 5.58 (1H, d, J=10Hz), 3.48 (3H, s), 2.28 (2H, m), 2.12 (2H, m), 1.66 (3H, broad s), 1.14 (3H, s); mass spectrum m/e 180.

Vinylmagnesium bromide (35)

In a 250 ml three-necked flask equipped with dry ice condenser and maintained under a nitrogen atmosphere was placed 100 ml of anhydrous tetrahydrofuran and 4.86 g (0.2 mol) of magnesium. The reaction was initiated with 0.5 ml of vinyl bromide and the remaining vinyl bromide (10.7 g, 7.1 ml, 0.1 mol) was added at a rate which permitted the maintenance of a gentle reflux. After 2 hr the solution was transferred to a double sealed dark bottle and its concentration determined by NMR to be approximately 1M: NMR (THF) δ 6.67 (1H, dd, J=17,21.5Hz), 6.14 (1H, dd, J=8.17Hz), 5.53 (1H, dd, J=8,21.5Hz).

(1,5-Dimethyl-4-methoxy-2,4-cyclohexadienyl)-2-hydroxybut-3-ene (42)

To a 1M solution of vinyl magnesium bromide 35 in tetrahydrofuran (3 ml) cooled to 0°C was added a solution of 323 mg (1.8 mmol) of aldehyde 41, in 1 ml of anhydrous tetrahydrofuran. Following the addition, the reaction mixture was allowed to warm to room temperature and was stirred for 15 min. The mixture was extracted from water with ethyl ether and the combined organic extracts washed with brine and dried over magnesium sulfate. Removal of the solvent in vacuo gave after column chromatography on silica gel [benzene:ethyl acetate (9:1)] 312 mg (83%) of unstable allylic alcohol 42: IR (film) 3425 cm^{-1}; NMR (CCl_4) δ 6.04-5.48 (3H, m), 5.14 (1H, dd, J=2,17Hz), 4.99 (1H, dd, J=2,12Hz), 4.26 (1H, bm), 3.48 (3H, s), 2.14 (2H, m), 1.56 (3H, s), 1.06 (3H, s); mass spectrum m/e 208.

(1,5-Dimethyl-4-methoxy-2,4-cyclohexadienyl)-but-3-en-2-one (36)

To a solution of 10 mg (0.095 mmol) of allylic alcohol 42 in 0.2 ml of anhydrous dimethyl sulfoxide and 0.2 ml of anhydrous triethylamine was added a solution of 70 mg (.11 mmol) of sulfur-trioxide-pyridine complex in 0.3 ml of anhydrous dimethyl sulfoxide.[40] After 1 h at room temperature the reaction mixture was diluted with ethyl ether and partitioned with water. The combined ethereal extracts were washed with water and brine and dried over magnesium sulfate. Removal of the solvent in vacuo yielded ketone 36: NMR (d_6-benzene) δ 6.13 (1H, dd, J=10,17Hz), 5.84 (1H, dd, J=3,17Hz), 5.83 (1H, d, J=10Hz), 5.62 (1H, d, J=10Hz), 5.21 (1H, dd, J=3,10Hz), 3.28 (3H, s), 2.48 (1H, d, J=15Hz), 2.20 (1H, d, J=15Hz), 2.04 (2H, m), 1.73 (3H, broad s), 1.08 (3H, s); mass spectrum m/e 206.

1,3-Dimethyl-9-methoxy-5-oxotricyclo[4.3.1.$0^{3,7}$]dec-8-ene (47)

A solution of 15 mg (0.073 mmol) of enone 36 in 0.3 ml of anhydrous benzene containing a trace of 2,6-di-t-butyl-p-cresol was placed in a sealed tube and heated to 60°C in an oil bath. The reaction was terminated after 1.5 h and the solvent removed in vacuo. Column chromatography on alumina (benzene:hexane (7:3)) gave 13 mg (87%) of 47: IR (film) 1740 and 1635 cm^{-1}; NMR (d_6-benzene) δ 4.47 (1H, d, J=7Hz), 3.18 (3H, s), 2.29-1.40 (4H, m), 1.34 (1H, m), 1.20-0.94 (3H, m), 1.03 (3H, s), 0.82 (3H, s); mass spectrum m/e 206.131 (M^+, calcd for $C_{13}H_{18}O_2$: 206.130).

2-Lithio-1-propene (51)

Utilizing the general procedure of Braude and Evans,[48] 968 mg (8 mmol, .711 ml) of 2-bromo-1-propene was added to a stirred mixture of 112 mg (16 mmol) of lithium (2% sodium) and 8 ml of anhydrous ethyl ether under a nitrogen atmosphere. When heat was no longer evolved, the reaction was stirred for an additional 30 min. The solution was transferred by canula to a reagent bottle and maintained under an argon atmosphere.

1,3-Dimethyl-5-isopropenyl-9-methoxytricyclo-[4.3.1.$0^{3,7}$]dec-8-en-5-ol (52)

To a solution of isopropenyllithium 51 (1 ml, 1M in ethyl ether) cooled to 0°C was added, dropwise, 18 mg (0.09 mmol) of ketone 47. The reaction mixture was maintained at 0°C for 30 min and then was allowed to come to room temperature. After an additional 30 min, the reaction was quenched with water-saturated ethyl ether and was partitioned with ethyl ether and water. The organic extract was dried over $MgSO_4$ and solvent was removed in vacuo. The crude product was subjected to column chromatography on alumina (benzene, Rf 0.41) to give 15.3 mg (69%) of alcohol 52: NMR (d_6-benzene) δ 5.04 (1H, broad s), 4.95 (1H, s), 4.79 (1H, q, J=2Hz), 4.59 (1H, d, J=7Hz), 3.28 (3H, s), 2.47 (1H, m), 2.14 (3H, m), 1.78 (3H, s), 1.86-0.92 (4H, m), 1.26 (3H, s), 0.89 (3H, s); mass spectrum m/e 248.

1,3-Dimethyl-5-isopropenyl-9-oxotricyclo[4.3.1.$0^{3,7}$]dec-4-ene (54)

A solution of 52 mg (.2 mmol) of alcohol 52 in 0.3 ml of dimethyl sulfoxide was heated in an oil bath to 150-160°C. The course of the reaction was monitored by NMR and after 11 h the reaction mixture was diluted with water and extracted with ethyl ether. The combined ethereal extracts were washed with water and brine and dried over $MgSO_4$. Removal of the solvent gave 43 mg (94%) of crude dienyl ketone 54 which was subjected to column chromatography on alumina (benzene:hexane (9:1)): IR (film) 1718 cm^{-1}; NMR ($CDCl_3$) δ 5.80 (1H, s), 4.94 (1H, broad d, J=6Hz), 2.78 (1H, m), 2.48 (2H, d, J=3Hz), 1.92 (3H, s), 1.78-1.01 (4H, m), 1.12 (3H, s) and 0.88 (3H, s); mass spectrum m/e 216.

1,3-Dimethyl-5-isopropyl-9-oxotricyclo[4.3.1.$0^{3,7}$]decane (5)

To a prereduced (H_2, 1 atm) suspension of 50 mg of PtO_2 in 3 ml of methanol was added 27 mg (.13 mmol) of diene 54. After 46 h the reaction mixture was diluted with ethyl ether and filtered through Celite to give 24 mg of a mixture of isopropyl ketone 5 and isopropylidene 55 in a 7:3 ratio. Subjecting the mixture to hydrogenation with iridium black as catalyst provided a sample of pure 5: NMR ($CDCl_3$) δ 2.34 (2H, d, J=3Hz), 1.71, 1.63, 1.56,1.49 (bs), 1.04 (3H, s), 0.91 (3H, s), 0.90 (6H, m); mass spectrum m/e 220.

D. BIBLIOGRAPHY

(1) T.E. Thompson, J. Mar. Biol. Assoc. U.K., 39, 123 (1960).

(2) R.E. Johannes, Veliger, 5, 104 (1963).

(3) J. Rixbec, Faul. Colon. Franc., 2, 1 (1928).

(4) B.J. Burreson, P.J. Scheuer, J. Finer and J. Clardy, J. Am. Chem. Soc., 97, 4763 (1975).

(5) R.H. Wittaker and P.P. Kenny, Science, 171, 3973 (1971).

(6) L. Minale, R. Riccio, and G. Sodano, Tetrahedron, 30, 1341 (1974).

(7) E. Fattorusso, S. Magno, L. Mayol, C. Santacroce, and D. Sica, Tetrahedron, 30, 3911 (1974).

(8) B.D. Blasio, E. Fattorusso, S. Magno, L. Mayol, C. Pedone, C. Santacroce, and D. Sica, Tetrahedron, 32, 473 (1976).

(9) B.J. Burreson, C. Christopherson and P.J. Scheuer, Tetrahedron, 31, 2015 (1975).

(10) F. Cafieri, E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, Tetrahedron, 29, 4259 (1973).

(11) J.T. Baker, R.J. Wells, W.E. Oberhansli, and G.B. Hawkes, J. Am. Chem. Soc., 98, 4010 (1976).

(12) B.J. Burreson, C. Christopherson, and P.J. Scheuer, J. Am. Chem. Soc., 97, 201 (1975).

(13) S.J. Wratten, D.J. Faulkner, K. Hirotsu, and J. Clardy, Tetrahedron Lett., 4345 (1978).

(14) I. Hagedorn and H. Tonjes, Pharmazie, 12, 567 (1957).

(15) M. Nobuhara, H. Tazima, K. Shido, A. Itai, T. Okamoto, and Y. Iitaka, Chem. Pharm. Bull., 24, 832 (1976).

(16) Hoffmann-LaRoche AG, Japan Pat. 100714 (August 25, 1976).

(17) H.O. House and T.H. Cronin, J. Org. Chem., 30, 1061 (1965).

(18) W. Oppolzer, Angew. Chem., Int. Ed. Engl., 16, 10 (1977).

(19) R.G. Carlson, Annu. Rep. Med. Chem., 9, 270 (1974).

(20) H. Yamamoto and H.L. Sham, J. Am. Chem. Soc., 101, 1609 (1979).

(21) A. Krantz, Ph.D. dissertation, Yale University, New Haven, Conn. 1967.

(22) F. Naf and G. Ohloff, Helv. Chim. Acta, 57, 1868 (1974).

(23) G. Frater, Helv. Chim. Acta, 57, 172 (1974).

(24) W. Oppolzer and R.L. Snowden, Tetrahedron Lett., 3505 (1978).

(25) R.D. Stipanovic and R.B. Turner, J. Org. Chem., 33, 3261 (1968).

(26) E.J. Cory, J.A. Katzenellenbogen, N.W. Gilman, S.A. Roman, and B.W. Erickson, J. Am. Chem. Soc., 90, 5618 (1968).

(27) J.M. Denis, G. Girard, and J.M. Conia, Synthesis, 549 (1972).

(28) E. Wenkert and D.A. Berges, J. Am. Chem. Soc., 89, 2507 (1967).

(29) J.C. Floyd, Tetrahedron Lett., 2877 (1974).

(30) D. Seyferth and M.A. Weiner, Chem. Ind. (London), 402 (1959).

(31) P.L. Pickard and T.L. Tolbert, J. Org. Chem., 26, 4887 (1961).

(32) T. Curtius, Chem. Ber., 31, 2489 (1898).

(33) S.H. Harper and K.C. Sleep, J. Sci. Food Agric., 6, 116 (1955).

(34) E. Winterfeldt, Synthesis, 617 (1975).

(35) J. Vit, Org. Chem. Bull., 42, 1 (1970).

(36) J.C. Collins, W.W. Hess, and F.J. Frank, Tetrahedron Lett., 3363 (1968).

(37) W.G. Dauben, M. Lorber, and D.S. Fullerton, J. Org. Chem., 34 3687 (1969).

(38) K.E. Pfitzner and J.G. Moffatt, J. Am. Chem. Soc., 87, 5661, 5670 (1965).

(39) M. Fetizon and M. Golfier, C.R. Acad. Sci., 267, 900 (1968).

(40) J. Parikh and W. von E. Doering, J. Am. Chem. Soc., 89, 5505 (1967).

(41) J. Gauthier and P. Deslongchamps, Can. J. Chem., 45, 297 (1967).

(42) L. Horner and A. Mentrup, Justus Liebigs Ann. Chem., 646, 65 (1961).

(43) N.L. Drake and G.B. Cocke, "Organic Synthesis", Collect. Vol. II, Wiley, New York, N.Y., 1943 p 406.

(44) H. Gilman, F.W. Moore and O. Baine, J. Am. Chem. Soc., 63, 2479 (1941).

(45) C.H. Heathcock, R.A. Badger, and J.W. Patterson, Jr., J. Am. Chem. Soc., 89, 4133 (1967).

(46) J.E. McMurry, J. Am. Chem. Soc., 90, 6821 (1968).

(47) E.A. Braude and E.A. Evans, J. Chem. Soc., 3333 (1956).

(48) V.J. Traynelis, W.L. Hergenrother, H.T. Hanson, and J.A. Valicenti, J. Org. Chem. 27, 2377 (1962).

(49) H. Yamamoto, private communication.

(50) E.J. Cory, M. Behforouz, and M. Ishiguro, J. Am. Chem. Soc., 101, 1608 (1979).

(51) N.E. Searle, "Organic Synthesis", Collect. Vol. IV, Wiley New York, N.Y., 1963 p 424.

(52) A.J. Birch, J. Chem. Soc., 596 (1946).

(53) D. Seyferth and F.G.A. Stone, J. Am. Chem. Soc., 79, 515 (1957).

(54) G. Fraenkel, D.G. Adams, and J. William, Tetrahedron Lett., 767 (1963).

(55) W.L. Mock and M.E. Hartman, J. Org. Chem., 42, 459 (1977).

(56) P. Baumgarten, Ber., 59, 1166 (1926).