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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES IN THE ELUCIDATION OF STRUCTURE OF MAMMOSIN, A DITERPENE LACTONE

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

ROBERT EUGENE MIDDLEBROOK

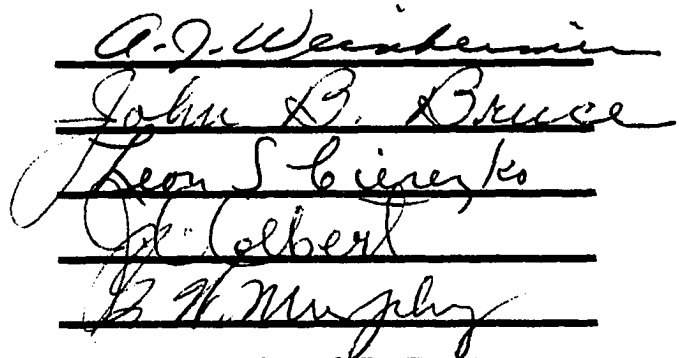
Norman, Oklahoma

1966

STUDIES IN THE ELUCIDATION OF STRUCTURE

OF MAMMOSIN, A DITERPENE LACTONE

APPROVED BY



DISSERTATION COMMITTEE

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
INTRODUCTION	1
RESULTS AND DISCUSSION	7
EXPERIMENTAL	46
SUMMARY	76
BIBLIOGRAPHY	77

LIST OF TABLES

Table		Page
I.	Spin Decoupling Results for Mammosin in $CDCl_3$ (60 Mc.)	44
II.	Spin Decoupling Results for Dihydro-mammosin in CCl_4 (60 Mc.)	45

STUDIES IN THE ELUCIDATION OF STRUCTURE OF MAMMOSIN, A DITERPENE LACTONE

INTRODUCTION

The diterpene lactone mammosin, formerly known as eunicin (1) is isolated from the gorgonian Eunicea mammosa Lamouroux, a marine invertebrate. The compound accounts for approximately 1% of the dry animal, and is known to occur at least in part, in the zooxanthellae of the parent gorgonian.

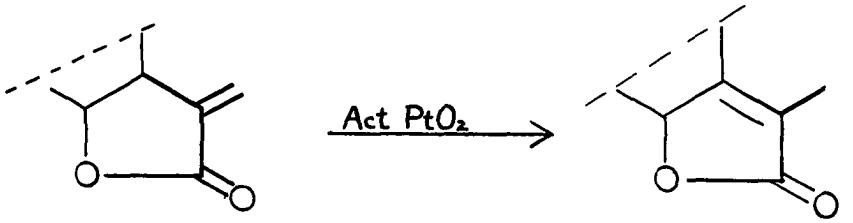
Mammosin, m.p. 154-155°, $[\alpha]_D^{25}$ -89°, has the composition $C_{20}H_{30}O_4$; the molecular weight of 334 was confirmed by mass spectral analysis. Preliminary infrared examination disclosed the presence of hydroxyl (3623 cm^{-1}), γ-lactone (1765 cm^{-1}), and double bond (1664 cm^{-1}). In the ultraviolet there was no absorption above 220 mu. The presence of methyl groups was detected by signals in the N.M.R. spectrum at 0.85 p.p.m. (doublet), 1.15 p.p.m. (singlet) and 1.52 p.p.m. (singlet) characteristic of secondary, tertiary, and vinyl methyl absorption respectively. Three vinyl hydrogens were indicated by absorption occurring at 5.07 p.p.m. (triplet), 5.70 p.p.m. (doublet), and 6.40 p.p.m. (doublet). The former hydrogen suggested the

presence of a trisubstituted double bond flanked by a methylene group, while the latter signals are characteristic of an unsaturated methylene conjugated with a carbonyl group (2) which can only be lactone carbonyl in mammosin.

The tertiary nature of the hydroxyl group was demonstrated by its appearance as a sharp singlet at 4.30 p.p.m. in the N.M.R. spectrum determined in dimethyl sulfoxide. Chemical confirmation was provided (3) by its failure to undergo oxidation on treatment with chromium trioxide and sulfuric acid in acetone (Jones reagent) (7), or with chromium trioxide in pyridine. Surprisingly, the hydroxyl function could be acetylated by treatment of mammosin with boron trifluoride and acetic anhydride (4), resulting in a paramagnetic shift in the N.M.R. absorption of the tertiary methyl to 1.45 p.p.m. Thus mammosin contains a tertiary methyl carbinol group.

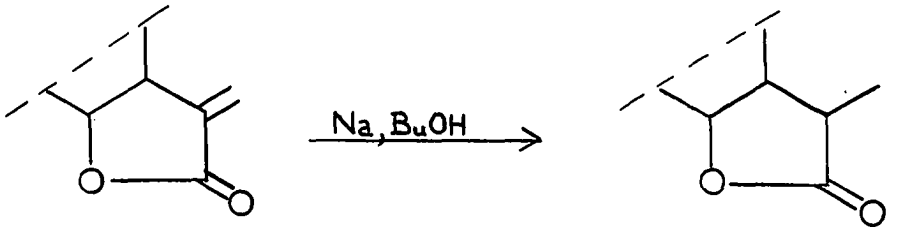
Catalytic hydrogenation of mammosin resulted in the uptake of one mole of hydrogen, disappearance of the 5.07 p.p.m. triplet, and appearance of a new secondary methyl at 1.00 p.p.m. as a doublet ($J = 6$ c.p.s.). The downfield doublets at 5.70 p.p.m. and 6.40 p.p.m. were also absent, and new vinyl methyl appeared at 1.85 p.p.m. Hydrogenation had occurred at the trisubstituted double bond of mammosin with concomitant isomerization of the exomethylenic double bond of the lactone to an <u>endo</u> position. This allylic isomerization was demonstrated independently,

as shown in partial structures (I) and (II), by stirring mammosin with activated catalyst under nitrogen, thus affording isomammosin (4).



(I) (II)

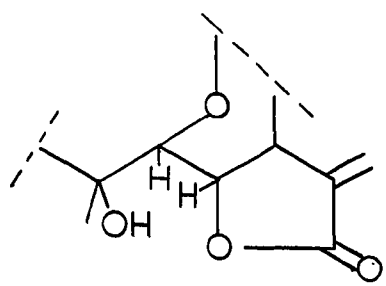
Treatment of mammosin with sodium in refluxing butanol provided dihydromammosin by reduction of the exo-methylenic double bond of the lactone (partial structures I and III) (4).



(I) (III)

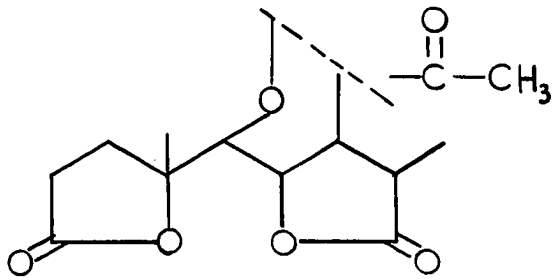
The nature of the fourth oxygen atom of mammosin was partially revealed by two proton absorptions in the N.M.R. spectrum at 3.25 p.p.m. (double doublet) and 2.85 p.p.m. (doublet). The signals represented two hydrogens, each alpha to an ether function. Although the chemical shifts are somewhat unusual for ether hydrogens, with the exception of oxide protons, nonetheless the presence of this kind of hydrogen was discounted after treatment

of mammosin with acidic reagents and lithium aluminum hydride failed to alter the N.M.R. signals. No chemical treatment was found which would rationally cleave the ether, or indeed, yield any information whatsoever concerning the two carbon atoms serving as sites of attachment for the ether. N.M.D.R. data, however, fixed one ether terminus (4) by showing that the ether hydrogen at 2.85 p.p.m. and lactone hydrogen at 4.43 p.p.m. were spin coupled. Since the 2.85 p.p.m. ether hydrogen appeared as a sharp doublet, the tertiary methyl carbinol was assigned to the adjacent carbon atom as shown in partial structure (IV) to accommodate the observed splitting.



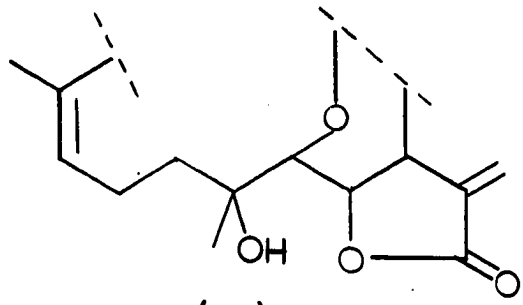
(IV)

Oxidative cleavage at the tri-substituted double bond of dihydromammosin produced a new γ-lactone by lactonization of the resulting acid with the tertiary hydroxyl function, thus affording a keto-dilactone [partial structure (V)] (4).



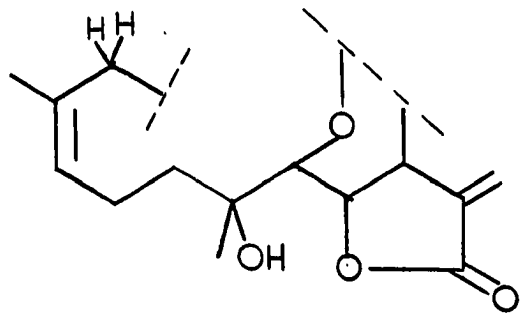
(V)

This compound establishes the relative positions of the trisubstituted double bond and tertiary hydroxyl in mammosin as in partial structure (VI).



(VI)

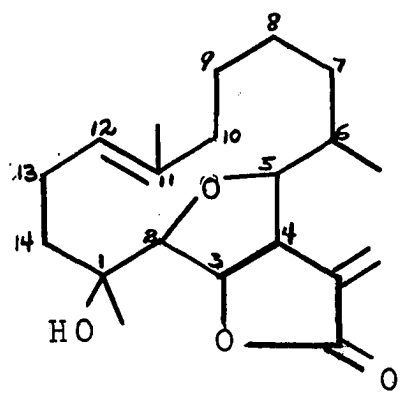
The acetate formed by Baeyer-Villiger oxidation of the methyl ketone of (V) showed two methylene hydrogens at 4.08 p.p.m. (triplet) on carbon adjacent to the carbon bearing acetate. Thus, partial structure (VII) can be written for mammosin.



(VII)

All efforts to establish a carbon skeleton for mammosin by dehydrogenation failed (3). This fact, taken with the molecular formula and knowledge of the functionality in mammosin, led to the conclusion that in addition to the γ-lactone, mammosin contained one large carbocyclic ring bridged by ether oxygen. A skeleton of this kind is found in the tobacco diterpenes (5) and in cembrene (6).

Thus, five of the remaining six carbon atoms in mammosin complete a fourteen-membered ring, and bear the secondary methyl group and remaining secondary ether terminus. These latter functions were positioned according to the most reasonable interpretation of the N.M.D.R. data available at the time, and led to the following structure for mammosin (structure A).



(A)

RESULTS AND DISCUSSION

Part I. The γ-Lactone

Attempts to hydrolyze the α,β-unsaturated γ-lactone of mammosin by the action of mild or strong base, followed by normal reacidification, always resulted in the isolation of starting material as the only product (3). That the lactone does open on treatment with aqueous base was shown by the dissolution of mammosin when it was refluxed for an hour or stirred overnight at room temperature. The free hydroxy acid can be isolated, however, by careful, dropwise acidification of the chilled, basic solution with cold, dilute acid to pH 5-6.

The N.M.R. spectrum of mammosin revealed a single hydrogen (4.43 p.p.m., double doublet) under the acyloxy oxygen atom of the lactone. In order to confirm the indicated degree of substitution at the gamma position of the lactone, a stable hydrolysis product was needed. Oxidation of the new alcohol to a ketone would have provided the necessary chemical evidence. Since the hydroxy acid itself underwent relactonization readily, it was converted to the methyl ester by reaction with ethereal diazomethane

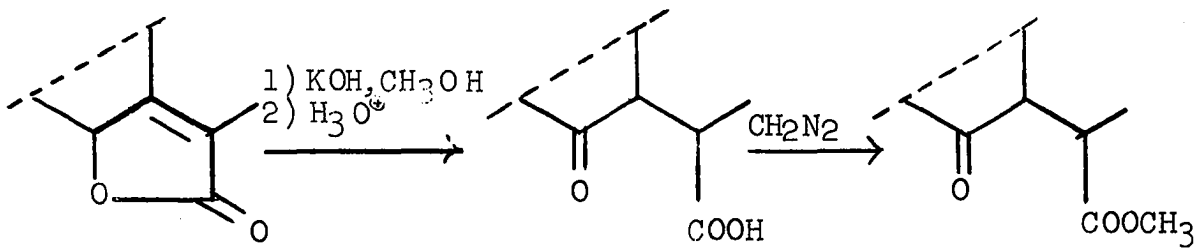
solution. However, the ester's ability to withstand acidic reaction conditions was only slightly better than that of the acid. Relactonization occurred on even decomposition of excess diazomethane with a drop or two of acetic acid, and attempts to oxidize the amorphous hydroxy ester with Jones reagent (7) or chromium trioxide in pyridine resulted in reclosure.

Michael addition of methanol to the α,β-unsaturated γ-lactone of mammosin was effected at room temperature by the action of sodium methoxide in methanol. The downfield doublets at 5.68 p.p.m. and 6.42 p.p.m. attributed to the exo-methylene conjugated with the lactone carbonyl, were absent in the product. Methyl ether absorption appeared at 3.36 p.p.m., and the former exo-methylene hydrogens now appeared as a doublet at 3.72 p.p.m. characteristic of ether protons also beta to the lactone carbonyl.

Since a new asymmetric carbon atom was formed, it was not surprising that the addition product was obtained as a mixture of the two possible epimers. One could be isolated, and it analyzed well. But the gas chromatogram of the pure epimer showed two peaks, one corresponding to mammosin. Loss of methanol by a reverse Michael reaction, thus affording mammosin, is not unexpected at the column temperatures employed (200-250°).

Although the hydroxyl group of mammosin hydroxy-

acid could not be successfully oxidized to a ketone before relactonization occurred, nonetheless the presence of a hydrogen at this center, and also the nature of the lactone ring, were chemically demonstrated by the conversion of isomammosin to its corresponding keto-ester. This transformation exhibited typical butenolide behavior as shown in partial structures (II), (VIII), and (IX) (8).



(II) (VIII) (IX)

It had been noted in earlier work (3) that isomammosin was hydrolyzed with much greater difficulty than mammosin itself, requiring a long period of reflux in strong methanolic base. Acidification of this solution with strong acid, followed by treatment of the resulting acidic product with diazomethane and attempted purification by chromatography on Florisil, afforded no product from the column.

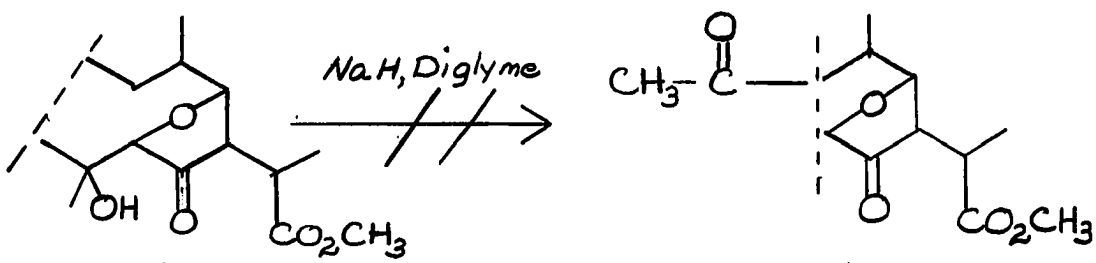
Subsequent interpretation of these facts suggested that the strongly acidic conditions used to regenerate the keto-acid resulted in lactol formation. Indeed, the infrared spectrum of the acidic product had shown γ-lactone carbonyl absorption. It was unusual, however, that

diazomethane did not react either with the pseudo-acid, or with free carboxylic acid, presumably readily available by reversal of the equilibrium between the two acids as the methyl ester is formed. The pseudo-acid itself would not be expected to emerge from the slightly basic Florisil column.

Careful acidification of the solution with cold, dilute acid to pH 5-6 afforded a gummy keto-acid, which was not isolated but treated in ether with diazomethane solution and chromatographed to give an oily keto-ester. The thin layer chromatogram revealed a single elongated spot suggesting the presence of more than one species. This could be anticipated since a new asymmetric carbon atom had been formed at the alpha position of the ester, but even further complications were indicated in the N.M.R. spectrum of the product. Absence of lactone vinyl methyl and appearance of new secondary methyl was observed as expected. However, the tertiary methyl, normally present at 1.15 p.p.m. in mammosin itself as a singlet, absorbed partly at 1.15 p.p.m. and also at 1.3 p.p.m. in approximately a 50:50 ratio. This deviation from normal absorption suggested that the methyl group was present both as methyl under tertiary hydroxyl and methyl under hemiketal, the hemiketal having been formed by the interaction of the tertiary hydroxyl and the newly formed ketone of the keto-acid. In the infrared spectrum of the oily

keto-ester, both ketone and ester carbonyls occur as a single absorption band centered at 1725 cm^{-1}. On the other hand, the anhydro keto-ester in which hemiketal formation is not possible, shows closely spaced but distinct ketone and ester absorptions at 1720 cm^{-1} and 1735 cm^{-1} respectively.

Evidence for the existence of the keto-ester, at least in part, as hemiketal requires revision of the formerly proposed structure for mammosin (structure A) since β-hydroxy ketones do not exhibit hemiketal formation. Further evidence against structure A is supplied by complete failure to effect a reverse aldol reaction on the keto-ester employing sodium hydride in diglyme (the dimethoxy ether of diethylene glycol) as in partial structures (X), and (XI).

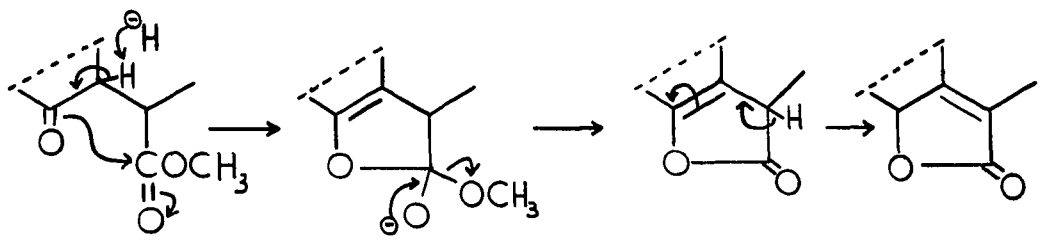


(X) (XI)

It might be argued that the attachment of the ether oxygen alpha to the ketone would, to some extent, hinder the progress of a reverse aldol if one chooses to regard this site as a bridgehead carbon atom and invokes Bredt's Rule (9). The mechanism of the reaction, however, would surely rule out any valid application of Bredt's Rule.

Therefore, the reverse aldol could be expected to proceed as shown, but there was no indication that methyl ketone, a reasonable reverse aldol product, had been formed.

Surprisingly, the product isolated from this reaction was isomammosin, this compound apparently having been produced from oily, originally acidic, material as outlined in partial structures (XII) and (II).



(XII) (II)

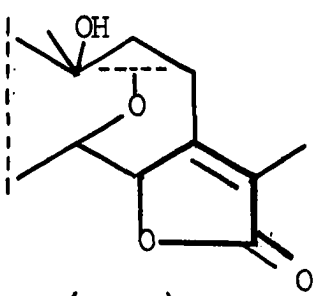
Although isomammosin was obtained in low yield, nonetheless it verifies the structure of the keto-ester portion of the molecule and provides further evidence for the existence of an epimeric carbon atom alpha to the carbomethoxy group of the ester. Destruction of the epimeric center by introduction of a double bond conjugated with the lactone thus led to the isolation of the crystalline product.

Additional information about the γ-lactone of mammosin and its derivatives has been provided by ozonolysis at the exo-methylenic position of mammosin itself (3)(4). One would expect that ozonolysis of isomammosin (II) would provide pyruvic acid by cleavage of the double bond and subsequent hydrolysis of the resulting α-keto ester.

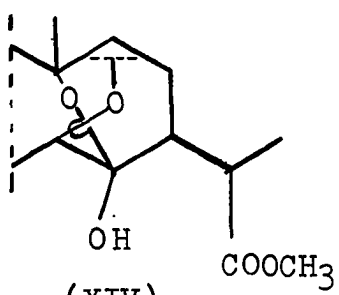
This has been observed in an ozonolysis of anhydrodihydro-isomammosin, pyruvic acid being obtained as its 2,4-dinitrophenylhydrazone, although in small yield.

Part II. Revision of Structure A

Since isomammosin keto-ester failed to undergo a reverse aldol reaction and its N.M.R. spectrum indicated partial interaction between the tertiary hydroxyl function and ketone, the tertiary hydroxyl is tentatively placed at C-7 as shown in partial structure (XIII). This assignment allows the formation of a hemiketal as indicated in partial structure (XIV) and is consistent with the failure of a reverse aldol reaction in the keto-ester. Chemical evidence of a more positive nature supporting C-7 as the site of the tertiary carbinol system will be presented in a later section.



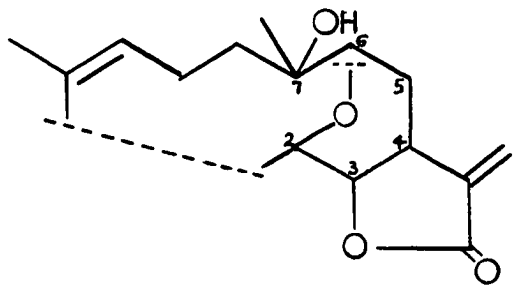
(XIII)



(XIV)

Relocation of the tertiary hydroxyl function requires a number of additional changes in the formerly proposed structure of mammosin (structure A). First, the relative positions of the trisubstituted double bond and tertiary hydroxyl have been firmly established both chemically and

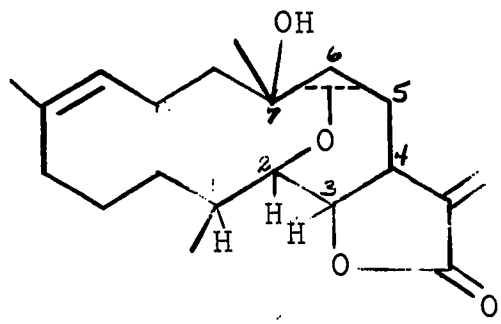
instrumentally (4). Thus, a shift in the position of the trisubstituted double bond is necessary and leads to partial structure (XV).



(XV)

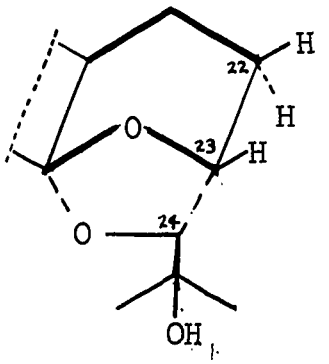
Secondly, extensive decoupling data have shown beyond question that the doublet ether hydrogen at C-2, 2.85 p.p.m., partial structure (XV) , is spin coupled to the lactone hydrogen at C-3, the 4.43 p.p.m. double doublet. Irradiation at either site will simplify the splitting at the other, a singlet occurring for C-2 or a doublet for C-3. The basic difficulty is that the splitting patterns of the two ether hydrogens in mammosin (2.85 p.p.m. doublet and 3.25 p.p.m. double doublet) indicate a total of only three neighboring hydrogens to be present. One must be the lactone hydrogen and the other two (as will be shown) are ordinary methylene hydrogens. The occurrence of the C-2 ether hydrogen as a simple doublet was accommodated in structure A by placement of the tertiary hydroxyl function at C-1, thus satisfying the requirement of a quaternary center adjacent to the C-2 ether hydrogen. However, since the tertiary hydroxyl function has now been reassigned to

C-7 as in partial structure (XV), and since there are no other quaternary centers in the molecule, there must be an additional hydrogen adjacent to the C-2 ether hydrogen, and the coupling constant between the two must be zero in order to observe a simple doublet at C-2. The secondary methyl group is thereby assigned to C-1, and the remaining three carbon atoms complete a fourteen membered carbocyclic ring leading to partial structure (XVI).



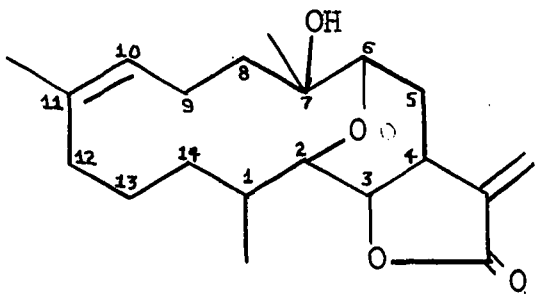
(XVI)

In order for the C-1 and C-2 hydrogens of partial structure (XVI) to have a J value of zero, the dihedral angle between the two must be approximately 90°. This explanation is not without precedent. Corsano et. al., (28) have pointed out in the structure of cimigenol [partial structure (XVII)] that the signal of the 23-H is a doublet (J = 10 c.p.s.). Provided the stereochemistry is as shown, dihedral angles of approximately 90° (J = 0) for the 24-H/23-H and for the 23-H/22-αH exist.



(XVII)

The necessity to reassign the other attachment of the ether oxygen to C-6 as shown in structure (XVIII) is the result of the examination of a number of pieces of instrumental data. Carbon atoms 3,4 and 10 bear hydrogens which

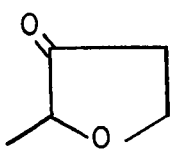


(XVIII)

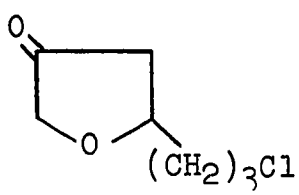
are clearly recognizable in the N.M.R. spectrum of mammosin. C-9 is excluded as a site of attachment for the ether because the vinyl hydrogen at C-10 is split to a triplet thus clearly requiring a methylene group at C-9. The ether cannot be attached at C-13 because the splitting pattern of the 3.25 p.p.m. ether hydrogen (double doublet) cannot be accommodated if it is flanked on both sides by methylene groups. The failure of the ether to react with acidic reagents and with lithium aluminum hydride prevents the

ether from residing at C-1 or C-14. In addition, C-1 is best excluded since it bears the secondary methyl, clearly a doublet in the N.M.R. spectrum of mammosin. C-12 is excluded because Baeyer-Villiger oxidation of the methyl ketone of (V) afforded a dilactone-acetate which showed two hydrogens under acetate which must be at C-12. The remaining possible sites for the ether terminus can only be at C-5, C-6, or C-8.

Recent studies (29, 30) have shown that 3-furanones such as 2-methyltetrahydrofuran-3-one (XIX) and 5-(3-chloropropyl)-dihydro-3-(2H)-furanone (XX) have ketone carbonyl absorption at 1770 and 1760 cm^{-1} respectively, significantly shifted from the 1740 cm^{-1} characteristic of cyclopentanones. If structure A were correct for mammosin,

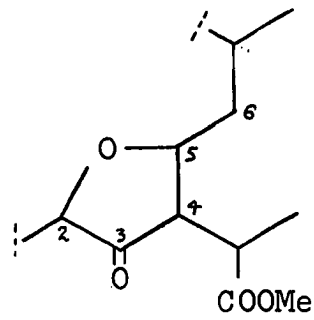


(XIX)



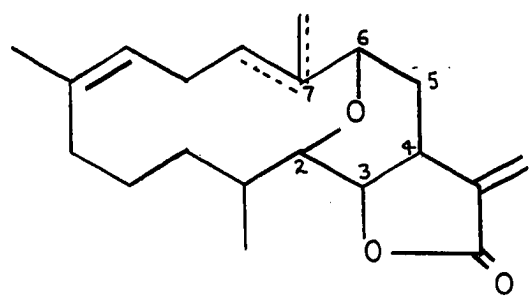
(XX)

the derived keto-ester [partial structure (XXI)] would contain a tetrahydrofuran-3-one, and ketone carbonyl absorption should occur around 1760 cm^{-1} or higher. The fact is that it does not. Ketone and ester absorption both occur at 1725 cm^{-1}, a position more compatible with the absorptions of larger ring β-keto-ethers (30). C-5 then cannot be an ether terminus.



(XXI)

Of the two carbon atoms (C-6 or C-8) remaining as possible sites for the ether terminus, C-6 is preferred, and the ether is tentatively assigned to that position. Additional chemical evidence favoring assignment of C-6 as the ether terminus is found in anhydromammosin, a mixture of two dehydration products. The C-6 proton of structure (XXII) is shifted significantly downfield in the N.M.R. spectrum when it becomes allylic as the result of dehydration, but this information in itself does not rule out C-8 as a possible ether terminus. Final exclusion of C-8 must await the presentation of additional evidence.



(XXII)

Part III. Dehydration Studies

Initially, dehydration studies were conducted on dihydroisomammosin employing hot formic acid as the dehydrating agent (3). A beige colored, solid product was obtained, but in such poor yield that milder conditions were desired for further work (4). Furthermore, the tertiary alcohol remained intact on treatment with potassium hydrogen sulfate in refluxing dioxane (10). Thus a new search was begun for an effective but relatively mild dehydrating agent.

The most satisfactory reagent, in terms of reaction time and yield of product, was thionyl chloride/pyridine acting on mammosin or a derivative at low temperature. The drawback to this procedure, a very big one indeed, is that the product is invariably a mixture of two double bond isomers having approximately a 1:1 distribution of exo and endo double bonds, the result of a two way dehydration. Analysis by gas chromatography showed this product distribution, and thin layer chromatography on silica gel H in a variety of solvents revealed two overlapping spots.

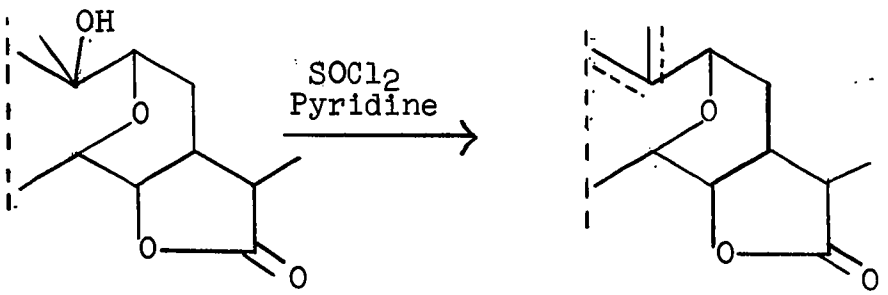
Nonetheless, an attempt was made to separate the mixture by conventional column chromatography on Florisil. A few milligrams of each product from the dehydration of dihydromammosin were obtained after much tedious effort. The majority of the material emerged from the column as

a mixture showing a preponderance of one isomer or the other by gas chromatography or thin layer chromatography.

Nor were the miseries of the chromatography well rewarded by the analyses of the compounds. The analyses of anhydro compounds, whether performed on the separated products or the mixture itself were frequently just outside the range of acceptability. Poor carbon and hydrogen analyses accompanied by high oxygen content are reminiscent of the work of Erdtman et. al., (11), and are attributable to air oxidation of dehydration products. Isomeric oils encountered in the studies of mammosin have, in general, analyzed poorly, although their analyses often are good enough so that when considered with their respective instrumental spectra, no serious doubt concerning their identity intrudes.

As expected, in the N.M.R. spectrum of the anhydro-dihydromammosin mixture, the tertiary methyl present in dihydromammosin is absent; an exo-methylene signal appears at 4.95 p.p.m. and new vinyl methyl absorption occurs at 1.7 p.p.m. Neither of these bands integrates fully for the expected number of hydrogens, as would be required by a mixture. Other significant absorption occurs at 3.97 p.p.m. which is assigned to hydrogen on carbon bearing ether oxygen. Since this hydrogen appears in dihydro-mammosin itself at 3.38 p.p.m., the observed downfield shift is interpreted as evidence for the now allylic

nature of this proton, as in partial structures (XXIII) and (XXIV).



(XXIII) (XXIV)

Since the oily dehydration products were not amenable to separation by ordinary chromatographic techniques, numerous attempts were made to isomerize the mixtures to single isomers employing a variety of acidic and basic reagents, but without success (12). However, possible utilization of silicic acid impregnated with silver nitrate as a column support was not overlooked (13). Considerable success had been achieved in this laboratory in the separation of unsaturated, isomeric sesquiterpene hydrocarbons using the silicic acid - silver nitrate chromatographic technique (14). Thin layer chromatography of anhydromammosin mixture on a plate spread with silica gel H - silver nitrate adsorbent (15) indicated that the two anhydro products would separate well on column chromatography, and they were obtained in this manner and characterized.

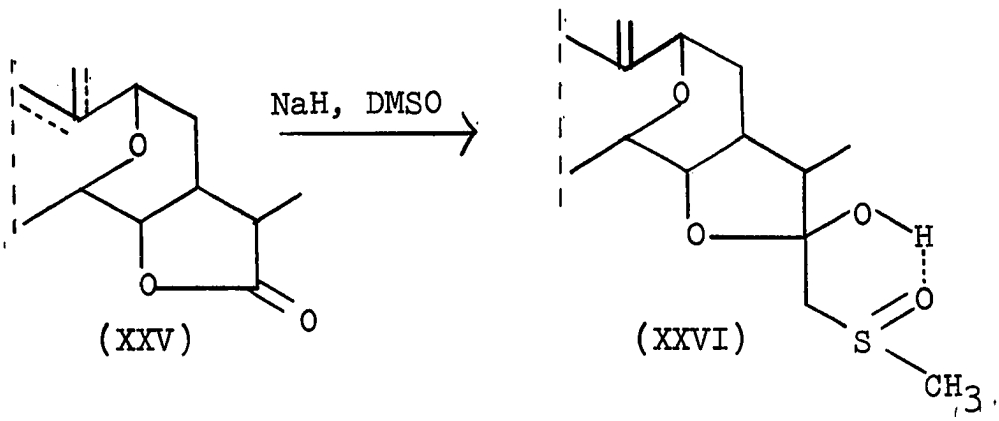
Exo-anhydromammosin, a crystalline compound, showed new exo-methylene absorption as a singlet occurring at 4.93 p.p.m. and integrating for two hydrogens. The tertiary methyl present in mammosin itself was absent. The double doublet characteristic of one ether terminus gave a signal centered at 3.8 p.p.m. having been shifted from its customary position at 3.25 p.p.m. in mammosin.

The endo isomer was an oily compound, possibly a mixture of cis and trans double bond isomers, which turned yellow rather quickly on standing in air. The N.M.R. spectrum of the freshly chromatographed product showed new vinyl methyl at 1.71 p.p.m. (singlet) along with the expected new vinyl hydrogen signal centered at 5.75 p.p.m. An even more dramatic shift of the ether hydrogen was apparent in the endo isomer, this hydrogen now appearing at 3.93 p.p.m. (double doublet).

An N.M.R. spectrum of the endo isomer after it had been exposed for a short time to air showed a singlet beginning to form at 1.28 p.p.m. characteristic of methyl under oxygen and possibly indicating slow formation of oxide at the newly created double bond.

Treatment of anhydrodihydromammosin mixture with sodium hydride in dimethylsulfoxide afforded an interesting compound, $C_{22}H_{36}O_4S$. The N.M.R. spectrum displayed an exo-methylene signal at 4.91 p.p.m. integrating for two

hydrogens. No vinyl methyl corresponding to an endo double bond created by the dehydration was visible. New methyl absorption did occur at 2.70 p.p.m. and is indicative of the introduction of dimethyl sulfoxide anion into the molecule. The exact position of the dimethyl sulfoxide residue was puzzling since the N.M.R. spectrum accounted well for all hydrogens observed in dihydromammosin itself, including the lactone hydrogen at 4.18 p.p.m. However, a glance at the infrared spectrum revealed a curious absence of any lactone carbonyl stretching as well as the presence of sulfoxide stretching at 1020 cm^{-1}. Consideration of these facts suggests that dimethyl sulfoxide anion has added across the lactone carbonyl thus creating a hemiacetal stabilized by a hydrogen bond to the sulfoxide oxygen. The somewhat lower than normal sulfoxide stretching in the infrared (1020 cm^{-1} as opposed to 1040 cm^{-1}) may also be rationalized on this basis (31). The reaction, in addition, apparently isomerized the dehydration mixture to a single exo-product as judged by the N.M.R. spectrum. The sequence is outlined in partial structures (XXV) and (XXVI).



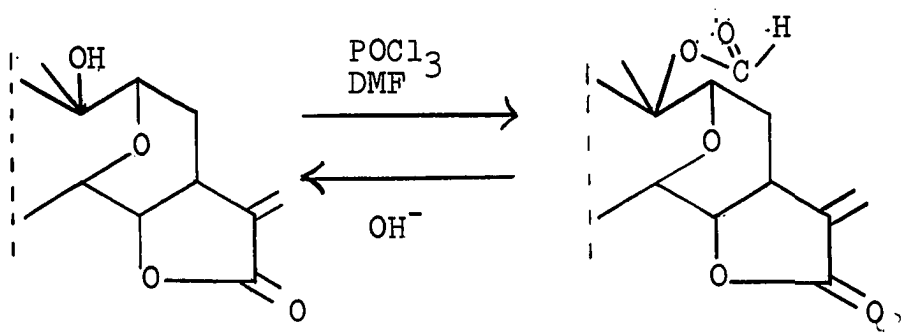
(XXV) (XXVI)

Still other reagents were employed in order to effect a dehydration providing a single crystalline product. Dehydration with phosphorus oxychloride in pyridine at room temperature afforded the same mixture of products as obtained with thionyl chloride in pyridine.

Treatment of mammosin with methane sulfonyl chloride provided not only some of the dehydration mixture but also a crystalline compound, $C_{21}H_{32}O_7S$. This compound has consistently analyzed for a single extra oxygen atom beyond the mesylate derivative which would be $C_{21}H_{32}O_6S$. The N.M.R. spectrum revealed tertiary methyl at 1.46 p.p.m. indicating this group had suffered a paramagnetic shift from its customary position at 1.14 p.p.m. in mammosin itself. An additional methyl group appeared at 4.25 p.p.m. Normal mesylate methyl appears at approximately 3 p.p.m. It appears that the data are best interpreted by considering the compound as a methyl sulfate derivative of mammosin since dimethyl sulfate itself exhibits proton absorption at 3.8 p.p.m. (16) and the methylene hydrogens of ethyl benzenesulfonate appear at 4.2 p.p.m. (17). The reagent used in the preparation of this compound was not distilled, and it is possible that the peculiar result stems from this. It is also possible that it is the result of an unusual oxidation, but no further work is planned on this compound.

Attempted dehydration with phosphorus oxychloride

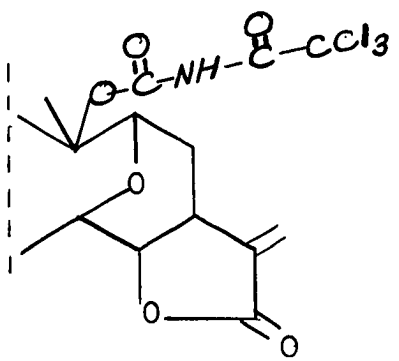
in dimethylformamide provided an interesting result. The reaction between N-substituted formamides or formanilides and certain Lewis acids provides a useful formylating agent, the Vilsmeier reagent (18). Still other Lewis acids, thionyl chloride for example, and dimethylformamide also have application as a dehydration medium (19). Treatment of mammosin dissolved in dimethylformamide with phosphorus oxychloride in the cold afforded another derivative of the tertiary alcohol. Mammosin formate was obtained in good yield but was not accompanied by any dehydration product. Again a downfield shift of the tertiary methyl occurred so that both vinyl methyl and tertiary methyl, now under formate, gave a singlet at 1.51 p.p.m. integrating for six hydrogens. The signal representing formate hydrogen itself arose at 8.11 p.p.m. Mammosin formate could be hydrolyzed to mammosin by the action of dilute base [partial structures (XXVII) and (XXVIII)].



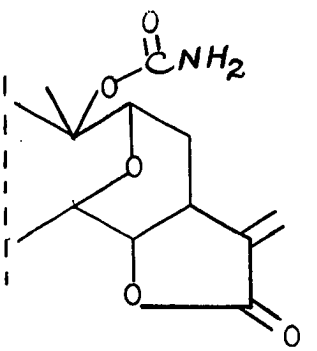
(XXVII) (XXVIII)

A precisely analogous compound has been prepared from isomammosin.

An attempt was made to prepare yet another derivative of the tertiary alcohol for X-ray study. Treatment of mammosin with trichloroacetylisocyanate afforded a solid compound, the trichloroacetylurethane of the tertiary alcohol. When this solid was chromatographed on Florisil, it underwent hydrolysis to the crystalline carbamate as seen in partial structures (XXIX) and (XXX). Being a



(XXIX)



(XXX)

β-dicarbonyl compound, the urethan could quite reasonably be expected to behave in this manner. The tertiary methyl of the carbamate was shifted downfield absorbing at 1.46 p.p.m. Hydrogens attached to nitrogen gave a signal at 4.86 p.p.m. as a broad singlet.

Additional comment is necessary concerning the apparent ease with which the tertiary alcohol forms derivatives. Since tertiary alcohols do not normally follow this reactivity pattern, one might conclude that the stereochemical requirements of the alcohol are such as to preclude facile dehydration; yet the dehydration may be

easily accomplished using quite ordinary reagents. In addition, an examination of a Dreiding model of mammosin does not yield any unique information about this anomalous behavior. However, at this point in the study of mammosin, it would not be unreasonable to suggest that the large ring system, bridged by an ether oxygen, probably provides a stereochemical condition at the alcohol that may result in either dehydration or derivative formation. And indeed, the fact that only two of the possible three anhydro compounds are formed indicates that there must be an effect exerted either by a bridgehead position or the presence of ether oxygen, which prevents double bond formation toward the six-membered ether ring.

Part IV. Studies of the Ether Linkage of Mammosin

The effort to obtain chemical information concerning the ether linkage of mammosin constitutes a turbulent chapter in the elucidation of structure of this diterpene lactone. Indeed, all recent work has been channeled toward the solution of this problem. Specifically, it has become necessary to define the sites of attachment of the ether, and to cleave it by a reasonable chemical approach, i.e., one that does not involve the molecule's destruction or extensive skeletal rearrangement.

In earlier work (3), mammosin had been subjected to

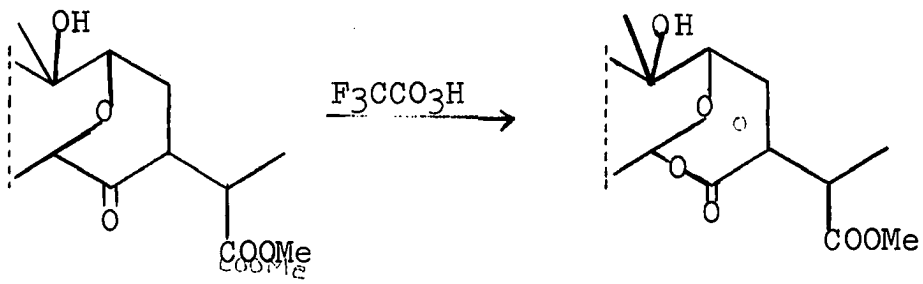
a variety of acidic reagents and conditions. This kind of treatment produced no indication that the ether linkage was responding to efforts to promote its cleavage. The existence of the ether as an epoxide, oxetane, or vinyl ether was considered but was discarded when acid treatment failed to produce any indication of ether cleavage. The alternatives, a tetrahydrofuran or pyran, or perhaps a larger oxygen containing ring emerged as the remaining possibilities.

The ether was recognized as a disecondary ether in later work (4) largely based on N.M.R. and N.M.D.R. spectra. Attempted cleavage with boron trifluoride etherate and acetic anhydride (20) afforded some interesting compounds (4) but no solid indication that the ether had been opened.

Not only does the ether display unusual resistance to chemical attack, but the ether protons also exhibit abnormal chemical shifts in the N.M.R. Signals representing one hydrogen each appear at 2.85 p.p.m. (doublet) and at 3.25 p.p.m. (double doublet). The former chemical shift is unusual for an ether proton, other than oxide or oxetane protons, and probably is indicative of a shielded condition existing in that part of mammosin in which the ether lies.

Since N.M.D.R. data had fixed one ether terminus at the carbon atom adjacent to the gamma position of the lactone, one obvious approach to the cleavage of the ether

involved a Baeyer-Villiger insertion of oxygen at the ketone of isomammosin keto-ester as outlined in partial structures (XXXI) and (XXXII).



(XXXI) (XXXII)

A simple acid hydrolysis would then conveniently open the ether. However, there was no indication the reaction proceeded as desired although it was performed with variations in conditions employing either meta-chloroperbenzoic acid or trifluoroperacetic acid. Since it is strongly suspected that the keto-ester itself exists in part as hemiketal, it is entirely possible that the dry, acidic conditions, required for the success of the Baeyer-Villiger reaction, may have enhanced hemiketal formation in the ester itself, thus dooming the reaction to failure from the start.

Still another approach involved treatment of isomammosin keto-ester with chromous chloride solution. The utility of this reagent in the cleavage of α, β-epoxy ketones (21) and lactones (22), the deacetoxylation of vinylogous keto-acetates (23) and cleavage of vinylogous keto-lactones (24), offered some hope that is might be an

effective reagent for the cleavage of the α-keto-ether function. Consequently, isomammosin keto-ester was treated with freshly prepared aqueous chromous chloride solution (25). The reagent itself is bright blue in color and slowly changes to green as chromous ion is oxidized to chromic ion. No such color change was noted, though the keto-ester was allowed to stand with the reagent several hours under nitrogen; only starting material was isolated. Another abortive effort yielded only starting material after treatment of mammosin with zinc in refluxing acetic acid (26).

Since all efforts to cleave the ether employing the keto-ester as starting material failed, attention was turned to the use of anhydromammosin. The other ether terminus is allylic to the dehydration positions and since allylic ethers are susceptible to cleavage by hydrogenolysis, anhydromammosin mixture was hydrogenated in a variety of solvents and catalysts, but in no case was an alcohol formed. The only product was tetrahydroanhydroisomammosin, a complex mixture of stereoisomers. Treatment of anhydromammosin with sodium in refluxing butanol gave only dihydroanhydromammosin. An attempt was made to isomerize the allylic ether to a vinyl ether (12) using potassium tertiary butoxide in dimethyl sulfoxide, but with no success.

It had been postulated (4) that the reaction of

mammosin with boron trifluoride etherate and acetic anhydride resulted in acylation of the trisubstituted double bond of mammosin as well as acetylation of the tertiary hydroxyl function. There are elemental analyses and some N.M.R. evidence to support this supposition. In order to determine whether the ether linkage itself was susceptible to attack by boron trifluoride and acetic anhydride, the reaction was carried out on the keto-dilactone obtained from cleavage of the double bond of dihydromammosin. Use of this compound prevented both the trisubstituted double bond and tertiary hydroxyl groups from reacting with the reagent since they were no longer present. However, the only product isolated proved to be the enol-acetate of the ketone.

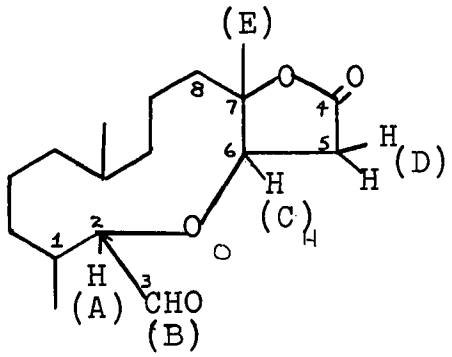
The failure of the preceeding experiments necessitated a fresh approach to the problem of cleaving the ether. Ozonolysis studies had, in general, offered little hope that a solution to the problem could be effected in this manner. Complex mixtures had been encountered in all of these reactions though they were carried out at low temperature and with mild workups. However, in earlier studies (4) a potentially useful compound had been isolated in very poor yield from an ozonolysis of dihydroisomammosin, the catalytic hydrogenation product of mammosin (3). An inherent limitation of dihydroisomammosin itself (and

therefore any compounds derived from it) is its existence as a mixture of stereoisomers. Hydrogenation of the trisubstituted double bond of mammosin may occur from front and back sides of the molecule so that the product is a mixture of stereoisomers, separable only in very poor yield and after arduous chromatography (4). Products obtained from reactions employing dihydroisomammosin as starting material have been difficult to crystallize and purify almost without exception and usually may be better described as solid rather than crystalline.

Such a compound was the aldehyde-lactone obtained by a low temperature ozonolysis of dihydroisomammosin in methanol (4) (see Scheme II). The yield of the compound ranged from 5 to 20 per cent. The N.M.R. data revealed two superimposed secondary methyl doublets centered at 0.91 p.p.m., and a tertiary methyl at 1.39 p.p.m. Two additional hydrogens occurred as a doublet at 2.77 p.p.m. ($J = 9$ c.p.s.); still another hydrogen appeared at 4.02 p.p.m. (broad singlet), and there was a signal for an additional hydrogen at 4.26 p.p.m. (triplet). An aldehyde proton appeared as a sharp singlet at 9.7 p.p.m. The compound, though twice analyzed, could not be fitted to a single formula, and since it occurred in such low yield, its isolation was simply reported (4) and attention turned in other directions.

To us the most reasonable interpretation of the N.M.R.

spectral data of the aldehyde-lactone is suggested by structure (XXXIII) a $C_{17}H_{28}O_4$ compound. The tertiary methyl



(XXXIII)

(E) (1.39 p.p.m.) had suffered a paramagnetic shift from its customary position in the spectrum of dihydroisomammosin at 1.22 p.p.m. The two hydrogens at 2.77 p.p.m. (D) could be rationalized by being alpha to the lactone carbonyl and beta to ether oxygen. In addition scission of the large ring probably would tend to normalize the signals of the ether protons by removing a shielding effect, possibly due to the α , β -unsaturated lactone of dihydroisomammosin, thus shifting ether signals from the 2.8-3.3 p.p.m. region down to the 4.0 p.p.m. area of the spectrum (A, 4.02 p.p.m. and C, 4.26 p.p.m.). In addition, coupling has been demonstrated between the 4.26 p.p.m. position (C) and the 2.77 p.p.m. doublet (D), the latter collapsing to a broad singlet on irradiation at the former, thus providing some confirmatory evidence for the assignment of the ether terminus at C-6.

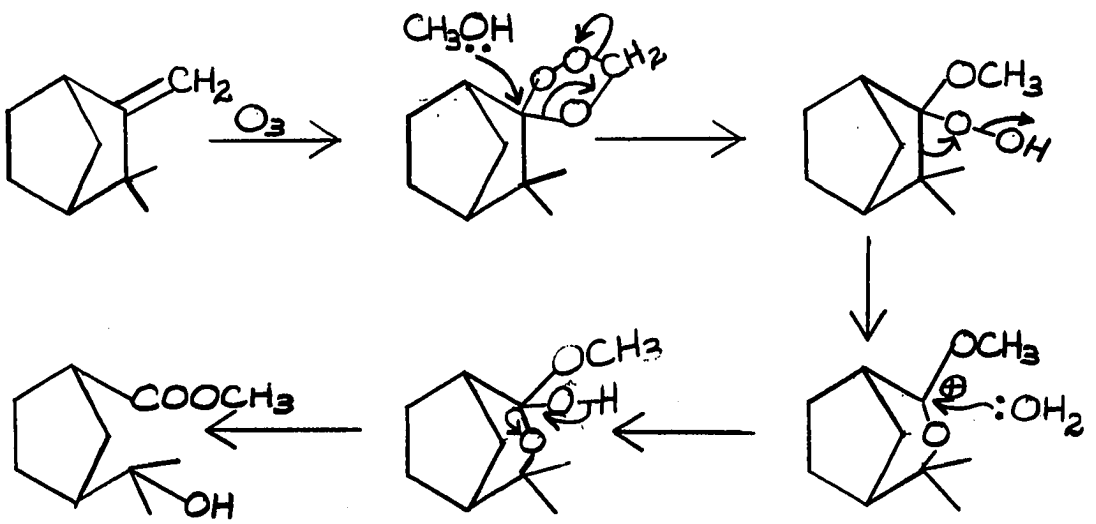
It appeared that the aldehyde-lactone might offer a route into the ether linkage if its identity could be firmly established to be a $C_{17}H_{28}O_4$ compound. The compound was prepared again, dried in vacuum, sealed and shipped for analysis; this third attempt provided a good analysis for $C_{17}H_{28}O_4$, and the compound is currently undergoing mass spectral analysis. In addition it has been found that when this compound is allowed to stand in air, it will gradually lose its solid character. The first two analyses of the aldehyde-lactone contained percentages of oxygen somewhat higher than acceptable, and it is possible that the compound suffered air oxidation in transit prior to analysis.

Aldehyde-lactone (XXXIII) provides confirmatory evidence for placing the tertiary methyl carbinol at C-7 in mammosin (structure B) since this group has been incorporated into the new γ-lactone of (XXXIII). The C-5 carbon atom of mammosin becomes carbon alpha to lactone carbonyl in (XXXIII) and clearly bears two hydrogens thus further ruling out C-5 as a possible ether terminus. Moreover, C-8 is excluded as a site of attachment for the ether by the decoupling of the C-5 protons and C-6 ether proton in (XXXIII).

Several attempts were made to increase the yield of the aldehyde-lactone, but to no avail. It was definitely not a normal ozonolysis product and probably was the result

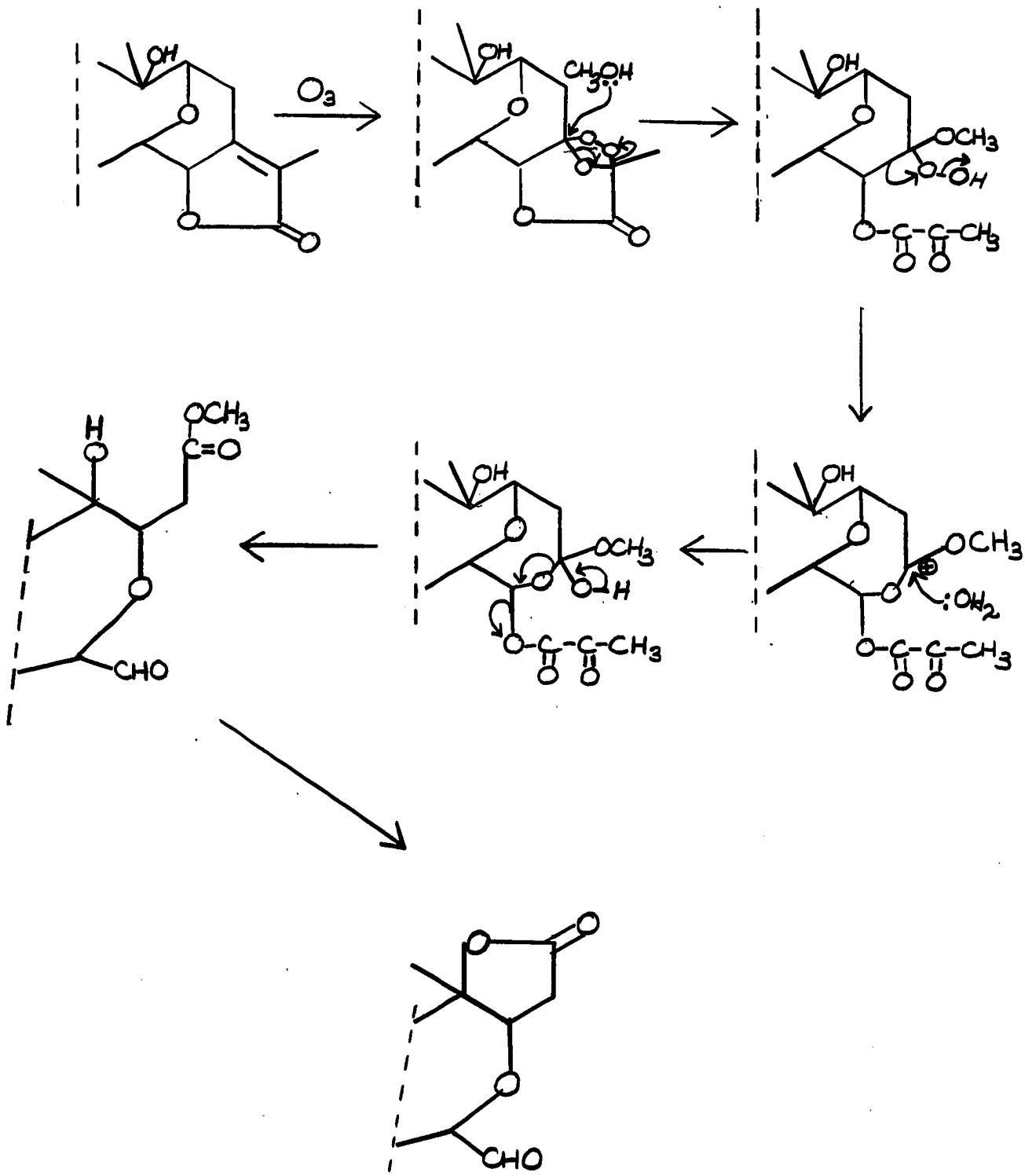
of an ozonide rearrangement. It might then be expected to occur in low yield.

A possible rationale for the production of the aldehyde lactone exists in the work of Bailey (27). Ozonolysis of camphene in methanol gives only a little of the expected camphenilone. The product is instead a methyl ester obtained by rearrangement of the intermediate alkoxy-hydroperoxide shown in Scheme I.



Scheme I

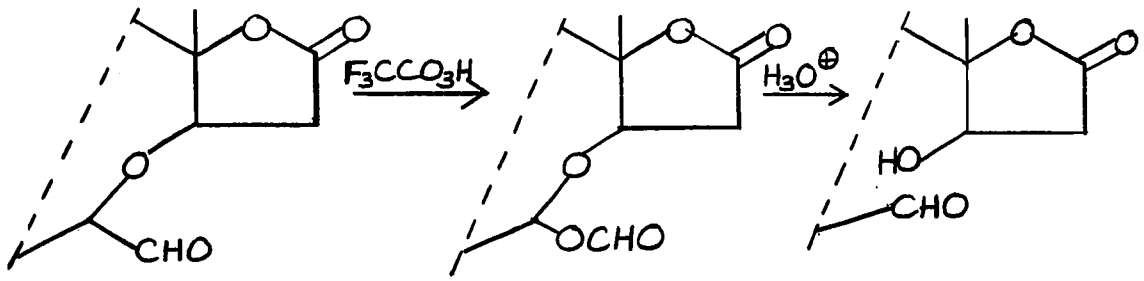
Decomposition of the intermediate ozonide of dihydro-isomammosin with water (or methanol) could produce aldehyde-lactone (XXXIII) by an analogous mechanism (Scheme II). Production of a methyl ester has also been noted in this reaction although it has not been isolated pure. It is possible that methanol could decompose the ozonide thus



Scheme II

leading to a methyl ester which could lactonize spontaneously or perhaps remain in part as methyl ester, the remainder going to lactone.

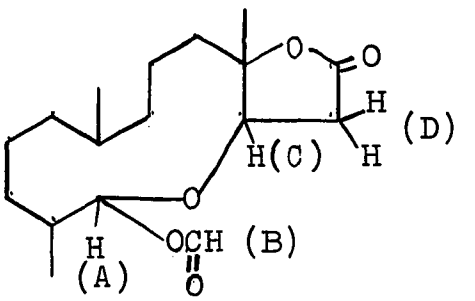
If structure (XXXIII) assigned to the aldehyde-lactone is correct, a Baeyer-Villiger insertion of oxygen would give a formate ester of a hemiacetal which would be easily hydrolyzable in dilute aqueous acid as shown in partial structures (XXXIV), (XXXV) and (XXXVI). Somewhat



(XXXIV) (XXXV) (XXXVI)

surprisingly, the peracid treatment gave exclusive formation of hemiacetal formate; no oxidation of the aldehydic function to an acid was observed. The hemiacetal formate appeared to have undergone partial hydrolysis during the reaction, but this behavior is not surprising. This product distribution was also reflected in the thin layer chromatogram. There were two spots on the silica gel H plate, developed in 8:2 benzene-ethyl acetate. R_f values of 0.52 and 0.09 were observed. The former value is assigned to the hemiacetal formate itself and the latter to its hydrolysis product. No attempt was made to separate

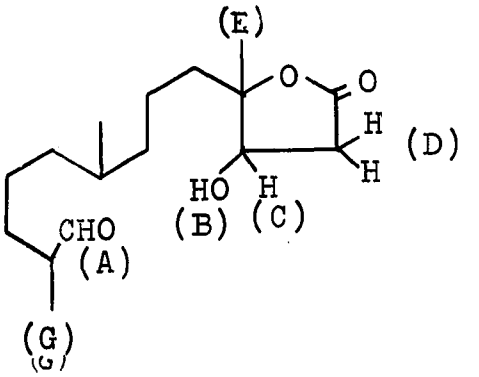
the two products because the hemiacetal formate appeared to be too sensitive to hydrolysis to survive chromatography. The N.M.R. spectrum of the oily product was best interpreted in terms of structure (XXXVII). Hydrogen A



(XXXVII)

appeared at 6.2 p.p.m., and its chemical shift could be expected for hydrogens of this type. Formate hydrogen B gave a signal at 8.12 p.p.m. as a singlet. Ether hydrogen C was centered at 4.32 p.p.m. Hydrogens at D appeared as a familiar doublet centered at 2.78 p.p.m.

Hydrolysis of (XXXVII) was completed in aqueous acidic ethanol. The product was an oil whose N.M.R. spectrum was consistent with structure (XXXVIII). Formate



(XXXVIII)

hydrogen and hydrogen under hemiacetal-formate were absent. New aldehyde hydrogen A was present at 9.6 p.p.m. as a doublet. Hydroxyl hydrogen B appeared as a low mound centered at 4.0 p.p.m., upon which was imposed a double doublet centered at 4.16 p.p.m., due to hydrogen C. Hydrogens alpha to lactone carbonyl occurred as a double doublet centered at 2.7 p.p.m. (J = 11 and 8 c.p.s.). Tertiary methyl E under lactone gave a signal at 1.38 p.p.m. as a singlet. Secondary methyl G, alpha to aldehyde, appeared at 1.08 p.p.m. (J = 7 c.p.s.) having been shifted downfield from its customary position at 0.90 p.p.m. in the spectrum of the original aldehyde lactone itself (XXXIII). An additional secondary methyl (F), somewhat smeared, gave a signal centered at 0.85 p.p.m. The thin layer chromatogram on silica gel H, developed with benzene-ethyl acetate, revealed a single major spot, R_f = 0.11. This value compares favorably with the R_f value of 0.09 assigned to the second product arising from Baeyer-Villiger treatment of aldehyde-lactone (XXXIII), and thought to be the result of partial hydrolysis of the hemiacetal-formate (XXXVII).

It must be pointed out that all compounds in the ether cleavage sequence were mixtures of epimers and non-crystalline. There are no analyses on hemiacetal formate (XXXVII) or ether cleavage product (XXXVIII). The N.M.R. spectra were frequently complicated by attendant doubling

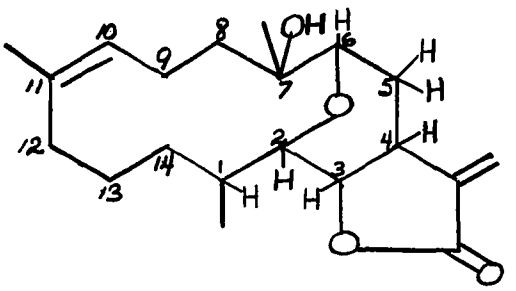
due to the non-identity of frequency in epimeric pairs. The observed chemical shifts, however, occur in those regions of the spectra where they would normally be expected. Coupling constants in some instances cannot be fully discerned. It is stated only that this work, from aldehyde-lactone (XXXIII) to the ether cleavage product (XXXVIII) provides a set of consistent chemical facts which could be predicted and were confirmed by experimentation.

In an attempt to avoid the problems introduced by the use of the epimeric mixture of dihydroisomammosins, an alternate starting material for the ether cleavage sequence was sought. The oxide of the trisubstituted double bond of isomammosin was prepared and one of the two possible epimers obtained pure by chromatography. This compound was ozonized, but the oxide failed to survive the work-up and resulted in the creation of an extremely complex mixture. An attempt was made to open the oxide with acetic acid in anticipation of future cleavage at this site after ozonolysis, but the oxide opened both ways providing yet another mixture.

Part V. Discussion of N.M.D.R. Data

Earlier work depended heavily on nuclear magnetic double resonance spectra for the assignments of certain features of mammosin that were very difficult to place otherwise (4). Some features had to be specified solely by instrumental techniques because there was no chemical

demonstration to confirm or reject the accuracy of an assignment. In the light of new evidence, largely already cited, the structure formerly proposed, which was based in part on unconfirmed spectral interpretations, is modified to structure B.



B

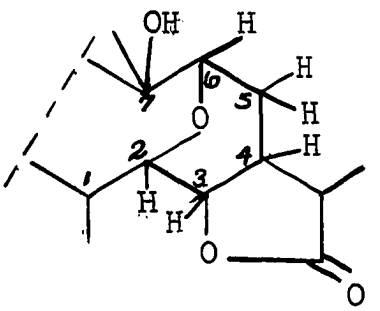
The decoupling data on mammosin itself has shown conclusively that the hydrogen at C-2, the 2.85 p.p.m. doublet is spin coupled to the lactone hydrogen at C-3, the 4.43 p.p.m. double doublet. Irradiation at either site will simplify the splitting at the other, the former hydrogen collapsing to a singlet and the latter to a doublet.

In structure B it is necessary that the coupling constant between the methine hydrogen at C-1 and the ether hydrogen at C-2 be zero. A Dreiding model of structure B shows that a dihedral angle of approximately 90° between the two hydrogens is certainly possible. Ample analogy (28) has been offered in support of this assignment.

The ether proton at C-6 in structure B appears as a

double doublet centered at 3.25 p.p.m. Irradiation at the methylene hydrogens of C-5, 70-80 c.p.s. upfield from C-6 at approximately 120 c.p.s. collapses the double doublet to a singlet. The two hydrogens at C-5 must have slightly different chemical shifts to split the C-6 proton to a double doublet, and they could certainly be expected to show this difference in structure B, with one being slightly more deshielded than the other. In addition the allylic C-4 hydrogen at 3.43 p.p.m. (206 c.p.s.) is coupled not only with the exo-methylene protons at C-16, but also with the methylene hydrogens at C-5, 65-90 c.p.s. upfield from it at approximately 128 c.p.s.

In N.M.D.R. studies of dihydromammosin [partial structure (XXXIX)] , irradiation at the C-4 proton collapses the C-3 lactone hydrogen (double doublet) to a doublet.

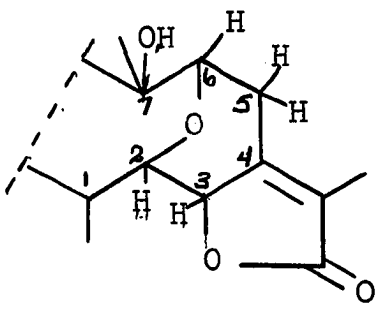


(XXXIX)

The ether proton at C-6 occurs at 3.36 p.p.m. as a double doublet. Irradiation 92 c.p.s. upfield at C-5 reduces the C-6 proton to a broad singlet.

The N.M.R. spectrum of isomammosin shows four protons

in the 3.0 p.p.m. region of the spectrum. Two are ether protons superimposed on an allylic proton signal. The fourth proton absorption is that of the tertiary hydroxyl function [partial structure (XL)].



(XL)

Structure (XL) would appear to demand the existence of five protons in the 3 p.p.m. region of the spectrum--two ether, two allylic and one hydroxyl proton. However, the pi system of the lactone can effectively deshield one of the germinal allylic protons. The six-membered ether ring selectively places the equatorial allylic proton in the deshielding cone of the α,β-unsaturated γ-lactone thus shifting it into the ether region of the spectrum. Unfortunately, the splitting patterns and coupling constants of the ether protons cannot be discerned because of complications imposed by allylic and hydroxyl protons. (All N.M.D.R. data is summarized in Tables I and II.)

In accord with biogenetic considerations, structure B satisfies the isoprene rule and incorporates the skeleton encountered in the tobacco diterpenes (29) and in cembrene (6).

TABLE I

SPIN DECOUPLING RESULTS FOR MAMMOSIN (A) IN $CDCl_3$ (60 Mc.)

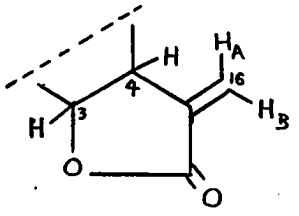
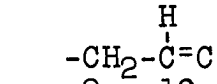
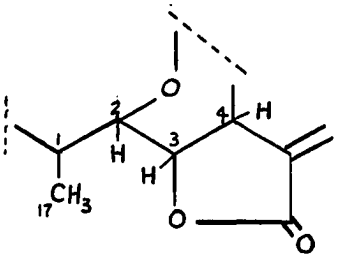
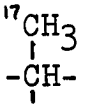
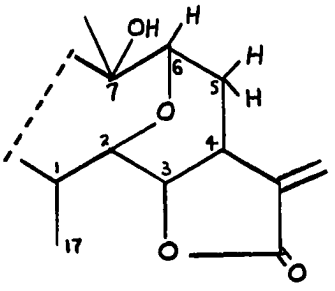
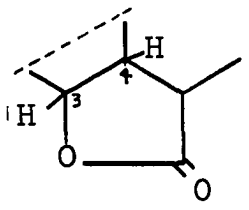
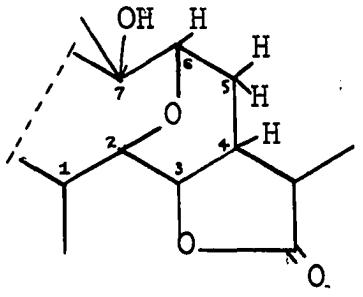
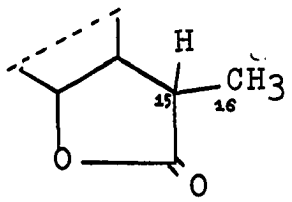
Irradiated	Proton, Assignment	Observed	Multiplicity Change
C-4, 3.43		6.42, C-16B	d to s
C-4, 3.43		5.68, C-16A	d to s
C-9, 2.04		5.07, C-10	t to s
C-3, 4.43		2.85, C-2	d to s
C-2, 2.85		4.43, C-3	dd to d
C-1, 1.83		0.85, C-17	d to s
C-5, 2.0		3.25, C-6	dd to s
C-5, 2.05		3.5, C-4	m-narrows

Table II

SPIN DECOUPLING RESULTS FOR DIHYDROMAMMOSIN
IN CCl_4 (60 Mc.)

Irradiated	Proton, Assignment	Observed	Multiplicity Change
C-4, 2.60		4.32, C-3	dd to d
C-5, 1.80		3.36, C-6	dd to broad s
C-15, 2.6		1.27, C-16	d to s

EXPERIMENTAL

All melting points are uncorrected. All solvents were redistilled before use. Methylene chloride was passed through Linde molecular sieve 4A and tetrahydrofuran was distilled from lithium aluminum hydride. Diazomethane was prepared from EXR-101 (N,N'-Dinitroso-N, N'-Dimethyl Terephthalamide from DuPont). Chromatographic supports were Merck (Darmstadt) alumina (active neutral), Florisil (100/200 mesh), and Mallinckrodt's silicic acid (powder).

Gas chromatographic analysis was conducted on a Micro-Tek, BC-1600 having a hydrogen flame detector. Thin layer chromatography was performed on 5 X 20 cm. glass plates coated with Merck (Darmstadt) silica gel H, or, in the chromatogram of anhydromammosin in particular, on silica gel H - silver nitrate coated plates (15). The plates were placed in iodine vapor for visualization of the chromatogram. Silver nitrate coated plates were first sprayed with Chromatospray bromcresol green before exposure to iodine vapor. The infrared spectra were produced on Perkin-Elmer, Model 21 and Beckman IR-8 spectrophotometers and run either in chloroform solution or as potassium bromide

pellets. N.M.R. spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as internal reference. Samples were run in varying concentrations in deuteriochloroform solution. Chemical shifts are reported in δ-values (p.p.m. from TMS) and are followed by the multiplicity of the signals and corresponding coupling constants. The multiplicity is denoted by the symbols: s, singlet; d, doublet; t, triplet; dd, double doublet; and m, multiplet.

Alfred Bernhardt Laboratories, Mulheim, Germany, carried out the analyses. The N.M.R. double resonance experiments were kindly performed by Dr. Pat W. Flannigan, Continental Oil Company, Ponca City, Oklahoma. Mr. David Cochran separated the dehydration products of mammosin.

Mammosin. Mammosin was isolated from the pentane extracts of Eunicea mammosa obtained near Turtle Rock, Bimini, Bahamas (1). Chromatography of the crude extracts on Florisil afforded a crystalline solid, which, after several recrystallizations from hexane-benzene (3:1) gave an analytical sample, $C_{20}H_{30}O_4$, m.p. 154-155.5° and $[\alpha]_D^{27.3}$ -89.4° (c = 0.75, $CHCl_3$) (3). Active hydrogen and C-methyl analyses indicated one active hydrogen and 2.2 C-methyl groups in mammosin (3). The molecular weight of 334 was confirmed by mass spectral analysis. In the ultraviolet spectrum mammosin displayed strong end absorption which had a shoulder with a maximum between 200

and 220 mu (4). The infrared spectrum ($CHCl_3$) showed significant bands at 3623 and 3497 cm^{-1} (hydroxyl), 1765 cm^{-1} (γ-lactone), and 1664 cm^{-1} (double bond). In the N.M.R. spectrum (deuteriochloroform) methyl groups were present at 0.85 d (J = 7 c.p.s.), 1.15 s, and 1.52 s. Olefinic protons appeared at 5.07 t, 5.70 d (J = 3 c.p.s.) and 6.40 d (J = 3 c.p.s.). The hydrogen on carbon bonded to lactone oxygen occurred at 4.43 dd (J = 10 c.p.s. and 10 c.p.s.). Two ether protons gave signals at 3.25 dd (J = 10.5 c.p.s. and 10.5 c.p.s.) and at 2.85 d (J = 10 c.p.s.). A proton allylic to the exo-methylene group appeared as a multiplet at 3.5 p.p.m.

Preparation of Mammosin Hydroxy Acid. To 575.4 mg. (1.7 mmoles) of mammosin in 25 ml. of distilled water was added 0.17 g. (3 mmoles) of potassium hydroxide. The mixture was refluxed with stirring until all mammosin had gone into solution (approximately one hour). The solution was then cooled to 0° in an ice bath. Five ml. of ethanol were added to the cold solution, and the stirred mixture was acidified dropwise with cold, dilute sulfuric acid to pH 6-7 smoothly precipitating mammosin hydroxy acid. The solid was filtered and washed with a few ml. of distilled water and dried in air to give 540.6 mg. (90%) of white, amorphous powder, m.p. 155-156°.

Anal. Calculated for $C_{20}H_{32}O_5$: C, 68.15; H, 9.15; O, 22.70. Found: C, 67.90; H, 9.35; O, 22.68.

Attempts to recrystallize the acid invariably resulted in partial reclosure to the lactone. Poor solubility prevented determination of the N.M.R. spectrum. Significant infrared absorption (KBr) occurred at 3480 cm^{-1} (carboxyl and hydroxyl), 1675 cm^{-1} (α , β-unsaturated carboxyl), and 162- cm^{-1} (double bond); γ-lactone absorption was absent.

Mammosin from Mammosin Hydroxy Acid. Dry mammosin hydroxy acid (115.1 mg.) was slowly heated under nitrogen to its melting point in an oil bath. At 158° a few bubbles could be seen slowly coming off the liquid. Heating was continued up to 173° for a total elapsed heating time of 30 minutes. The sample was allowed to cool to room temperature and then triturated with hexane, affording 77.8 mg. of crude material, m.p. 142-155°. The infrared spectrum of the product was identical with that of mammosin itself. Recrystallization from hexane - benzene (8:2) gave pure mammosin, m.p. 156°. There was no depression of the mixed melting point.

Preparation of the Methyl Ester of Mammosin Hydroxy Acid. Excess ethereal diazomethane solution was added to 285 mg. (0.8 mmole) of mammosin hydroxy acid and the mixture placed in the freezer at -25° for twenty minutes. All hydroxy acid was in solution at the end of this time, and a faint yellow color of excess diazomethane was visible. The ethereal solution was evaporated on a

rotary distillation unit without heating to give 298.5 mg. of solid, amorphous product. The solid was dissolved in benzene and filtered through Florisil with 50:50 benzene - ethyl acetate. The solvent was removed with gentle heat affording 233.7 mg. of solid which was triturated with hexane giving 115.5 mg. of amorphous mammosin hydroxy ester, m.p. 73-74°.

The infrared spectrum (KBr) of the product revealed significant absorption at 3540-3100 cm^{-1} (hydroxyl), 1720 cm^{-1} (α , β -unsaturated ester), 1625 cm^{-1} (double bond) and 1138 cm^{-1} (methyl ester). In the methyl region of the N.M.R. spectrum peaks appeared at 0.87 d, 1.12 s, and 1.66 s; methoxyl appeared at 3.79 s. Vinyl hydrogens gave signals at 5.18 t, 5.9 s and 6.43 s. Ether hydrogens were present at 3.6 dd and 3.25 d. Hydrogen under secondary hydroxyl appeared at 3.9 (broad). Thin layer chromatography of the hydroxy ester on silica gel H with 8:2 benzene - ethyl acetate showed a single spot, R_f = 0.20.

Preparation of Mammosin Methyl Ether. To 0.56 g. (1.7 mmoles) of mammosin in 15 ml. of absolute methanol was added 0.11 g. (2 mmoles) of powdered sodium methoxide dissolved in 15 ml. of methanol. After standing at room temperature overnight the contents of the flask were brought briefly to a boil and then cooled to room temperature.

A few ml. of distilled water were added, and the solution was acidified to pH-4 by dropwise addition of dilute sulfuric acid. Gummy material which had precipitated at this point was removed from the flask with a glass stirring rod. Further cooling of the solution in an ice bath accompanied by agitation with a stirring rod resulted in further precipitation of 166 mg. of yellowish white, solid material. This solid was taken up in ether, washed with dilute aqueous bicarbonate and water, then dried over sodium sulfate. The ether solution was filtered through a Florisil column having a thin layer of Norite at the top. The material coming off the column was colorless but non-crystalline. The product was passed through Florisil again affording 125.6 mg. of solid, m.p. 95-96°. Two recrystallizations from hexane left 57.6 mg. of crystalline solid m.p. 100-102.5°.

Anal. Calculated for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35; O, 21.83. Found: C, 69.36; H, 9.24; O, 21.64.

Thin layer chromatography of the methyl ether on silica gel H developing with benzene - ethyl acetate (8:2) showed a single spot, R_f = 0.13. The infrared spectrum (KBr) revealed bands at 3540-3420 cm^{-1} (hydroxyl), and 1765 cm^{-1} (γ-lactone). N.M.R. signals characteristic of methyl groups appeared at 0.85 d, 1.13 s, 1.62 s, and 3.36 s. A single vinyl proton signal was present at 5.1 t, and the lactone hydrogen occurred at 4.3 dd. A doublet,

attributed to the two new ether hydrogens beta to lactone carbonyl, appeared at 3.72 p.p.m. (J = 2 c.p.s.). The original ether bands appeared from 2.9 to 3.4, partially covered by the methoxyl signal. The downfield doublets characteristic of the conjugated exo-methylenic hydrogens of mammosin itself were absent.

Preparation of Isomammosin Keto-Ester. Isomammosin (438 mg.) was dissolved in 5 ml. of 1 N methanolic potassium hydroxide and refluxed overnight. Water was added and the clear solution cooled in an ice bath. Dropwise acidification with 5% aqueous sulfuric acid to pH 4 precipitated a white, tacky solid which was filtered, washed and dried, affording 307 mg. of amorphous white powder. An additional 100 mg. of the same material was extracted from the aqueous acidic solution with ether.

The infrared spectrum showed bands at 3600-3300 cm^{-1} (hydroxyl and carboxyl) and 1720 cm^{-1} (ketone and carboxyl).

Ethereal diazomethane solution was added dropwise to 407 mg. (1.16 mmoles) of isomammosin keto-acid. Immediate evolution of nitrogen occurred. Addition of diazomethane was continued until evolution of gas ceased and the solution was slightly yellow in color. After standing one-half hour at room temperature, the ether was removed on the rotary evaporator with slight heating to afford a viscous oil which could not be crystallized.

Thin layer chromatography of the oil on silica gel H with 8:2 benzene - ethyl acetate showed a single elongated spot (R_f = 0.36) with faint streaking from the origin.

Chromatography of the oil on 5 g of Florisil and elution of the column with 2% ethyl acetate in benzene afforded 350 mg. of the keto-ester as an oil. Attempts to crystallize the oil failed as did efforts to form 2,4-dinitrophenylhydrazone and oxime derivatives. Thin layer chromatography of the product on silica gel H with benzene - ethyl acetate (8:2) showed only a single elongated spot, R_f = 0.36. (The analysis of the keto-ester was performed on a small amount of crystalline material recovered from a reaction employing the keto-ester as starting material.)

Anal. Calculated for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35. Found: C, 68.50; H, 9.15.

The infrared spectrum ($CHCl_3$) of the oil displayed bands at 3610 (sharp) and 3500 cm^{-1} (hydroxyl), 1725 cm^{-1} (ketone and ester), and 1165 cm^{-1} (methyl ester). The N.M.R. spectrum revealed methyl absorption at 0.76 d, 1.15-1.26 (secondary methyl superimposed on tertiary methyl), 1.54 s and 3.7 s. Vinyl hydrogen appeared as a broad peak at 5.2.

Treatment of Isomammosin Keto Ester with Sodium Hydride in Diglyme. To a slurry of 2 g. of sodium hydride (a 51% oil dispersion previously washed with hexane) in

4 ml. of anhydrous diglyme was added 215.2 mg. (5.9 mmoles) of isomammosin keto-ester dissolved in 4 ml. of anhydrous diglyme. The mixture was stirred under nitrogen for 24 hours at room temperature. The slurry was then chilled in an ice bath to 0° and water added dropwise to hydrolyze excess hydride. An initial extraction of the aqueous mixture with hexane served to remove residual mineral oil. A second extraction with ether followed by several water washes of the organic phase and thorough drying over sodium sulfate gave 146.5 mg. of neutral material. The thin layer chromatogram showed a single major spot with a few lighter spots trailing it. The product was chromatographed on 5 g. of Florisil and eluted with 3% ethyl acetate in benzene affording 23.6 mg. of isomammosin. The product was identified by melting point (125°) and infrared spectrum. There was no depression of the mixed melting point on admixture of authentic isomammosin.

Ozonolysis of Anhydrodihydroisomammosin. Isolation of Pyruvic Acid. A stream of ozone was passed through a solution of 374 mg. of anhydrodihydroisomammosin dissolved in 50 ml. of methanol at -70°. Ozonolysis was continued until the blue color of excess ozone had persisted approximately five minutes. The reaction mixture was then worked up reductively by hydrogenation over palladium on charcoal. The solution was filtered from the catalyst, which was washed well with methanol, and the solvent gently removed

on the rotary evaporator until only a few milliliters of solution were left. The contents of the flask were transferred to a three-neck flask, and steam distillation commenced. After collection of approximately 200 ml. of distillate, the distillation flask was cooled to room temperature and its contents extracted with ether. The residual aqueous solution was then treated with 2.4-dinitrophenyhydrazone reagent and allowed to stand in the refrigerator. A yellow precipitate formed and settled to the bottom of the flask. Filtration afforded 28.8 mg. of pyruvic acid 2,4-dinitrophenylhydrazone, m.p. 214.5-215°. The infrared spectrum (KBr) was identical with that of authentic pyruvic acid 2,4-dinitrophenylhydrazone, and the mixed melting point was undepressed.

Preparation of Anhydrodihydromammosin. A solution of 160.8 mg. (0.48 mmole) of dihydromammosin in 2 ml. of pyridine was chilled in a mixture of dry ice, acetone, and water to -45°. Thionyl chloride (1.5 ml.) was added and the mixture stirred one hour at -45°. The contents of the flask were then poured over ice and extracted well with ether. The ether layer was thoroughly washed with dilute sulfuric acid followed by dilute bicarbonate and distilled water. After drying over sodium sulfate the ether was removed leaving a clear oil which was filtered through Florisil using benzene as the eluant. Analysis of the oily product by gas chromatography on 5% SE-30 at a column

temperature of 230° showed two peaks, slightly overlapping with a retention time of approximately one minute.

Significant infrared absorption occurred at 1770 cm^{-1} (γ-lactone), 1642 cm^{-1} (double bond), and 905 cm^{-1} (exo-methylene). The N.M.R. spectrum clearly showed the product to be a mixture of exo and endo isomers. The spectrum revealed new exo-methylene absorption at 4.95 s having intensity less than two hydrogens; the vinyl hydrogen of the trisubstituted double bond was present at 5.25 t. In the methyl region, part of a new vinyl methyl was visible at 1.7 s as well as original vinyl methyl at 1.6 s. Secondary methyl signals occurred at 1.29 d (J = 6 c.p.s.) and at 0.95 d (J = 6 c.p.s.). The tertiary methyl present in dihydromammosin itself was absent. A single hydrogen at the gamma position of the lactone was visible at 4.37 dd. Additional bands were centered at 3.97 t and are attributed to hydrogen on carbon bearing ether oxygen; a second ether hydrogen appeared at 3.28 as a doublet with fine splitting at the tops of the peaks (J = 10 c.p.s.). These bands apparently are those originally found at 3.36 and 3.1. (Since this product is a mixture of isomers, the fine splitting was ascribed to slight differences in chemical shifts in the two isomers.)

Florisil chromatography of 97 mg. of the oily anhydro mixture using benzene as the eluant separated the products into distinct fractions each containing a majority

of one product. Gas chromatographic analysis (5% SE-30 at 235°) of one fraction containing 59 mg. showed two peaks in a 10:90 product distribution. This fraction was rechromatographed on 6 g. of Florisil initially employing hexane as the eluant. By gradually increasing the polarity of the solvent to 35% benzene in hexane, 29.7 mg. (m.p. 94-96°) of recrystallized exo-anhydrodihydromammosin was obtained.

Anal. Calculated for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.91; H, 9.46.

Only a few milligrams of the endo dehydration product were obtained by laborious chromatography. The analysis of this compound, m.p. 132-135°, was unacceptable, being 1.3% low in carbon, 0.73% high in oxygen and 0.08% high in hydrogen.

Treatment of Mammosin with Methane Sulfonyl Chloride in Pyridine. A solution of 500 mg. (1.37 mmoles) of mammosin in 3 ml. of dry pyridine was chilled to -20° in the freezer. One gram of methane sulfonyl chloride was added to the solution which was allowed to warm to room temperature and stand for 43 hours. The dark brown mixture was poured over ice and extracted well with ether. The combined ether extracts were washed first with dilute aqueous hydrochloric acid, then with dilute sodium bicarbonate and distilled water, and finally thoroughly dried over sodium sulfate. Removal of the solvent afforded a yellow

gum which crystallized on trituration with hexane. The thin layer chromatogram of the crude product (silica gel H, 8:2 benzene - ethyl acetate) showed four spots having R_f values of 0.67, 0.49, 0.40, and 0.35. The latter two were minor components.

The solid was dissolved in benzene and chromatographed on Florisil. Elution with benzene gave 53.4 mg. of clear oil identical in infrared spectrum to anhydromammosin mixture. Continued eluction with benzene - ethyl acetate (99:1) afforded 110.4 mg. of crystalline solid which was recrystallized from hexane - benzene prqviding an analytical sample, m.p. 175-176°.

Anal. Calculated for $C_{21}H_{32}O_7S$: C, 58.86; H, 7.31. Found: C, 59.00; H, 7.53.

The N.M.R. spectrum revealed vinyl hydrogens at 6.44 d, 5.68 d and 5.08. Ether hydrogens occurred at 3.48 dd and 2.89 d (J = 10 c.p.s.). In the methyl region signals appeared at 0.90 s, and at 1.45 s and 1.56 s. An additional methyl group gave a signal at 4.26 s. The infrared spectrum (KBr) displayed significant bands at 1771 cm^{-1} (α , β -unsaturated γ-lactone), and 1666 cm^{-1} (double bond).

Treatment of Mammosin with Phosphorus Oxychloride in Dimethylformamide. Preparation of Mammosin Formate. A solution of 604 mg. (1.8 mmoles) of mammosin in 5 ml. of dimethylformamide was chilled in the freezer to -25°. Two

ml. of phosphorus oxychloride were added dropwise to the solution and the mixture allowed to warm to room temperature. The solution was poured over ice, precipitating a white solid immediately. The solid was taken up in ether, washed well with small portions of distilled water, and then dried over sodium sulfate. Removal of the ether afforded a crystalline solid which was recrystallized from hexane - ethyl acetate to give 482.4 mg. of white needles, m.p. 129.5-131°.

Anal. Calculated for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34. Found: C, 69.76; H, 8.33.

The infrared spectrum (KBr) revealed bands at 1769 cm^{-1} (α , β -unsaturated γ-lactone), 1725 cm^{-1} (formate ester), 1669 cm^{-1} (double bond) and 1180 cm^{-1} (formate). In the methyl region of the N.M.R. spectrum absorption occurred at 0.88 d and 1.51 s (methyl under formate superimposed on vinyl methyl). Ether hydrogens were present at 2.89 d and 3.42 dd. Hydrogen at the gamma position of the lactone absorbed at 4.47 dd, and vinyl hydrogens gave signals at 5.08 t, 5.7 d, and 6.46 d. A lone formate hydrogen appeared at 8.11 s.

The thin layer chromatogram on silica gel H, eluting with chloroform - hexane (8:2), revealed a single spot, R_f = 0.72.

Hydrolysis of Mammosin Formate. Mammosin formate (107 mg.) in 6 ml. of tetrahydrofuran and 2 ml. of water

was stirred overnight at room temperature with a pinch of sodium carbonate. The solution was then flooded with water, extracted well with ether, washed with distilled water, and thoroughly dried over sodium sulfate. Removal of the solvent and trituration of the product with hexane afforded 71 mg. of crystalline mammosin having infrared spectrum and melting point (156°) identical to that of authentic mammosin. There was no depression of the mixed melting point.

Treatment of Isomammosin with Phosphorus Oxychloride in Dimethylformamide. Preparation of Isomammosin Formate. A solution of 184 mg. (0.55 mmole) of mammosin in 3 ml. of dimethyl formamide (dried over CaH) was chilled to -25°. Phosphorus oxychloride (1.5 ml.) was added dropwise to the chilled solution and the mixture allowed to warm to room temperature. The mixture was then poured over ice and the organic product taken up in ether; the ether phase was washed well with distilled water and dried over sodium sulfate. Removal of the solvent left a crystalline solid which was recrystallized from hexane - ethyl acetate affording 134 mg. of needles, m.p. 143-144.5°.

Anal. Calculated for $C_{21}H_{30}O_5$: C, 69.59; H, 8.34; O, 22.07. Found: C, 69.67; H, 8.35; O, 21.82.

Methyl groups were apparent in the N.M.R. spectrum at 0.96 d, 1.59 s, (tertiary and vinyl methyls superimposed) and 1.92 s. Ether hydrogens appeared between 2.9 and 3.5

and the hydrogen atom at the gamma position of the lactone occurred at 4.58 d. A single vinyl hydrogen was present at 5.12 t and formate hydrogen gave a signal at 8.12 s. The infrared spectrum ($CHCl_3$) revealed absorption bands at 1755 cm^{-1} (α,β -unsaturated γ-lactone), 1725 cm^{-1} (formate ester), 1685 cm^{-1} (double bond), and 1180 cm^{-1} (formate).

The thin layer chromatogram on silica gel H using 8:2 chloroform - hexane as eluant showed a single spot, R_f = 0.69.

Treatment of Anhydrodihydromammosin with Sodium Hydride in Dimethylsulfoxide. Sodium hydride (0.5 g.) was added to 20 ml. of dimethylsulfoxide and the slurry stirred under nitrogen 1 hour at 35°. Anhydrodihydromammosin (256 mg.) dissolved in 5 ml. of dimethylsulfoxide was added dropwise over 5 minutes to the slurry under a slow stream of nitrogen. Immediately after addition the mixture turned yellow, then reddish brown, and finally after 3 1/2 hours purple. The contents of the reaction flask were cooled in an ice bath and water added to destroy excess hydride. The organic product was extracted several times with ether and the combined ether extracts thoroughly washed with water. After drying over sodium sulfate the ether was removed leaving 153.8 mg. of neutral material. Recrystallization from hexane - benzene provided an analytical sample, m.p. 139.5-140.5°.

Anal. Calculated for $C_{22}H_{36}O_4S$: C, 66.64; H, 9.15. Found: C, 66.34; H, 9.43.

The N.M.R. spectrum showed methyl signals at 0.92 d, 1.11 d, 1.6 s and 2.7 s. Vinyl hydrogens were present at 4.95 (two protons) and 5.3 (broad) (one proton). Hydrogen at the gamma position of the original lactone appeared at 4.18 dd. The infrared spectrum (KBr) showed absorption bands 3600-3100 cm^{-1} (hydroxyl), 1645 cm^{-1} (double bond) and a shoulder at 1020 cm^{-1} [sulfoxide is shifted (31) from its customary 1060-1040 cm^{-1} position by virtue of being hydrogen bonded]. A band at 895 cm^{-1} indicated the presence of an exo-methylene. No carbonyl absorption appeared.

The thin layer chromatogram on silica gel H with ethyl acetate as solvent showed a single spot, R_f = 0.39.

Treatment of Mammosin with Trichloroacetylisocyanate. Preparation of Mammosin Carbamate. Mammosin (175.5 mg.) was gently heated on the steam bath with 1.5 ml. of trichloroacetylisocyanate until dissolution occurred (about 5 minutes). The reaction mixture was allowed to cool to room temperature and was then flooded with hexane and the contents heated in hexane. The flask was then cooled in a dry ice - acetone bath and its contents rubbed with a glass stirring rod. A white solid precipitated which was filtered off and washed with hexane. The product was chromatographed on Florisil using benzene. A small

amount of solid product emerged from the column in several fractions, and it was necessary to increase the eluting power of the solvent by addition of ethyl acetate up to 5% ethyl acetate in benzene. Recrystallization of combined solids in benzene afforded 179.5 mg. of white crystals which decomposed at 170-180°.

Anal. Calculated for $C_{21}H_{31}NO_5$; C, 66.82; H, 8.28 N, 3.71. Found: C, 67.06; H, 8.42; N, 3.92.

The N.M.R. spectrum showed methyl groups at 0.88 d, 1.46 s. and 1.53 s. Ether protons were visible at 2.89 d (J = 10 c.p.s.), and 3.39 dd. A signal centered at 4.45 dd represented hydrogen at the gamma position of the lactone. Hydrogens attached to nitrogen occurred at 4.86 s (broad). Signals at 5.06 t, 5.68 d and 6.42 d indicated vinyl hydrogens. The infrared spectrum (KBr) exhibited absorption bands at 3430-3200 cm^{-1} (amide), 1762 cm^{-1} (α, β-unsaturated γ-lactone), 1725 cm^{-1} (carbamate), 1660 cm^{-1} (double bond), and 1660 and 1620 cm^{-1} ("amide II band").

Thin layer chromatography of the product on silica gel H with 1:1 benzene - ethyl acetate showed a single spot, R_f = 0.34.

Dehydration of Mammosin. Preparation of Anhydromammosin Mixture. Mammosin (1.1 g.) dissolved in 5 ml. of pyridine was chilled to -25° and treated with 2 ml. of thionyl chloride. Immediate precipitation of pyridine

hydrochloride occurred. The mixture was kept at -25° for twenty minutes, then water was added dropwise and the organic product extracted with ether. The ether phase was washed several times with small portions of dilute hydrochloric acid, and finally with distilled water, followed by thorough drying over sodium sulfate. Removal of the solvent afforded an oil which was twice passed through alumina columns with hexane containing a trace of ethyl acetate. The product (860.8 mg.) remained oily and displayed two peaks of nearly identical retention times on gas chromatography (5% SE-30 at 250° with helium as carrier gas). The oily product was stirred with a spatula and placed in a vacuum oven for several hours in order to remove traces of solvent. The oil slowly solidified to a gummy solid whose thin layer chromatogram displayed two overlapping spots, R_f = 0.56 and 0.51 (silica gel H developed with benzene).

Anal. Calculated for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.55; H, 8.56.

In the N.M.R. spectrum methyl groups were visible at 0.92 d and 1.52 s. A partial vinyl methyl was present at 1.67 s. Lactone hydrogen occurred at 4.38 dd, and olefinic hydrogens at 4.89 s (intensity less than that of two hydrogens required by the exo isomer), 5.1 t, 5.53 d, 5.6 (broad peak due to new endo isomer and partially obscured by 5.53 doublet) and 6.29 d. Ether

hydrogens appeared at 2.8 and 3.8. The infrared spectrum ($CHCl_3$) revealed absorption bands at 1768 cm^{-1} (α , β - unsaturated γ-lactone), 1668 cm^{-1} and 1645 cm^{-1} (double bonds).

Treatment of Anhydromammosin Mixture with Potassium Tertiary Butoxide in Diglyme. Anhydromammosin mixture (225.1 mg.) was dissolved in 5 ml. of dry diglyme; to this solution was added 0.16 g. of potassium tertiary butoxide, and the solution was refluxed for an hour. Addition of water produced no precipitate at this point, thus requiring the solution to be acidified with dilute sulfuric acid to pH 1. Extraction with ether followed by thorough washing of the organic phase with distilled water and drying over sodium sulfate gave a product whose infrared spectrum was identical with that of starting material.

Separation of Exo and Endo Isomers of Anhydromammosin Mixture. Anhydromammosin (680 mg.) was chromatographed on 70 g. of silicic acid impregnated with silver nitrate (25% by weight). Elution of the column with benzene (40 ml. fractions taken every 30 minutes) afforded 184 mg. of endo-anhydromammosin in fractions 7-11. Continued elution of the column gave 195 mg. of exo-anhydromammosin in fractions 18-23. Each isomer had to be filtered through Florisil (using benzene as eluant) in order to remove a slight yellowish color.

Exo-anhydromammosin was obtained as a crystalline

solid, m.p. 131-132°, showing sifnificant infrared absorption at 1768 cm^{-1} (α , β-unsaturated γ-lactone), 1666 cm^{-1} (exo-methylenic double bond conjugated with lactone carbonyl), 1640 cm^{-1} (exo-methylenic double bond created by dehydration) and 902 cm^{-1} (exo-methylene double bond). Methyl groups in the N.M.R. spectrum were present at 0.92 d and 1.54 s. Olefinic hydrogens appeared at 4.92 s (two exo-methylenic hydrogens created by dehydration), 5.2 (broad) and 5.58 d and 6.36 d. Ether hydrogens gave signals at 3.05 d (J = 10 c.p.s. with the peaks very slightly split at the top), and at 3.8 dd. Hydrogen at the gamma position of the lactone occurred at 4.47 dd and hydrogen allylic to the exo-methylenic unsaturation of the lactone appeared at 3.35 m. The thin layer chromatogram of this isomer on silica gel H using 8:2 benzene - ethyl acetate showed a single spot R_f = 0.65.

Endo-anhydromammosin was an oily compound which turned yellow on standing in air. The infrared spectrum ($CHCl_3$) of a sample of the compound (which had been filtered through Florisil with benzene to remove color) showed significant absorption bands at 1770 cm^{-1}, (α , β - unsaturated γ-lactone), and 1670 cm^{-1} (exo-methylenic double bond conjugated with lactone carbonyl.) The N.M.R. spectrum showed methyl groups at 0.85 d, 1.55 s and 1.70 s (new vinyl methyl created by dehydration). Olefinic hydrogens appeared at 5.07 (broad), 5.57 d, superimposed on

a broad peak centered at 5.7 (new vinyl hydrogen created by dehydration and 6.4 d. Hydrogen at the gamma position of the lactone showed absorption at 4.45 dd. Ether hydrogens appeared at 3.93 dd and 2.74 d (J = 10 c.p.s.). Hydrogen allylic to the exo-methylenic unsaturation of the lactone occurred as a broad mound centered at 3.4 m. The thin layer chromatogram of the endo isomer on silica gel H with 8:2 benzene - ethyl acetate showed a single spot, R_f = 0.63.

Ozonolysis of Anhydrodihydroisomammosin. Isolation of Formaldehyde as Dimedone Derivative. Ozone was passed through a solution of 825.3 mg. (2.58 mmoles) of anhydrodihydroisomammosin dissolved in methanol at -70°. After the blue tinge of excess ozone had persisted for one minute, the solution was swept free of ozone and then hydrogenated over platinum oxide. After the solution had been filtered from the catalyst, the solvent was gently distilled into aqueous dimedone solution which was allowed to stand for several days. White needles of formaldehyde dimedone derivative (162.6 mg.) were slowly deposited, m.p. 188-189°. The yield was 21 per cent.

Treatment of Dihydromammosin with Phosphorus Oxychloride in Pyridine. Dihydromammosin (92 mg.) was dissolved in 3 ml. of pyridine and chilled to -25°. Two ml. of phosphorus oxychloride was added and the temperature maintained at -25° for 6 hours. Water was added to the reaction mixture and the organic product extracted

with ether. The organic phase was washed with small portions of dilute hydrochloric acid, then distilled water, and dried over sodium sulfate. Distillation of the solvent afforded an oil which crystallized on trituration with hexane and proved to be starting material m.p. 149-153°. The infrared spectrum (KBr) was identical to that of authentic dihydromammosin.

Treatment of Isomammosin Keto Ester with Chromous Chloride. Freshly prepared chromous chloride solution (4.6 ml. of approximately 0.8 N solution) was added by syringe to a stirred solution of 349.5 mg. (1.0 mmole) of isomammosin keto-ester in 10 ml. of acetic acid at room temperature. A slow stream of nitrogen was bubbled continuously through the solution for 11 hours. No change of color was noticed during the course of the treatment, the solution remaining bluish violet. Water was added and the product taken up in ether. The ether phase was washed well with dilute sodium bicarbonate, then with distilled water, and finally thoroughly dried over sodium sulfate. Removal of the solvent afforded 313.7 mg. of oil. The oil gave a single spot on thin layer chromatography which had an R_f value identical to that of starting material. The infrared spectrum ($CHCl_3$) of the oil was also identical to that of starting material.

Treatment of Anhydromammosin Mixture with Sodium in n-Butanol. Preparation of Anhydrodihydromammosin.

Anhydromammosin mixture (374 mg.) was dissolved in 50 ml. of refluxing normal butyl alcohol. Finely cut sodium (2 g.), previously washed with hexane, was added quickly to the refluxing solution and the mixture stirred 1 1/2 hours. The solution was cooled, distilled water was added, and butanol and water were removed on the rotary evaporator. When almost all butanol had been removed, the solution was cooled and extracted with ether. The aqueous phase remaining was acidified with concentrated hydrochloric acid to pH 1. The organics were taken up in ether, and the organic phase washed with dilute sodium carbonate and distilled water; the ether solution was then thoroughly dried over sodium sulfate. Distillation of the ether afforded 191.3 mg. of neutral material. Chromatography of this oil on Florisil afforded an analytical sample of anhydrodihydromammosin mixture, m.p. 123-132.5°.

Anal. Calculated for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.17; H, 9.59.

The infrared spectrum ($CHCl_3$) showed absorption at 1770 cm^{-1} (γ-lactone) 1645 cm^{-1} (double bond) and 905 cm^{-1} (double bond). The thin layer chromatogram of this product showed two overlapping spots, R_f = 0.35 and 0.31 (silica gel H eluting with benzene).

Treatment of Mammosin Keto-Dilactone with Boron Trifluoride and Acetic Anhydride. Preparation of the Enol Acetate. Mammosin keto-kilactone (100 mg.) was

dissolved in 3 ml. of acetic anhydride and 1 ml. of dry ether and chilled to -20° in the freezer. Distilled boron trifluoride etherate (0.7 ml.) was added and the mixture allowed to stand at -20° for 7 1/2 hours. The reaction mixture was then poured over 20 ml. of ice water containing 1 ml. of pyridine. After standing 1/2 hour the product was taken up in ether, washed well with dilute hydrochloric acid, then with distilled water, and dried over sodium sulfate. Thin layer chromatography of the oil remaining after removal of ether showed two spots having R_f values, 0.64 and 0.47. The latter value corresponded to that of starting material. The procedure was repeated on the mixture, but the thin layer chromatogram still showed some starting material remaining. The crude product was chromatographed on Florisil; elution of the column with 2% ethyl acetate in benzene provided white crystalline solid which was recrystallized from hexane - benzene affording 32.5 mg. of mammosin keto-dilactone enol acetate, m.p. 82-84.5°.

Anal. Calculated for $C_{22}H_{32}O_7$: C, 64.68; H, 7.90. Found: C, 64.56; H, 8.09.

The infrared spectrum (KBr) showed absorption bands at 1765-1755 cm^{-1} (γ-lactone and enol acetate), 1700 cm^{-1} (double bond) and at 1205 cm^{-1} (enol acetate). Thin layer chromatography of the product on silica gel H with 1:1 benzene ethyl acetate showed a single spot, R_f = 0.63.

The second product from the reaction was eluted from the column and identified as starting material by its infrared spectrum.

Ozonolysis of Dihydroisomammosin. Preparation of Dihydroisomammosin Aldehyde-Lactone. Dihydroisomammosin, a mixture of stereoisomers, (1.74 g.) was ozonized in 70 ml. of methanol for 5 minutes until the blue color of excess ozone persisted. Distilled water was then added to the ozonide, and water and methanol were removed on the rotary evaporator. The product was taken up in ether, washed well with distilled water, and dried over sodium sulfate. Removal of the solvent afforded 1.85 g. of white glassy solid. Chromatography of the glass on 40 g. of Florisil with 2% ethyl acetate in benzene failed to produce a solid product, but did achieve a crude separation. Fractions 3-13 of the Florisil chromatography were combined (674.6 mg.) and rechromatographed on 20 g. of silicic acid using 20% ethyl acetate in benzene as eluant. Fractions 6-10 of this chromatography were solidified by addition of hexane and chilling thus providing 178.5 mg. of product, melting at 93-100°--the melting range reflecting the product's existence as an epimeric mixture.

Anal. Calculated for $C_{17}H_{28}O_4$: C, 68.89; H, 9.52. Found: C, 68.74; H, 9.31.

The N.M.R. spectrum showed methyl absorption at 0.91 d (two secondary methyls), and 1.39 s. Methylene

hydrogens alpha to γ-lactone occurred at 2.77 d, and ether hydrogens appeared at 4.02 (broad) and 4.26 t. Aldehyde hydrogen showed at 9.75 s. The infrared spectrum (KBr) showed significant bands at 1780 cm^{-1} (γ-lactone) and 1735 cm^{-1} (aldehyde).

Treatment of Dihydroisomammosin Aldehyde-Lactone with Trifluoroperacetic Acid. Hydrolysis of the Product. Dihydroisomammosin aldehyde-lactone (71 mg.), (0.24 mmole), was dissolved in 5 ml. of dry methylene chloride to which 0.2 g. of sodium dihydrogen phosphate had been added. Trifluoroperacetic acid (0.36 mmole), prepared from trifluoroacetic anhydride and 90% hydrogen peroxide (32) was added and the slurry stirred 2 1/2 hours at room temperature. The salts were then filtered and washed well with ether. The combined ether washings were extracted with dilute, aqueous sodium bicarbonate solution, then washed well with distilled water and dried over sodium sulfate. Distillation of the solvent afforded 69.2 mg. of clear oil. The thin layer chromatogram of the oil on silica gel H, eluting with 8:2 benzene - ethyl acetate showed two spots, R_f = 0.52 and 0.09. Secondary methyls appeared in the N.M.R. spectrum at 0.92 and tertiary methyl at 1.4. Two hydrogens gave a signal centered at 2.78 d. An ether hydrogen appeared at 4.0-4.5. A single hydrogen was present at 6.2 (hydrogen under hemiacetal formate ester). Formate hydrogen occurred at 8.12 s. The

peaks of this spectrum were badly split on the sides and tops due to the mixture of hydrolyzed and unhydrolyzed product and also the presence of epimeric pairs. (Some aldehyde hydrogen was present, the result of partial hydrolysis of hemiacetal formate). The infrared spectrum ($CHCl_3$) of the mixture displayed significant absorption bands at 1778 cm^{-1} (γ-lactone) and 1726 cm^{-1} (formate).

Hydrolysis of the mixture was carried to completion by stirring the oil, dissolved in 25% aqueous ethanol containing several drops of 10% aqueous sulfuric acid, at room temperature for 6 1/2 hours. Most of the ethanol was then removed on the rotary evaporator, distilled water was added, and the product extracted with ether. The ether extract was washed well with distilled water and dried over sodium sulfate. Removal of the solvent afforded 59 mg. of oil. The thin layer chromatogram on silica gel H eluting with 8:2 benzene - ethyl acetate showed a single major spot, R_f = 0.11. Two very faint spots occurred ahead of this compound. The oil was chromatographed on Florisil and the leading impurities removed. In the methyl region of the N.M.R. spectrum of the product, signals occurred at 1.38 s, 1.08 d, and 0.85 d (smeared). Aldehyde hydrogen was present at 9.6 d; two methylene hydrogens alpha to lactone carbonyl appeared at 2.7 dd. Hydrogen under hydroxyl showed absorption at 4.16 dd. An hydroxyl

hydrogen was centered at 4.0 (broad). The infrared spectrum ($CHCl_3$) of the oily product showed absorption bands at 3600-3400 cm^{-1} (hydroxyl), 2720 cm^{-1} (aldehyde), 1765 cm^{-1} (γ-lactone) and 1725 cm^{-1} (aldehyde).

Preparation of Isomammosin Oxide. Isomammosin (1.6 g., 5 mmoles) dissolved in 50 ml. of distilled chloroform was stirred for ten hours at room temperature with 3.3 g. (16 mmoles) of 85% meta-chloroperbenzoic acid. The excess peracid was then extracted from the chloroform solution with cold dilute sodium hydroxide solution. Ether was added and the ether - chloroform solution washed repeatedly with distilled water and finally dried over sodium sulfate. After the solvent had been removed the semi-solid product was passed through Florisil with 2% ethyl acetate in benzene. The first fraction off the column contained 520 mg. of crystalline oxide, which was recrystallized from hexane - benzene, m.p. 174-177°.

Anal. Calculated for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.37; H, 8.40.

The methyl region of the N.M.R. spectrum showed signals at 0.99 d and 1.26 s (superimposed tertiary methyls under hydroxyl and under oxide). A vinyl methyl was present at 1.88 s. Ether and oxide hydrogens appeared between 2.5 and 3.5. A lactone hydrogen showed at 4.58 d. In the infrared spectrum (KBr) absorption bands appeared at 3460 cm^{-1} (hydroxyl), 1767 cm^{-1} (γ-lactone), and 1685

cm^{-1} (double bond).

Continued elution of the column afforded 361.7 mg. more of the oxide. Material coming off the column after this was crystalline, but gummy, thus suggesting the presence of more than one oxide.

SUMMARY

Mammosin, a diterpene lactone isolated from the pentane extracts of the gorgonian Eunicea mammosa, has the molecular formula $C_{20}H_{30}O_4$, confirmed by mass spectral analysis. The compound has been shown to contain an α-methylenic-γ-lactone, a tertiary methyl carbinol system, an isolated trisubstituted double bond, an inert disecondary ether, and a secondary methyl group. Consideration of the functionality, molecular formula, and all available chemical and instrumental data on mammosin and its derivatives, leads to the conclusion that the compound contains a 14-membered carbocyclic ring, bridged by ether oxygen, and fused to the α-methylenic-γ-lactone as shown in structure B.

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