THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

I. CONVERSION OF α-OXIMINO ESTERS TO NITRILES IN ALKALINE DIETHYLENE GLYCOL

II. SYNTHESIS OF α-IMINO, α-KETO AND α-AMINO ACETALS FROM GRIGNARD-DIMETHOXYACETONITRILE ADDUCTS

III. STUDIES DIRECTED TOWARD THE CHARACTERIZATION OF MAMMOSIN, A DITERPENE LACTONE ISOLATED FROM THE GORGONIAN, EUNICEA MAMMOSA LAMOUROUX

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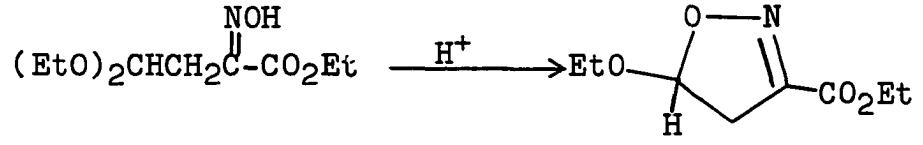
I. CONVERSION OF α-OXIMINO ESTERS TO NITRILES IN ALKALINE DIETHYLENE GLYCOL

INTRODUCTION

In connection with the synthesis of an amino acid analogue, we prepared ethyl α-oximino-β,β-diethoxybutyrate (I). This compound subsequently was found to undergo two very interesting chemical transformations, of which one is unique to (I), and the other may offer a new synthetic application for oximino esters.

When treated with dilute aqueous acid, (I) gave a compound, $C_8H_{13}NO_4$, amounting to the loss of ethanol. It was assigned the structure (II) on the basis of its infrared spectrum and interpretation of its N.M.R. spectrum. This structure is mechanistically reasonable on the basis of hydroxyl attack on the carbonium ion resulting from loss of a molecule of ethanol from the acetal function.



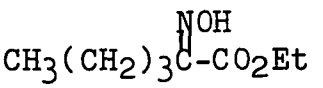
(I) (II)

The second unusual reaction of (I) was encountered in an attempted saponification with potassium hydroxide in diethylene glycol at 110°. Instead of obtaining the expected oximino acid, cyanoacetaldehyde diethylacetal was isolated in 75% yield, apparently the result of a novel "second-order" Beckmann type of reaction. The nitrile is ordinarily derived from the oximino acid by dilute aqueous acid (1, 2), by the usual Beckmann conditions (3), or by heating to their melting points (4-10). Beckmann reactions of this type have also been carried out using the combined action of base and an acylating or sulfonylating agent (11-13). There is no mention in the literature, however, of the promotion of this type of reaction by base alone. There is also no reported one-step conversion of oximino esters to nitriles.

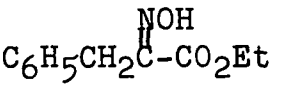
Oximino esters usually are hydrolyzed by aqueous base to the corresponding acids, which are reported to be stable in aqueous base (14). The oximino acids are then converted to nitriles by heating with dilute aqueous acid (2). The purpose of this study was then to determine whether oximino esters could be readily converted to nitriles in basic solution. Not only would a one-step process be more convenient, but it would also be useful for compounds having acid-sensitive functional groups. For instance, cyanoacetaldehyde diethylacetal could not have been obtained by treatment of the corresponding acid of (I) with aqueous acid because of the instability of the acetal function to acid; but it

was conveniently and readily obtained directly from the oximino ester by reaction in base.

To determine the potential synthetic value of this reaction, it was briefly investigated with two simpler and readily available (15) compounds, ethyl α-oximinocaproate (III) and ethyl α-oximino-β-phenylpropionate (IV).



(III)



(IV)

RESULTS AND DISCUSSION

Ethyl ∝-oximinocaproate (III) when heated at 110° for one hour with four equivalents of potassium hydroxide in alkaline diethylene glycol gave valeronitrile in 62% yield; but ethyl ∝-oximino-β-phenylpropionate (IV) under the same conditions was converted quantitatively to ∝-oximino-β-phenylpropionic acid (V). At 185°, however, (IV) gave toluonitrile in 59% yield and phenylacetic acid, arising from hydrolysis of the nitrile, in 39% yield. By using only one equivalent of base, (IV) then gave the nitrile in a satisfactory 77% yield. Further, when (IV) was heated at 180° in the absence of base, no reaction occurred. These results, which are summarized in Table 1, clearly implicate the oximino acid as the reactive species producing the nitrile.

$$R\text{-}\overset{\overset{NOH}{\|}}{C}\text{-}CO_2Et \xrightarrow{^-OH} R\text{-}\overset{\overset{NOH}{\|}}{C}\text{-}CO_2^- \longrightarrow RCN \xrightarrow{^-OH} RCO_2^-$$

(I), R = $(EtO)_2CHCH_2$-

(III), R = $CH_3(CH_2)_3$-

(IV), R = $C_6H_5CH_2$-

TABLE 1

RESULTS OF A STUDY OF THE EFFECT OF TEMPERATURE AND BASE ON (III), (IV), AND (V) IN DIETHYLENE GLYCOL

Base/ Oxime	Temp. °C	Time Hrs.	Product(s)	Yield %
			$CH_3(CH_2)_3C(=NOH)-CO_2Et$ (III)	
4	110	1	nitrile	62
			$C_6H_5CH_2C(=NOH)-CO_2Et$ (IV)	
4	110	1	(V)	92
0	180	1	(IV)	99*
4	185	1	nitrile	59
			phenylacetic acid	39
1	185	1	nitrile	77
			$C_6H_5CH_2C(=NOH)-CO_2H$ (V)	
1	150	0.5	nitrile	94
0	125	0.5	nitrile	66
			(V)	24*
1	125	0.5	nitrile	43
			(V)	53*

*recovered material

In order to determine the influence of base, if any, and temperature on the nitrile formation from the oximino acid, a brief study was carried out using α-oximino-β-phenylpropionic acid (V). When (V) was heated at 150° for one-half hour in the presence of a stoichiometric amount of base, the nitrile was quantitatively produced. Under conditions of incomplete reaction (125°), the nitrile was obtained in 66% yield without using base and in only 43% yield when a stoichiometric amount of base was used. It is not clear from these results why the yields with and without base are not very different. The presence of base slightly retards the reaction, yet temperature exerts a significant influence on the rate of the reaction.

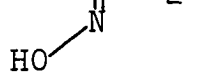
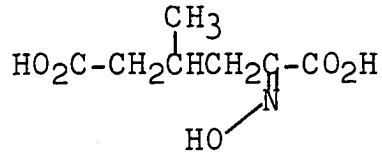
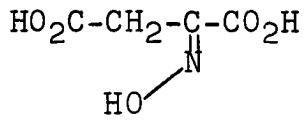
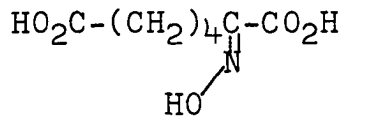
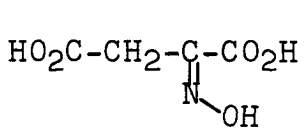
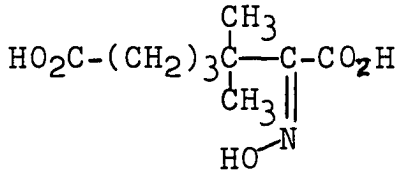
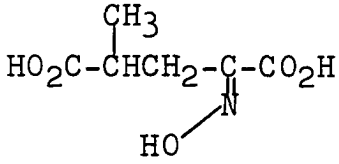
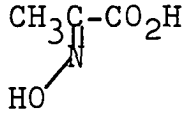
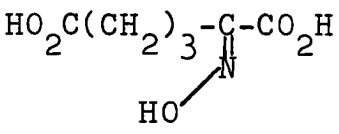
Our results in Table 1 indicate that, despite the reported (14) stability of oximino acids in aqueous base, one can take advantage of their thermal instability, in order to obtain the desired nitrile under neutral or basic conditions, by employing a solvent that allows temperatures above 100°. Since base is required only for the hydrolysis of the ester and only slightly retards formation of the nitrile, this method employing stoichiometric quantities of base in diethylene glycol offers a useful, one-step conversion of oximino esters to nitriles.

This considerable influence of temperature on the rate of the reaction is reasonable from the evidence in the literature of pyrolytic decomposition of oximino acids (4-10). Most

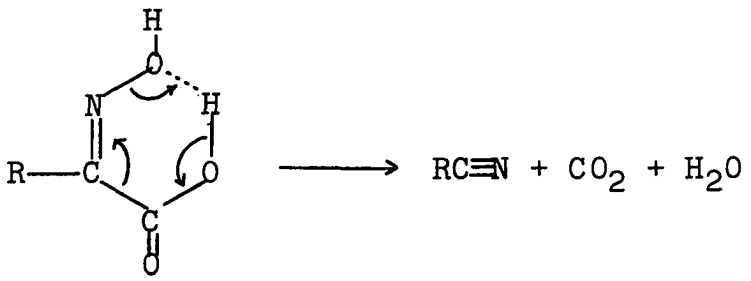
oximino acids described are reported to decompose at their melting point with evolution of gas. This gas was recognized as carbon dioxide in some cases while in others the second product of pyrolysis was identified as the nitrile. The structures of oximino acids reported to yield nitrile on decomposition are shown in Table 2.

TABLE 2

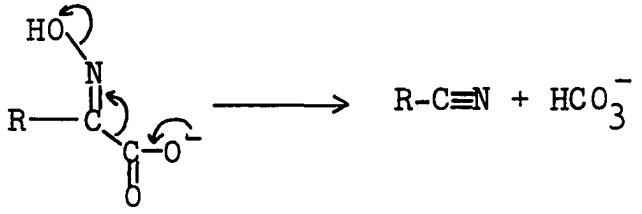
OXIMINO ACIDS REPORTED TO YIELD NITRILE ON HEATING TO THEIR MELTING POINTS

Compound	Ref.	Compound	Ref.
	(5)		(8)
	(4)		(7)
	(4)		(9)
	(6)		(10)
	(7)		

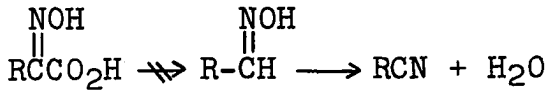
The pyrolytic decomposition of oximino acids could reasonably proceed by a cyclic concerted mechanism, which would be favored by strong intramolecular hydrogen bonding and would require the <u>syn</u> configuration.



However, the mechanism for the reaction in base would clearly involve the anion of the oximino acid and may proceed by a concerted <u>trans</u> elimination, which requires the <u>anti</u> configuration (16).



From our limited examples we are unable to discuss further the above mechanisms for the conversion of oximino acids to nitriles. We can, however, eliminate the mechanistic possibility of initial thermal decarboxylation of the oximino acid followed by thermal dehydration of the resulting aldoxime. Hauser reported that benzaldoxime and several substituted benzaldoximes when heated at 110° in 2N aqueous sodium hydroxide gave the corresponding acids (17). More



recently, Rapoport and Nilsson showed the base-catalyzed dehydration of aldoximes to be a general reaction (18). Their reaction conditions consisted of a 4:1 ratio of potassium hydroxide to aldoxime in diethylene glycol at about 190° and gave the corresponding acid of the nitrile. The results of both groups showed that base was necessary for nitrile formation and Rapoport pointed out that thermal dehydrations usually require much higher temperatures. In our experiments with the oximino acid (V), base was not required for nitrile formation and in fact retarded the reaction slightly. We also were unable to isolate any aldoxime under conditions of incomplete reaction.

EXPERIMENTAL

All melting points and boiling points are uncorrected. The infrared spectra were run in chloroform solutions on a Perkin-Elmer, Model 21 spectrometer. N.M.R. spectra were taken on a Varian A-60 spectrometer using tetramethylsilane as an internal reference. Samples were run in deuteriochloroform solutions, and the chemical shifts are reported in δ-values (p.p.m. from TMS).

Elemental analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, Germany.

Preparation of Diethyl 2,2-Diethoxyethylmalonate. This compound was prepared as described by Perkin and Pink (19). To 14.2 g. (0.627 mole) of sodium in 200 ml. of absolute ethanol was added all at once 100 g. (0.625 mole) of diethyl malonate and 80 g. (0.406 mole) of bromoacetal. The mixture was immediately sealed in an autoclave and heated to 180° with stirring for 4 hrs. The cooled mixture was filtered and the solids washed with ethanol. The filtrates were concentrated on the rotary evaporator, diluted with water and, after extraction with ether, gave a yellow oil, which upon fractionation gave 67 g. (60%) of product, b.p.

134° at 3.5 mm., n_D^{25} 1.4256; reported (20), b.p. 108-109° at 0.7 mm., n_D^{18} 1.4282.

The infrared spectrum displayed bands at 1725 cm^{-1} (ester) and 1058 and 1120 cm^{-1} (acetal).

Nitrosation of Diethyl 2,2-Diethoxyethylmalonate. To 55.2 g. (0.2 mole) of diethyl 2,2-diethoxyethylmalonate in a 500 ml. flask fitted with a stirrer, reflux condenser, and a dropping funnel was added 30 g. (0.40 mole) of ethyl nitrite (21). The mixture was kept at 0° for 0.5 hr. with stirring, then cooled to -40° and a solution of 5 g. (0.22 g. atom) of sodium in 120 ml. of absolute ethanol added dropwise over a period of one hour. After keeping the reaction mixture at -20° overnight, the solvent was then removed *in vacuo* without heating. Dilution with 100 ml. of water and extraction with ether removed diethyl carbonate. Addition of concentrated ammonium chloride solution to the aqueous phase precipitated the oxime as an oil, which was isolated by extraction with ether. The dried ether extracts were distilled to give 46.6 g. (89.6%) of crude ethyl α-oximino-β,β-diethoxybutyrate (I). Recrystallization from cold hexane gave hygroscopic needles, m.p. 37-38°.

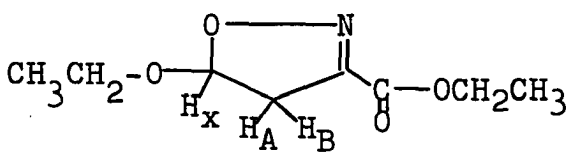
Anal. Calcd. for $C_{10}H_{19}NO_5$: C, 51.49; H, 8.21; N, 6.01. Found: C, 51.46; H, 8.22; N, 6.04.

The infrared spectrum ($CHCl_3$) showed bands at 3200 cm^{-1} (hydroxyl) and 1720 cm^{-1} (ester).

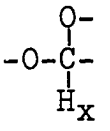
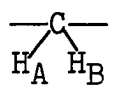
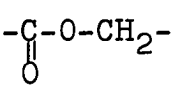
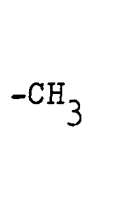
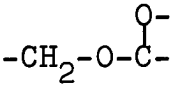
Treatment of Ethyl α-Oximino-γ,γ-Diethoxybutyrate (I) with Aqueous Acid. To 2.0 g. of (I) in 10 ml. of ethanol was added 50 ml. of 2% aqueous hydrochloric acid. This solution was warmed at steam bath temperatures for 1.5 hrs., cooled, and extracted with ether. The combined ether extracts were washed with dilute sodium bicarbonate solution, water, and dried over sodium sulfate. Removal of the ether left a light yellow oil, which upon fractionation through a spinning band column gave a major fraction, b.p. 94-95° at 1.1 mm. Redistillation gave 1.2 g. (75%) of 3-carbethoxy-5-ethoxy-2-isoxazoline (II) as a colorless oil, b.p. 79° at 0.5 mm.

Anal. Calcd. for $C_8H_{13}NO_4$: C, 51.33; H, 7.00; N, 7.48. Found: C, 50.83; H, 6.94; N, 7.72.

The infrared spectrum ($CHCl_3$) showed bands at 1738 cm^{-1} (ester) and 1596 cm^{-1} (C=N). The peaks displayed in the N.M.R. spectrum are listed below, followed by their multiplicity and integral values, and their respective structural assignments. The most significant feature is the ABX system composed of the acetal proton and the methylene protons in the isoxazoline ring. From the twelve line ABX system, J_{AB}=18 c.p.s. can be readily measured, and J_{ax}= 3 c.p.s. and J_{BX}=5 c.p.s. can be estimated (22).



(II)

δ, p.p.m.	assignment	δ, p.p.m.	assignment
5.70q(1H)		3.08 octet(2H)	
4.28q(2H)		1.31t(3H)	
3.68q(2H)		1.16t(3H)	

Treatment of Ethyl α-Oximino-β,β-Diethoxybutyrate (I) with Alkaline Diethylene Glycol. To a mixture of 30 ml. of diethylene glycol, 5 g. of potassium hydroxide and 5 ml. of water was added 10 g. of (I). The mixture was heated to 110° for 1.5 hrs., then cooled and diluted with ammonium chloride solution. Extraction with ether gave a yellow oil, whose infrared spectrum showed a band at 2250 cm^{-1} (nitrile). Distillation of this oil gave 4.6 g. (75%) of cyanoacetaldehyde diethylacetal, b.p. 87-88° at 10.7 mm., n_D^{25} 1.4136; reported (23), b.p. 91-93.5° at 11 mm., n_D^{25} 1.4153.

Nitrosation of Diethyl n-Butylmalonate. The procedure employed was essentially that of Shivers and Hauser (15). To 43.2 g. (0.2 mole) of diethyl n-butylmalonate was added 30 g. (0.40 mole) of ethyl nitrite (21). The mixture was cooled to -10° and 5 g. (0.22 g. atom) of sodium in 120 ml. of absolute ethanol was added dropwise over a period of one hour. After keeping the reaction mixture at -20° overnight, it was concentrated on the rotary evaporator, then diluted with 100 ml. of water, and extracted with ether to remove diethyl

carbonate. The aqueous phase was acidified to pH 5 with cold concentrated hydrochloric acid and the oily product extracted with ether. Distillation of the ether left a yellow solid, which after crystallization from hexane, gave 27 g. (80%) of ethyl ∝-oximinocaproate (III), m.p. 53-55°; reported (15) m.p. 53-55°.

Nitrosation of Diethyl Benzylmalonate. To 50 g. (0.2 mole) of diethyl benzylmalonate was added 30 g. (0.4 mole) of ethyl nitrite (21). The mixture was cooled to -10° and 5 g. (0.22 g. atom) of sodium in 120 ml. of absolute ethanol was added dropwise over a period of one hour, then kept at -20° overnight. After concentration on the rotary evaporator, the solution was diluted with water, and the diethyl carbonate extracted with ether. Acidification of the aqueous phase and extraction with ether gave, after recrystallization from hexane, 31 g. (75%) of ethyl ∝-oximino β-phenyl propionate (IV), m.p. 57-59°; reported (15) m.p. 56-58°.

The infrared spectrum ($CHCl_3$) showed bands at 3560 and 3260 cm^{-1} (hydroxyl) and 1720 cm^{-1} (ester).

Hydrolysis of Ethyl ∝-Oximinocaproate (III). A solution of 1.0 g. of ethyl ∝-oximinocaproate (III) in 30 ml. of 10% sodium hydroxide solution was heated at steam bath temperatures for ten minutes. The cooled solution was acidified with dilute hydrochloric acid and extracted with ether. The dried ether extracts were distilled to give 0.81 g.

(97%) of crude crystalline α-oximinocaproic acid. Recrystallization from benzene gave colorless crystals, m.p. 135-136°; reported (15) m.p. 136°.

The infrared spectrum showed significant bands at 3400-3000 cm^{-1} (hydroxyl), 1690 cm^{-1} (acid), and 1650 cm^{-1} (oxime).

Alkaline Degradation of Ethyl α-Oximinocaproate (III). A mixture of 3.7 g. (0.021 mole) of (III), 15 ml. of diethylene glycol, 2.5 g. (0.045 mole) of potassium hydroxide, and 2.5 ml. of water was heated for one hour at 110°. The cooled mixture was diluted with water, acidified with dilute hydrochloric acid (gas evolution), and the oily product extracted with ether. Distillation of the dried ether extracts left an oil, which after distillation, furnished 1.10 g. (62%) of valeronitrile, b.p. 134° at 735 mm., n_D^{25} 1.3955; authentic material, b.p. 137° at 735 mm., n_D^{25} 1.3946; reported (24) b.p. 141° at 764 mm., $n_D^{?}$ 1.3969.

Hydrolysis of Ethyl α-Oximino-β-Phenylpropionate (IV). A solution of 1.0 g. of ethyl α-oximino-β-phenylpropionate (IV) in 30 ml. of 10% sodium hydroxide solution was heated at steam bath temperatures for ten minutes. The cooled solution was acidified with dilute hydrochloric acid and extracted with ether. The dried ether extracts were distilled to give 0.85 g. (98%) of crude crystalline α-oximino-β-phenylpropionic acid (V). Recrystallization from aqueous ethanol gave colorless needles, m.p. 168°;

reported (25) m.p. 168°.

Degradations of Ethyl α-oximino-β-phenylpropionate (IV). (a). A mixture of 2.07 g. (10 mmoles) of ethyl α-oximino-β-phenylpropionate (IV), 15 ml. of diethylene glycol and 2.24 g. (40 mmoles) of potassium hydroxide was heated at 110° for one hour. The cooled solution was diluted with water and extracted with ether. The ether extracts, after being dried over sodium sulfate and distilled, furnished no neutral product. The aqueous phase was acidified with dilute hydrochloric acid and extracted with ether. The dried ether extracts furnished 1.65 g. (92%) of α-oximino-β-phenylpropionic acid (V), m.p. 168°.

(b). A solution of 2.07 g. (10 mmoles) of ethyl α-oximino-β-phenylpropionate (IV) in 15 ml. of diethylene glycol was heated at 180° for one hour. The cooled solution was diluted with water and extracted with ether. Removal of the ether from the dried extracts gave a quantitative recovery of the starting material, m.p. 168°.

(c). To 2.07 g. (10 mmoles) of ethyl α-oximino-β-phenylpropionate (IV) dissolved in 15 ml. of diethylene glycol was added 2.24 g. (40 mmoles) of potassium hydroxide. After the mixture had been heated at 185° for one hour, the solution was cooled, diluted with water and extracted with ether. The dried ether extracts were distilled to give crude oily material, which after distillation gave 0.50 g. (43%) of phenylacetonitrile, b.p. 98° at 10 mm., n_D^{25} 1.5201; reported

(26) b.p. 107° at 12 mm., n_D^{25} 1.5210. Acidification of the aqueous phase gave, after extraction with ether and crystallization with hexane, 0.53 g. (39%) of phenylacetic acid, m.p. 82-83°; authentic sample, m.p. 82°; reported (27) m.p. 76°. The infrared spectra of phenylacetonitrile and phenylacetic acid were identical with the spectra of authentic materials.

(d). A solution of 2.07 g. (10 mmoles) of ethyl α-oximino-β-phenylpropionate (IV), 15 ml. of diethylene glycol and 0.56 g. (10 mmoles) of potassium hydroxide was heated at 185° for one hour. The cooled solution was diluted with water, extracted with ether, and the extracts dried over sodium sulfate. Distillation of the ether gave crude phenylacetonitrile. Distillation of the crude product furnished 0.90 g. (77%) of phenylacetonitrile, b.p. 96° at 8 mm. The phenylacetonitrile and phenylacetic acid were further identified by comparing their infrared spectra with those of authentic samples.

Reactions of α-Oximino-β-phenylpropionic Acid (V) in Diethylene Glycol. (a). A mixture of 1.79 g. (10 mmoles) of α-oximino-β-phenylpropionic acid (V), 15 ml. of diethylene glycol, and 0.56 g. (10 mmoles) of potassium hydroxide was heated at 150° for one-half hour. After the solution had been cooled, it was diluted with water, extracted with ether, and the ether extracts dried over sodium sulfate. Removal of the ether left an oil, which on distillation furnished 1.1 g. (94%) of phenylacetonitrile, b.p. 97° at 9 mm.

(b). A solution of 0.90 g. (5 mmoles) of α-oximino-β-phenylpropionic acid (V) in 8 ml. of diethylene glycol was heated at 125° for one-half hour. The cooled solution was diluted with water, extracted with ether, and the ether extracts were washed with dilute sodium bicarbonate solution. The dried ether phase was distilled to give crude oily material. Distillation of this oil gave 0.38 g. (66%) of phenylacetonitrile, b.p. 98° at 10 mm. The bicarbonate washings were acidified with dilute hydrochloric acid and extracted with ether. These dried ether extracts were distilled to give 0.22 g. (24%) of recovered (V), m.p. 167°.

(c). A solution of 0.90 g. (5 mmoles) of α-oximino-β-phenylpropionic acid (V), 8 ml. of diethylene glycol, and 0.28 g. (5 mmoles) of potassium hydroxide was heated at 125° for one-half hour. The cooled solution was diluted with water, extracted with ether, and the ether extracts were washed with sodium bicarbonate solution. Removal of the ether left an oily material, which on distillation furnished 0.35 g. (43%) of phenylacetonitrile, b.p. 98° at 10 mm. The bicarbonate washings were acidified with dilute hydrochloric acid and extracted wtih ether. The dried ether extracts were distilled to give 0.47 g. (53%) of recovered (V), m.p. 168°.

SUMMARY

Ethyl α-oximino-γ,γ-diethoxybutyrate, ethyl α-oximinocaproate, and ethyl α-oximino-β-phenylpropionate were degraded in good yields to the corresponding nitriles by the action of base in diethylene glycol at temperatures of 110° to 185°. The corresponding oximino acid is clearly implicated as the reactive species producing the nitrile, apparently by a novel "second-order" Beckmann type of reaction. The usual Beckmann conditions employ acid catalysts or the combined action of base and an acylating or sulfonylating agent. There is no mention in the literature, however, of the promotion of this type of reaction by base alone. It was found that base slightly retards the formation of nitrile, but temperature exerts a significant influence on the rate of the reaction. Thus, by using a stoichiometric quantity of base to saponify the oximino ester to the salt of the acid, which apparently is converted to the nitrile by a thermal process, the ester is conveniently converted to the nitrile in a single step. This method is especially useful for compounds having acid-sensitive functional groups.

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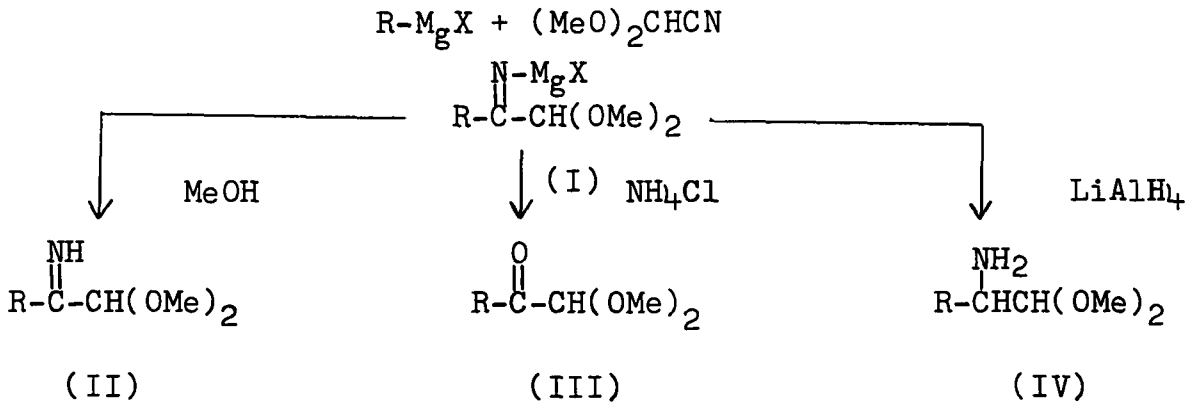
II. SYNTHESIS OF α-IMINO, α-KETO AND α-AMINO ACETALS FROM GRIGNARD-DIMETHOXYACETONITRILE ADDUCTS

INTRODUCTION

The synthesis of dimethoxyacetonitrile by Erickson (1) in 1951 offered a simple but interesting new starting material for preparing potentially useful compounds. In this present work we studied the reaction of dimethoxyacetonitrile with Grignard reagents and conversion of the resultant adducts (I) to α-imino, α-keto and α-amino acetals. Mild hydrolysis or methanolysis (2) of (I) gave the α-imino acetals (II), and hydrolysis with ammonium chloride or aqueous sodium hydroxide gave the expected α-keto acetals (III). Reduction of (I) with lithium aluminum hydride in tetrahydrofuran (3) furnished the α-amino acetals (IV).



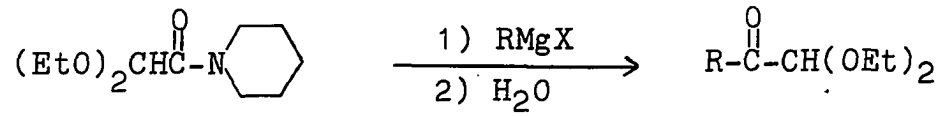
(II) (III) (IV)

These chemical transformations constitute an improvement and simplification of other synthetic approaches to these important vicinally difunctional compounds, which are of interest themselves, or as intermediates in the synthesis of a variety of heterocyclic systems. ∝-Keto acetals have been implicated in plant and animal cell metabolism (4) and more recently methylglyoxal bis-guanylhydrazone produced the first significant remissions in adult myelocytic leukemia (5). This finding stimulated others (6) to prepare homologous alkylglyoxal guanylhydrazones in hope of finding more effective drugs. They were faced, however, with the problem of the lack of a convenient and general synthesis of the required intermediate ∝-keto aldehydes or the corresponding acetals.

A variety of methods for the synthesis of ∝-keto acetals or aldehydes have been developed; yet few have been general. Such methods include those suitable for preparing aryl ∝-keto aldehydes such as the oxidation of aryl methyl ketones with selenium dioxide. Phenylglyoxal has been prepared from acetophenone by this method (7). Phenacyl bromides have been treated at room temperature with dimethyl sulfoxide to furnish yields of 71 to 95% of the phenylglyoxals (8). The reaction of nitrosyl chloride with acetophenone in ethanol at approximately 25-60° has produced phenylglyoxal diethylacetal in yields approaching 53% (9).

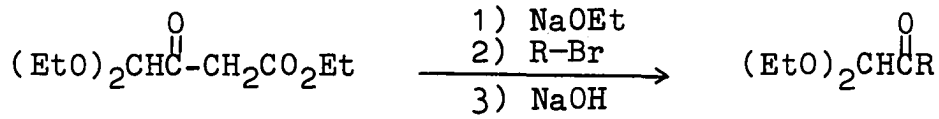
A more general synthesis of ∝-keto acetals has

involved the reaction of diethoxyacetylpiperidine (V) and Grignard reagents (10-12). t-Butylglyoxal diethylacetal was prepared by this method in 82% yield and phenylglyoxal diethylacetal was prepared in 56% yield (12).



(V)

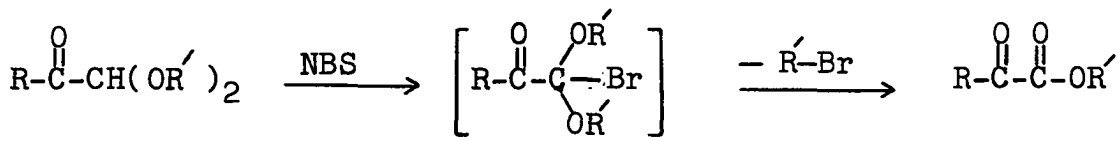
Another convenient and general synthesis has employed condensations with ethyl γ-diethoxyacetoacetate (VI) (4). This method has the advantage of not being limited to the Grignard reaction.



(VI)

Though these latter two methods have been very useful, the starting materials (V) and (VI) are not readily available.

One recent and important use of α-keto acetals is their conversion to α-keto esters on treatment with N-bromosuccinimide. This transformation has been briefly explored by Wright and represents a convenient two-step synthesis of α-keto esters from Grignard reagents (12).



Though ∝-amino aldehydes themselves are generally unstable, they have been prepared and isolated as their acetal derivatives. ∝-Amino acetals have been synthesized by several methods; yet none are particularly satisfactory. Aminoacetal itself has been prepared by the ammonolysis of bromoacetal in 32-39% yield (13). Potential anti-histaminic and spasmolytic compounds of the N-substituted amino acetal type have been prepared by the analogous amminolysis reaction (14).

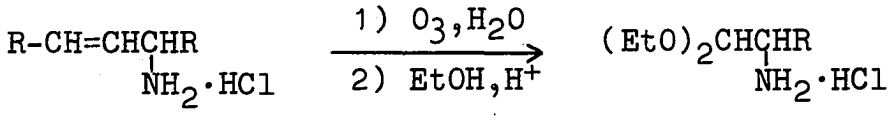
Two methods have employed ∝-amino acids and are thus obviously limited to the availability of the amino acids. The first, and one of the most widely used methods, has involved the sodium amalgam reduction of hydrochloride derivatives of ∝-amino esters (VII) (15-18). The second method has utilized phthalimide derivatives of ∝-amino acids and has involved the Rosenmund reduction of the corresponding acid chlorides to the protected amino aldehydes, which were then converted to the acetals (19). Saponification of the phthalimide group finally produced the amino acetal.

$$\underset{\text{(VII)}}{\text{R-CH(NH}_2\cdot\text{HCl)-CO}_2\text{Et}} \xrightarrow[2)\ \text{EtOH, H}^+]{1)\ \text{Na-Hg}} \text{R-CH(NH}_2\text{)-CH(OEt)}_2$$

Another method involves the ozonolysis of aqueous solutions of allyl amine hydrochlorides (VIII) and is also restricted to a rather specialized class of compounds (20).

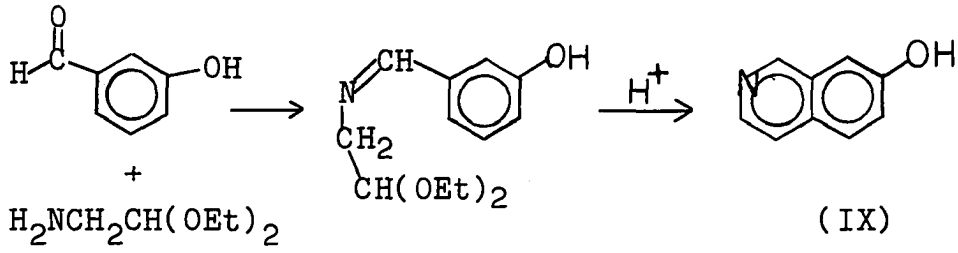
Amino acetals have been useful for the synthesis of a

number of heterocyclic systems.



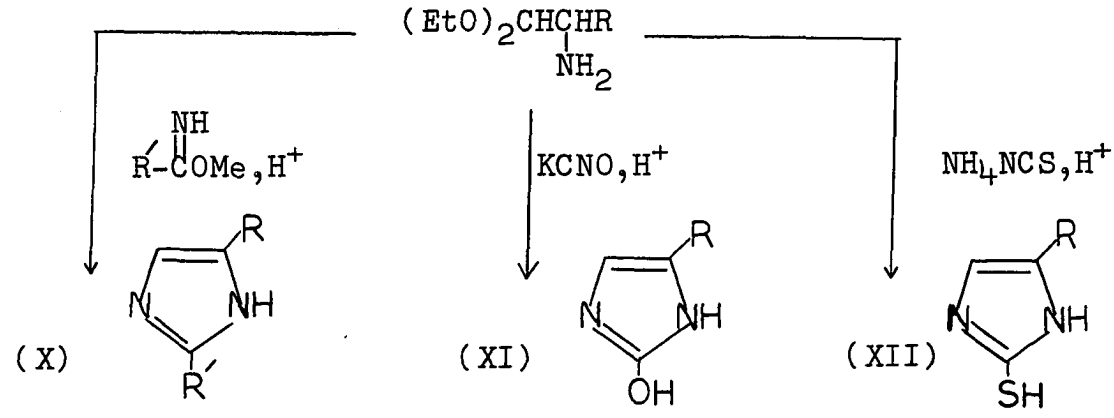
(VIII)

In the synthesis of quinine, Woodward and Doering condensed aminoacetal with m-hydroxybenzaldehyde to prepare 7-hydroxy-isoquinoline (IX) (21).

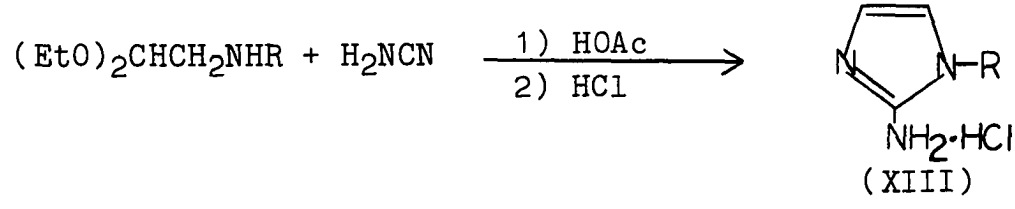


(IX)

Lawson used ∝-amino acetals and ∝-amino aldehydes to prepare 2,5-disubstituted imidazoles (X) by reactions with imidates (22). He also prepared 5-substituted 2-hydroxy-imidazoles (XI) in 27-57% yields using potassium cyanate (23). Akabori and Numano likewise synthesized a number of 5-substituted 2-mercaptoimidates (XII) by the action of ammonium thiocyanate on the solutions of amino aldehydes (24).

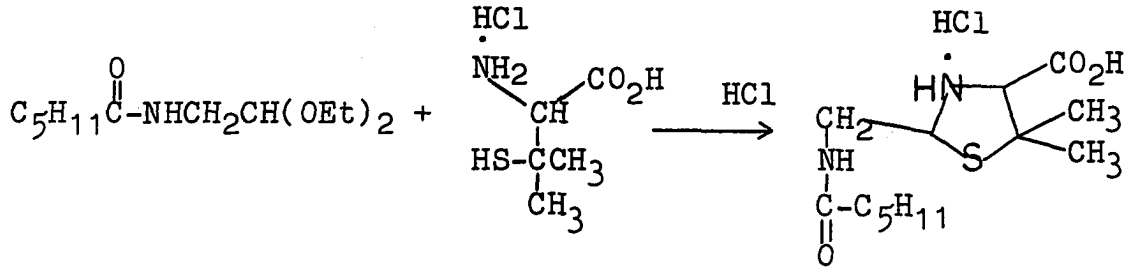


The condensation of amino acetals with cyanamide also produced 2-aminoimidazoles (XIII) (25).



(XIII)

Thiazolidine compounds similar to a portion of the penicillin molecule and adapted for use in the synthesis of compounds chemically related to penicillin were prepared by condensing β,β-dimethylcysteine hydrochloride with acetals containing amino or substituted amino groups (26). The following reaction shows the synthesis of 2-(caproylaminomethyl)-4-carboxy-5,5-dimethylthiazolidine hydrochloride (XIV).



(XIV)

RESULTS AND DISCUSSION

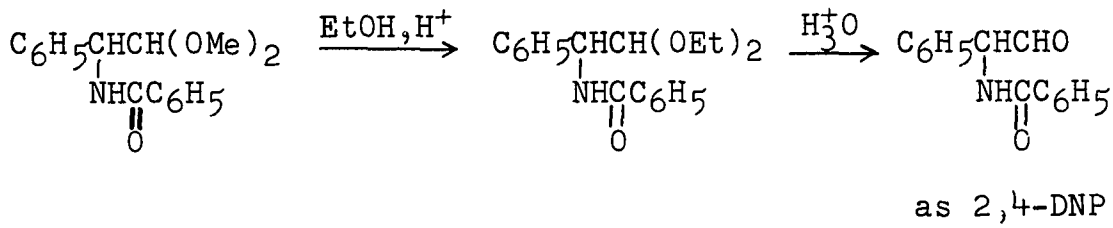
Initial studies of Grignard reactions with dimethoxyacetonitrile were conducted using phenylmagnesium bromide. After preparation of the Grignard reagent, an ethereal solution of dimethoxyacetonitrile was added slowly at room temperature, and the reaction proceeded smoothly with mild evolution of heat. Hydrolysis of the complex (Ia) (a,R=phenyl) with aqueous ammonium chloride gave a yellow oily material which showed ketimine absorption bands in the infrared (27). The N-H band appeared at 3240 cm^{-1} and the C=N band at 1620 cm^{-1}. Attempted distillation of this product resulted in vigorous evolution of ammonia and decomposition of the material. Hydrolysis of the crude ketimine with aqueous sodium hydroxide converted it to the corresponding ketone (IIIa), as indicated by the appearance of an infrared band at 1695 cm^{-1}. Phenylglyoxal dimethylacetal (IIIa) was also prepared in 72% yield by direct alkaline hydrolysis of (Ia). Its N.M.R. spectrum showed the acetal proton as a sharp singlet at 4.65 p.p.m. and the two acetal methyl groups as superimposed singlets at 2.82 p.p.m. Two aromatic protons, those ortho to the ring substituent, appeared at 7.67 p.p.m., and the

remaining three aromatic protons were centered at 6.91 p.p.m.

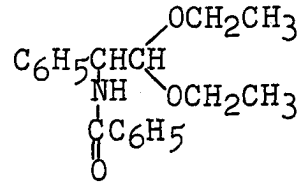
Reduction of (Ia) to α-aminophenylacetaldehyde dimethylacetal (IVa) in 69% yield was carried out by the addition of lithium aluminum hydride in tetrahydrofuran. In work by Pohland and Sullivan with Grignard adducts of simple nitriles, optimum yields of primary amines were obtained using 1.2 moles of lithium aluminum hydride per mole of the complex (3). If less than 1.2 moles of the reducing agent were used, the desired amine was contaminated with an undesirable imine formed in a side reaction. In the present work, we assumed a yield of approximately 80% of the adduct (Ia), based on the 72% yield of (IIIa) after distillation, and calculated the amount of lithium aluminum hydride required on this basis. When tetrahydrofuran was used as the solvent in preparing (Ia), reduction to (IVa) gave essentially the same yield. The N.M R. spectrum of (IVa) exhibited methyl group singlets at 3.16 p.p.m. and 3.34 p.p.m. The two protons of the amino group appeared as a broad singlet at 1.57 p.p.m. The acetal and benzylic protons exhibited an AB pattern, $J_{AB}/(\delta_B-\delta_A)=0.3$, which displayed a quartet with a peak intensity ratio of 1:2:2:1 (28). The estimated chemical shifts of the A and B protons were 3.97 p.p.m. and 4.20 p.p.m., respectively; and $J_{AB}=6$ c.p.s. was measured from the spectrum. A multiplet for the five aromatic protons was centered at 7.34 p.p.m.

The hydrochloride of (IVa) was prepared quantitatively

by adding dry hydrogen chloride to an ether solution of the amine. The acetamide derivative was prepared by adding acetyl chloride to a solution of the amine in pyridine and benzene (29). Both derivatives gave satisfactory analyses. The benzamide derivative was prepared by the same method as for the acetamide derivative and showed a chemical composition corresponding to a hemiacetal structure rather than the acetal; although its infrared spectrum did not confirm the presence of an hydroxyl group. Despite the unsatisfactory analysis, we converted the benzamide derivative to the amide diethylacetal by employing absolute ethanol and a catalytic amount of hydrogen chloride. The benzamide diethylacetal derivative had a satisfactory analysis and was hydrolyzed with aqueous acid to the corresponding aldehyde, which was isolated as the 2,4-dinitrophenylhydrazone.



The N.M.R. spectrum of the benzamide diethylacetal derivative furnished additional confirmation of its structure, which is summarized in Figure 1. The chemical shifts of the spectral peaks are followed by their multiplicity and integral values.



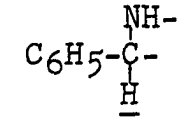
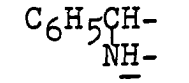
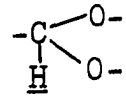
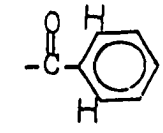
δ, p.p.m.	assignment	δ, p.p.m.	assignment
1.15 t (3H) 1.20 t (3H)	$-OCH_2\underline{CH_3}$	5.39 dd (1H)	
3.66 m (4H)	$-OC\underline{H_2}CH_3$	7.12 d (1H)	
4.71 d (1H)		7.42 m (8H)	aromatic
		7.85 dd (2H)	

Fig. 1.--N.M.R. spectral data and assignments for N-Benzoylaminophenylacetaldehyde dimethylacetal.

After characterization of the products derived from the reaction of phenylmagnesium bromide and dimethoxyacetonitrile, the general utility of the reaction for synthesis of homologous α-imino, α-keto and α-amino acetals was investigated. E. D. Parker (30) then prepared the Grignard-dimethoxyacetonitrile adducts (I), where R represents methyl, ethyl, i-propyl, sec-butyl, i-butyl and benzyl; he converted them also to the related products (II), (III) and (IV). Methanolysis of (I) gave the α-imino acetals (II), which, unlike the phenyl Grignard adduct, were readily distilled. Hydrolysis of (I) with ammonium chloride produced the

expected ∝-keto acetals (III); and reduction of (I) with lithium aluminum hydride gave the ∝-amino acetals (IV). These results are summarized in Table 1.

TABLE 1

RESULTS OF THE SYNTHESIS OF COMPOUNDS OF THE TYPE (II), (III) AND (IV)

R	$RC(=NH)CH(OMe)_2$ (II)	$R\text{-}C(=O)CH(OMe)_2$ (III)	$RCH(NH_2)CH(OMe)_2$ (IV)
methyl	-	45	31
i-propyl	75	59	63
sec-butyl	75	87	66
i-butyl	64	73	73
benzyl	50	45	67

The ∝-keto acetals were converted to bis-semicarbazone derivatives, and the ∝-amino acetals were converted to the benzamide derivatives. The low yields of ∝-keto- and ∝-aminopropionaldehyde dimethylacetals were possibly due to the loss of material due to solubility and loss during distillation of these volatile materials.

The possibility of a side reaction occurring by abstraction of the ∝-hydrogen of dimethoxyacetonitrile was also considered. Abstraction of the ∝-hydrogen by the Grignard reagent would result in a different course of reaction and thus lower the yields of products expected from

condensation with the nitrile group. In a study of the influence of structure on the reactions of Grignard reagents with nitriles having α-hydrogen, Hauser showed that the extent of abstraction of the α-hydrogen depended on how strongly the α-hydrogen was activated by the nature of the R group of the nitrile (31). The methoxyl groups of dimethoxyacetonitrile should reasonably be expected to exert a negative inductive effect thus increasing the activation of the α-hydrogen. In the present work, however, the satisfactory yields of most products warranted no suspicion of any significant reaction occurring via abstraction of the hydrogen atom. Failures in several attempts to alkylate dimethoxyacetonitrile also support this view.

EXPERIMENTAL

All melting points are uncorrected. Tetrahydrofuran was distilled from lithium aluminum hydride. The infrared spectra were run in chloroform solutions on a Perkin-Elmer, Model 21 spectrometer. N.M.R. spectra were taken on a Varian A-60 spectrometer using tetramethylsilane as an internal reference. Samples were run in varying concentrations in deuteriochloroform solutions, and the chemical shifts are reported in δ-values (p.p.m. from TMS).

Analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, Germany.

Preparation of Dimethoxyacetonitrile (1). To a mixture of 424 g. (4 moles) of methyl orthoformate and 236 ml. (6 moles) of hydrocyanic acid was added 1.62 g. (12 mmoles) of zinc chloride. After the mixture had stood at room temperature for one week, the catalyst was neutralized with methanolic potassium hydroxide. Removal of the hydrocyanic acid at the water aspirator left an oil which was distilled to give 343 g. (85%) of dimethoxyacetonitrile, b.p. 137° at 736 mm.; n_D^{20} 1.3878; reported (1) b.p. 139.5° at 772 mm., n_D^{25} 1.3818.

Reaction of Phenylmagnesium Bromide with Dimethoxyacetonitrile. To 2.88 g. (0.12 g. atom) of magnesium in a 1-L. flask fitted with a condenser and drying tube, a mechanical stirrer, and an addition funnel was added dropwise 18.8 g. (0.12 mole) of bromobenzene in 100 ml. of dry ether. After addition of the bromobenzene was complete, the mixture was stirred for an additional one-half hour. Dropwise addition of 10.1 g. (0.10 mole) of dimethoxyacetonitrile in 50 ml. of dry ether at room temperature took approximately one hour. A small amount of a gummy residue deposited on the sides of the flask. Shortly after the addition was complete, ammonium chloride solution was carefully added to the ice-cooled mixture; the gummy residue readily dissolved. The ether phase was then separated. The yellow oil remaining after removing the ether from the dried solutions showed ketimine absorptions in the infrared at 3240 cm^{-1}(NH) and 1620 cm^{-1} (C=N). Attempted distillation of this oil at reduced pressure resulted in evolution of ammonia and decomposition of the oil. Hydrolysis of the oil remaining in the flask gave a substance that exhibited infrared bands at 1695 cm^{-1} (aryl ketone) and 1120 and 1066 cm^{-1} (acetal).

Preparation of Phenylglyoxal Dimethylacetal. To 2.88 g. (0.12 g. atom) of magnesium in a 1-L. flask fitted with a condenser and drying tube, a mechanical stirrer, and an addition funnel was added dropwise 18.8 g. (0.12 mole) of bromobenzene in 100 ml. of dry ether. After addition of

bromobenzene was complete, the mixture was stirred for an additional one-half hour. Dropwise addition of 10.1 g. (0.10 mole) of dimethoxyacetonitrile in 50 ml. of dry ether at room temperature took approximately one hour. Near the end of the addition of dimethoxyacetonitrile, the solution became cloudy and a gummy residue deposited on the sides of the flask. Dilute sodium hydroxide was added, and the ether phase was separated and dried over sodium sulfate. Removal of the solvent left a yellow oil, which showed ketimine absorptions in the infrared spectrum at 3240 cm^{-1} (NH) and 1620 cm^{-1} (C=N). This oil was shaken with dilute sodium hydroxide solution until the evolution of ammonia ceased. Extraction with ether gave an oil which lacked the ketimine infrared absorption bands. Distillation of this oil gave 13 g. (72%) of phenylglyoxal dimethyl acetal, b.p. 108° at 4.7 mm., n_D^{25} 1.5161; reported (9) b.p. 109° at 5 mm., n_D^{20} 1.5157.

The infrared spectrum exhibited bands at 1695 cm^{-1} (aryl ketone), 1120 and 1066 cm^{-1} (acetal).

The N.M.R. spectrum displayed peaks at 2.82 s (6H), 4.65 s (1H), 6.91 m (3H), and 7.67 dd (2H).

Preparation of α-Aminophenylacetaldehyde Dimethylacetal. To 2.88 g. (0.12 g. atom) of magnesium was added dropwise 18.8 g. (0.12 mole) of bromobenzene in 100 ml. of dry ether. After addition of bromobenzene was complete, the mixture was stirred for an additional 1.5 hr. Addition of

10.1 g. (0.10 mole) of dimethoxyacetonitrile in 50 ml. of dry ether was performed dropwise at room temperature over a period of one hour; and a gummy residue deposited on the sides of the flask. A slurry of 3.8 g. (0.10 mole) of lithium aluminum hydride in 200 ml. of dry tetrahydrofuran was carefully added with cooling to the Grignard intermediate. After all the lithium aluminum hydride was added, the reaction mixture was allowed to stir overnight at reflux. Hydrolysis of the ice-cooled mixture was effected by careful addition of 4 ml. of water, 3 ml. of 20% sodium hydroxide, and finally 14 ml. of water, which resulted in the precipitation of inorganic matter. The mixture was filtered, the solid washed with ether, the combined filtrates dried over sodium sulfate, and the solvent removed in vacuo. Distillation of the residual oil gave 13.7 g. of a main fraction, b.p. 75-82° at 0.6 mm. Redistillation through a spinning band column gave 12.5 g. (69%) of colorless oil, b.p. 67.5° at 0.5 mm., n_D^{25} 1.5107; reported (32) b.p. 134-136° at 18 mm.

Anal. Calcd. for $C_{10}H_{15}NO_2$: C, 66.27; H, 8.34; N, 7.73. Found: C, 65.75; H, 8.33; N, 8.03.

The N.M.R. spectrum displayed peaks at 1.57 s (2H), 3.16 s (3H), 3.34 s (3H), 3.97 and 4.29 (2H), AB quartet, and 7.34 m (5H).

An experiment in which tetrahydrofuran was used as the solvent in place of ether gave the amino acetal in

essentially the same yield.

Preparation of ∝-Aminophenylacetaldehyde Dimethylacetal Hydrochloride. Dry hydrogen chloride was bubbled into a solution of 0.5 g. (2.8 mmoles) of ∝-aminophenylacetaldehyde dimethylacetal in 10 ml. of ether. The colorless product precipitated and was filtered and washed with ether. Recrystallization from absolute ethanol gave 0.55 g. (92%) of colorless needles, m.p. 170-171°.

Anal. Calcd. for $C_{10}H_{16}ClNO_2$: C, 55.17; H, 7.41; N, 6.43. Found: C, 55.17; H, 7.19; N, 6.21.

Preparation of N-Acetylaminophenylacetaldehyde Dimethylacetal. The procedure employed for the N-benzoyl derivative below was used. When 5 g. (28 mmoles) of aminophenylacetaldehyde dimethylacetal was treated with 2.16 g. (28 mmoles) of acetyl chloride, a yellow oil, which crystallized on standing, was produced. Recrystallization from water gave 2.0 g. of the N-acetyl derivative, m.p. 116.5-117°.

Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.55; H, 7.68; N, 6.27. Found: C, 64.42; H, 7.52; N, 6.39.

Preparation of N-Benzoylaminophenylacetaldehyde Dimethylacetal. To 5.0 g. (0.028 mole) of aminophenylacetaldehyde dimethylacetal in a solution of 100 ml. of dry benzene and 25 ml. of pyridine was added 2.88 g. (0.028 mole) of benzoyl chloride in 10 ml. of benzene (29). The mixture was warmed to 65°, stirred for 0.5 hr., then poured into 100 ml.

of 5% sodium carbonate solution, the benzene phase separated, washed well with water, dried over sodium sulfate and concentrated. The solid product was precipitated by adding hexane. Recrystallization from aqueous methanol gave 6.7 g. (85%) of crystalline amide, m.p. 144-145°.

Anal. Calcd. for $C_{17}H_{19}NO_3$: C, 71.56; H, 6.71; N, 4.91. Calcd. for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.64; H, 5.83; N, 5.15.

The infrared spectrum (CCl_4) showed significant bands at 3472 cm^{-1} (NH) and 1675 cm^{-1} (amide).

Preparation of N-Benzoylaminophenylacetaldehyde Diethylacetal. Dry hydrogen chloride was passed briefly into a solution of 1.0 g. of N-benzoylaminophenylacetaldehyde dimethylacetal in 200 ml. of absolute ethanol. The solution was then allowed to stand at room temperature for 10 hrs. Sufficient ethanolic potassium hydroxide was then added to neutralize the acid. Concentration of the solution, dilution with water, and extraction with ether gave 0.97 g. (88%) of the crystalline product. Recrystallization from aqueous methanol gave needles, m.p. 125-127°. This material was resubmitted to the above conditions and the resulting product recrystallized from aqueous methanol giving an analytical sample, m.p. 117°.

Anal. Calcd. for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40. Found: C, 72.88; H, 7.45.

The infrared spectrum (CCl_4) showed significant bands

at 3470 cm^{-1} (NH) and 1675 cm^{-1} (amide).

The N.M.R. spectrum displayed peaks at 1.15 t (3H), 1.20 t (3H), 3.66 m (4H), 4.71 d (1H) (J=3 c.p.s.), 5.39 dd (1H), 7.12 d (1H), 7.42 m (8H), and 7.85 dd (2H).

The 2,4-dinitrophenylhydrazone prepared in the usual manner (33) had m.p. 183-184° after recrystallization from ethanol.

Anal. Calcd. for $C_{21}H_{17}N_5O_5$: C, 60.14; H, 4.09. Found: C, 60.19; H, 4.05.

SUMMARY

The reaction of Grignard reagents (R = phenyl, methyl, i-propyl, sec-butyl, i-butyl, and benzyl) with dimethoxyacetonitrile provides intermediate adducts (I) which readily furnish the related α-imino, α-keto and α-amino acetals. Methanolysis of (I) produces the α-imino acetals (II); and hydrolysis of (I) with ammonium chloride gives the α-keto acetals (III). Reduction of (I) with lithium aluminum hydride furnishes the α-amino acetals (IV). In the six examples investigated thus far, no significant difficulties or side reactions were encountered.

This method constitutes an improvement and simplification of synthetic approaches to these vicinally difunctional compounds, which are of interest themselves as well as for intermediates for potential drugs useful in cancer chemotherapy and for intermediates in the synthesis of a variety of heterocyclic systems.

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III. STUDIES DIRECTED TOWARD THE CHARACTERIZATION OF MAMMOSIN, A DITERPENE LACTONE ISOLATED FROM THE GORGONIAN, EUNICEA MAMMOSA LAMOUROUX

INTRODUCTION

Mammosin is the name given to a diterpene lactone which has been isolated from the pentane extracts of Eunicea mammosa Lamouroux, a marine invertebrate commonly classified as a gorgonian (1). Preliminary studies of the structure of this compound have disclosed the functional groups present (2). Mammosin, $C_{20}H_{30}O_4$, was found from spectral and chemical data to contain a γ-lactone group, a tertiary hydroxyl group, three methyl groups, two double bonds, and an inert oxygen, presumed to exist as an ether group. Based on the above formula and functional groups, it was concluded that mammosin contains one carbocyclic ring. Failures in attempts to obtain an aromatic compound on dehydrogenation led to the conclusion that mammosin possesses a large carbocyclic ring.

Since the aid of N.M.R. spectroscopy was not readily available during these preliminary studies, determination of structural features was limited only to information gained

from the various chemical transformations and to the interpretation of the U.V. and I.R. data of the various products obtained. It will be seen from the present work that N.M.R. spectroscopy greatly assisted in determining most of the structural features of mammosin.

RESULTS AND DISCUSSION

The molecular weight of 334, determined by mass spectrometry, confirmed the molecular formula $C_{20}H_{30}O_4$ for mammosin. Despite the presence of two double bonds, mammosin absorbed only one mole of hydrogen and gave a mixture of two diastereoisomeric dihydro derivatives. After chromatography on Florisil, one diastereomer, m.p. 195°, was obtained pure. Comparisons of the U.V., I.R., and N.M.R. spectra of mammosin and the dihydro derivative could be interpreted in terms of saturation of an isolated double bond and isomerization of an α,β-unsaturated γ-lactone grouping. Since double bond isomerization occurs during the catalytic hydrogenation, the product is named dihydroisomammosin (II).

Although mammosin exhibits no characteristic U.V. absorption above 220 mμ, the catalytic dihydro compound showed a maximum at 226 mμ (ϵ 16,500). The lactone carbonyl and double bond absorptions in the I.R. spectrum of mammosin (1765 cm^{-1} and 1667 cm^{-1}, respectively), shown in Figure 1, were shifted to 1740 cm^{-1} and 1680 cm^{-1}, respectively, in the dihydro compound. The N.M.R. spectrum of mammosin exhibited a pair of low field doublets at 5.7 p.p.m. (J=3 c.p.s.) and

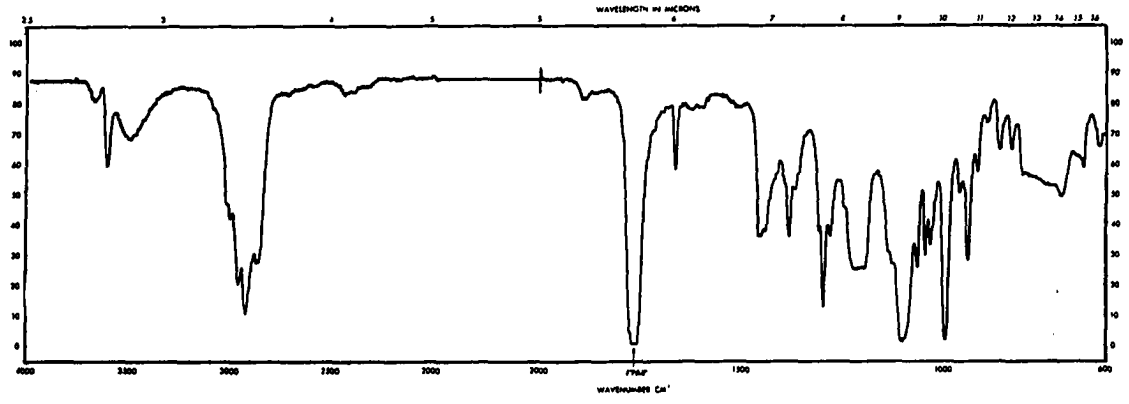


Fig. 1.--Infrared Spectrum of Mammosin

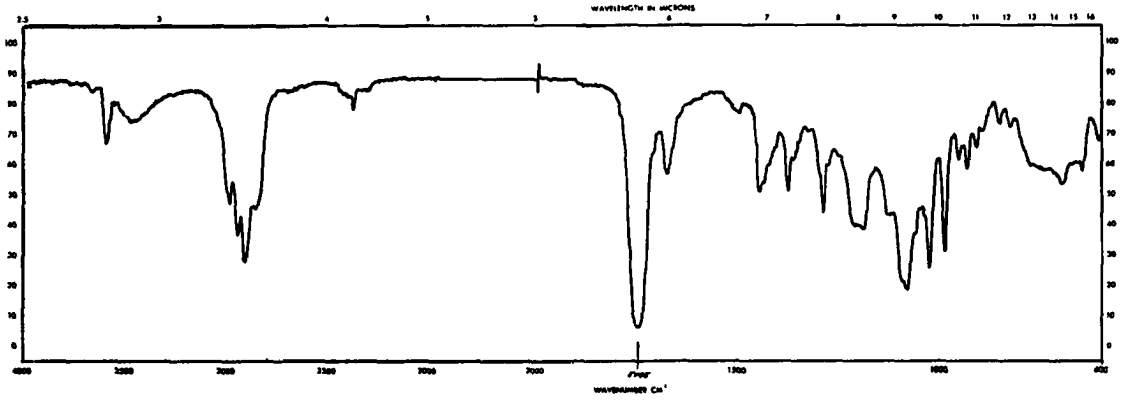


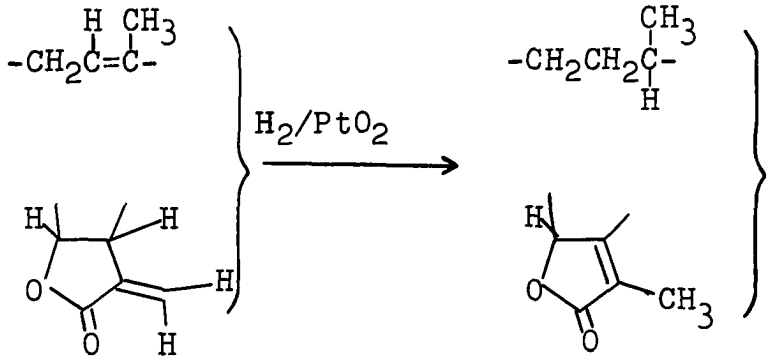
Fig. 2.--Infrared Spectrum of Isomammosin

6.4 p.p.m. (J=3 c.p.s.), each integrating for one proton. Another olefinic proton appeared as a triplet at 5.1 p.p.m., indicative of an olefinic proton on a carbon atom adjacent to a methylene group. A vinyl methyl group singlet appeared at 1.5 p.p.m. The N.M.R. spectrum of the catalytic dihydro compound displayed a new methyl group singlet at 1.85 p.p.m., which is characteristic of a vinyl methyl group on a carbon atom adjacent to a carbonyl, and a methyl group doublet at 1.00 p.p.m. (J=6 c.p.s.). The three olefinic protons in mammosin, as well as the vinyl methyl group at 1.5 p.p.m., were missing in this spectrum.

The nature of the double bonds was inferred from the spectral data. The infrared absorption at 1740 cm^{-1} for the lactone carbonyl in the dihydro compound is characteristic of intracyclic α,β-unsaturated γ-lactones (3). The U.V. data is also consistent with such a structure as van Tamelen has pointed out in his work with α-methylenic γ-lactones (4, 5). The I.R. absorption for the lactone carbonyl in this system appears in the region (1770 cm^{-1}) of the spectrum characteristic of saturated γ-lactones (6-8).

The low field doublets in the N.M.R. spectrum of mammosin are characteristic of an exomethylene group conjugated with a γ-lactone, as shown in partial structure (I).
W. Herz and others have studied numerous sesquiterpene lactones containing this α-methylenic γ-lactone system (6, 9-17).

With the α-methylenic γ-lactone system established in mammosin, the remaining olefinic proton at 5.1 p.p.m. and the vinyl methyl group at 1.5 p.p.m. must reside on adjacent carbon atoms of a second double bond. If they were on the same carbon, the splitting pattern of the olefinic proton would appear more complicated than the observed triplet. Furthermore, the appearance of a new secondary methyl group at 1.00 p.p.m. (J=6 c.p.s.) confirms the partial structure (II) for the dihydro compound.

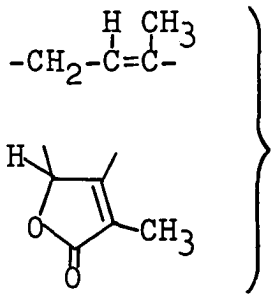


(I) (II)

Catalysts such as platinum and palladium frequently cause such double bond migrations. This is the case when the endocyclic double bond is difficultly reduced, as is frequently the case. In most instances mixtures occur and the composition of the mixture, composed of the reduced and isomerized exomethylene double bond products, depends usually on the hydrogenation conditions employed (10). In the hydrogenation of mammosin very little reduction of the conjugated exomethylene group takes place and that which does probably

occurs before isomerization, since dihydroisomammosin (II) resists further hydrogenation even under a pressure of 1300 pounds (2).

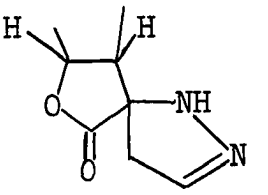
Double bond isomerization without concurrent saturation of the trisubstituted double bond was achieved by hydrogenating platinum oxide in absolute ethanol and then replacing the hydrogen atmosphere with nitrogen. Mammosin dissolved in absolute ethanol was carefully added, so as not to let air enter the system, to the activated catalyst and stirred for about twelve hours. Mammosin (I) was quantitatively converted to isomammosin (III) by this procedure. Isomammosin has a U.V. absorption at 227 mμ (ϵ 25,800) and significant I.R. absorptions at 1745 cm^{-1} and 1681 cm^{-1}, shown in Figure 2. The N.M.R. spectrum showed the exomethylene protons missing and displayed the new vinyl methyl group singlet at 1.85 p.p.m.



(III)

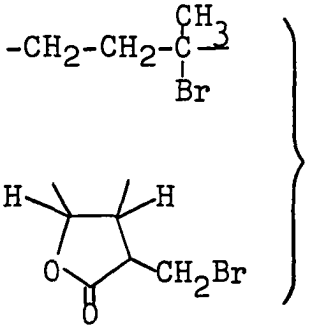
The presence of the exomethylene group was further established by ozonolysis of mammosin to give formaldehyde as the dimedone derivative and also by reaction with diazomethane to give the pyrazoline, (IV). Pyrazoline derivatives have

been used by van Tamelen to characterize the ∝-methylenic ɣ-lactone group (5).



(IV)

Further characterization of the two double bonds in mammosin was performed by the preparation of a dihydrobromide derivative (V) without the formation of a new hydroxyl group, which might occur if the ether were an epoxide and had been opened by hydrogen bromide to the bromohydrin. The regeneration of mammosin, although in low yield, by dehydrobromination with pyridine suggests that no skeletal isomerization occurred during the formation of the dihydrobromide derivative.

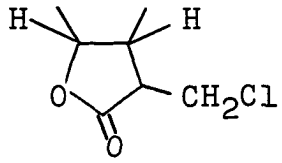


(V)

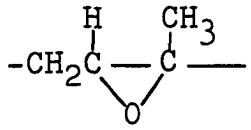
Quantitative epoxidation of mammosin with monoperphthalic acid gave a mono-epoxide derivative, while reaction with hydrogen chloride gave a mono-hydrochloride derivative. The N.M.R. spectra of the two compounds revealed

that they were derivatives of different double bonds.

The hydrochloride derivative displayed the olefinic proton triplet at 5.2 p.p.m. and the vinyl methyl group singlet at 1.5 p.p.m. The absence of the downfield exomethylene protons indicated the occurrence of Michael addition of hydrogen chloride to the α-methylenic γ-lactone group to give the partial structure (VI). No reaction occurred at the relatively hindered trisubstituted double bond; and a positive tetranitromethane test also confirmed its presence. However, the more reactive hydrogen bromide did add also to this double bond to give the dihydrobromide (V), although dihydroisomammosin (II) was unreactive toward hydrogen bromide.



(VI)



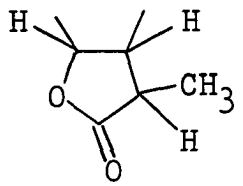
(VII)

Formation of an epoxide having the partial structure (VII) is indicated on the basis that the isolated double bond is reactive toward the electrophilic reagent; while the conjugated exomethylene group would be expected to be unreactive (18). Appearance of a methyl group singlet at 1.25 p.p.m. in the N.M.R. spectrum with disappearance of the vinyl methyl group substantiates this structural assignment.

Also, the intact α-methylenic γ-lactone group, now

in the absence of the strong U.V. end absorption displayed by the trisubstituted double bond, exhibits a maximum at 206 mμ (ϵ 10,300), which is characteristic of this type of group (10).

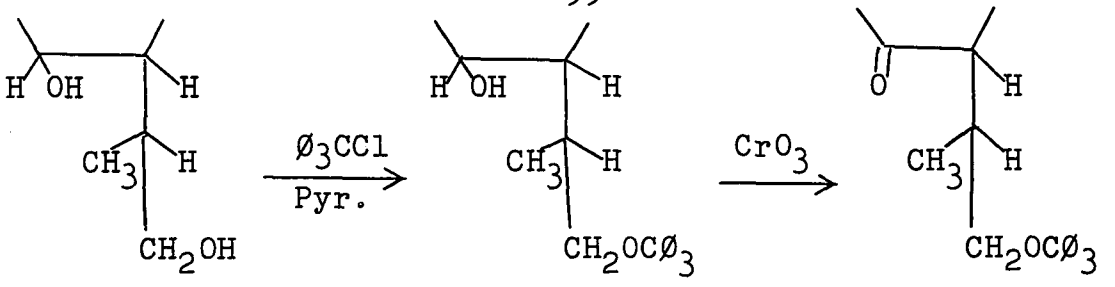
Mammosin was converted to dihydromammosin, represented by partial structure (VIII, by chemical reduction with sodium in refluxing butanol. This method reduces double bonds of unsaturated esters but not isolated double bonds (19).



(VIII)

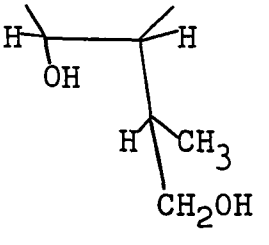
The N.M.R. spectrum showed the exomethylene group missing and the appearance of a new methyl group doublet at 1.25 p.p.m. (J=6 c.p.s.), consistent with the partial structure (VIII).

Reduction of dihydromammosin (VIII) with lithium aluminum hydride produced a diol (IX), which formed the trityl ether (X) on treatment with trityl chloride in pyridine. Oxidation of this trityl ether with chromium trioxide and pyridine gave a ketone (XI) with an I.R. absorption at 1717 cm^{-1}. This sequence of reactions confirmed the presence of a single proton on the carbon atom bonded to the lactone oxygen.



(IX) (X) (XI)

Reduction of mammosin (I) with lithium aluminum hydride gave a gummy solid that gave a positive tetranitromethane test and absorbed one mole equivalent of hydrogen on catalytic hydrogenation. This hydrogenated product gave a negative tetranitromethane test. It was concluded that the hydrogenated product had the partial structure (XII) and consisted of a mixture of diastereoisomers. Lithium aluminum hydride apparently reduced the exomethylene group as well as the lactone group. In creating the new asymmetric center on reduction of the exomethylene group, a mixture of diastereomers resulted; catalytic reduction of this mixture created still another asymmetric center, thus giving an even more complicated mixture.

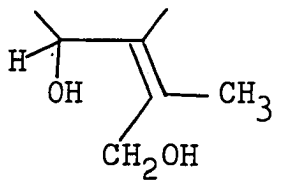


(XII)

Reduction of (I) with sodium borohydride, surprisingly, also reduced both the carbonyl and double bond of the

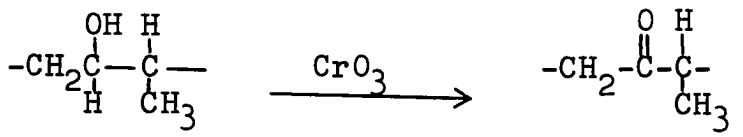
lactone grouping and gave a mixture of stereoisomers. There are several examples of reduction of the conjugated double bond in butenolide systems (20, 21) using sodium borohydride, one (21) of which is an α-methylenic γ-lactone system; but reduction of both the conjugated double bond and lactone carbonyl groups was unexpected.

Dihydroisomammosin (II) on reduction with lithium aluminum hydride earlier (2) had given a product, whose analysis suggested the partial structure (XIII). Apparently, the very hindered intracyclic conjugated double bond was not reduced by lithium aluminum hydride.



(XIII)

Hydroboration (22) of isomammosin (III) with bis-(3-methyl-2-butyl) borane readily occurs at the trisubstituted double bond. Alkaline hydrogen peroxide oxidation of the intermediate alkyl borane produced the alcohol (XIV), which was further oxidized to (XV) with chromium trioxide and pyridine.

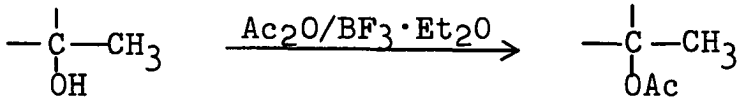


(XIV) (XV)

This ketone failed to dehydrate in alkaline ethanol and it was concluded that the tertiary hydroxyl group was therefore not beta to the ketone carbonyl.

The nature of the hydroxyl group in mammosin was shown to be tertiary by its unreactivity toward reagents expected to furnish derivatives of primary or unhindered secondary hydroxyl groups (2). The possibility of a hindered secondary hydroxyl was ruled out by the N.M.R. spectrum of mammosin in dimethyl sulfoxide. A sharp singlet at 4.34 p.p.m. for the hydroxyl proton indicated the absence of a proton on the carbon bonded to the hydroxyl group (23).

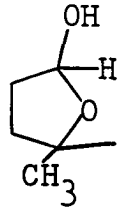
Mammosin, isomammosin, or dihydromammosin failed to dehydrate under mild conditions employing potassium hydrogen sulfate in anhydrous dioxane (24). Dehydration with thionyl chloride and pyridine, however, gave a mixture of products (25). An N.M.R. spectrum of this mixture showed part of a vinyl methyl group and part of an exomethylene group, indicating the dehydration of a methyl carbinol system (XVI). Further verification of this grouping was provided by acetylation of the tertiary hydroxyl group with acetic anhydride and boron trifluoride etherate at -20° for 1.5 hrs. Mammosin acetate (XVII) thus produced did not exhibit a paramagnetic



(XVI) (XVII)

shift of a proton bonded to a carbinol carbon, ordinarily observed in the N.M.R. on acylation of a primary or secondary hydroxyl group (26). However, the tertiary methyl group at 1.15 p.p.m. did exhibit a paramagnetic shift to 1.45 p.p.m. on acylation of the hydroxyl group.

The relationship between the trisubstituted double bond and the tertiary hydroxyl group in mammosin was partly revealed by ozonolysis. Selective ozonolysis of the trisubstituted double bond was carried out under controlled conditions (27) in methylene chloride-pyridine (99:1) at -70°. The progress of the reaction was followed by thin layer chromatography. The gummy product isolated exhibited I.R. absorptions corresponding to γ-lactone, ketone and double bond; and its N.M.R. spectrum revealed the intact α-methylenic γ-lactone system as well as a methyl group corresponding to a methyl ketone. The absence of the expected aldehyde group suggested the hemiacetal structure (XVIII). The presence in the N.M.R. spectrum of a proton at

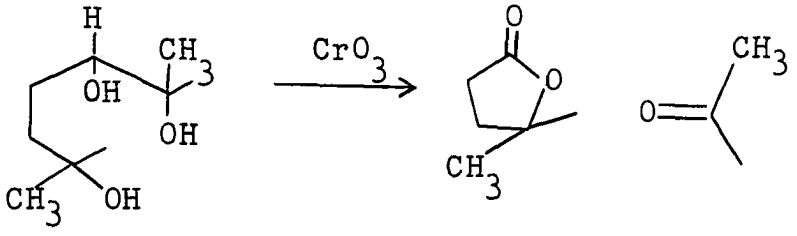


(XVIII)

5.5 p.p.m., attributed to an acetal proton (28), supports this cyclic hemiacetal structure. However, the elemental analysis of this gummy material was not in good agreement

with this structure.

A more direct proof was obtained in the following way. Dihydromammosin (VIII) on hydroxylation with potassium permanganate in acetone readily afforded dihydroxydihydromammosin (XIX). Oxidation of (XIX) with excess chromic acid produced a neutral compound (XX), which had I.R. absorptions corresponding to two γ-lactone groups and a ketone group. A methyl singlet at 2.18 p.p.m. in the N.M.R. spectrum confirmed the presence of the methyl ketone group. A paramagnetic shift of 0.25 p.p.m. for the tertiary methyl group



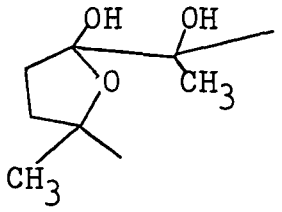
(XIX) (XX)

was also observed. The spontaneous lactonization observed in the above reaction demonstrates that the carbon atom bearing the olefinic proton of the trisubstituted double bond is gamma to the tertiary hydroxyl group.

(XX) could also be prepared directly in satisfactory yield from (VIII) without isolating the intermediate (XIX). This direct procedure has been very convenient for preparing keto lactones from such systems, as in the case of α-terpineol (29).

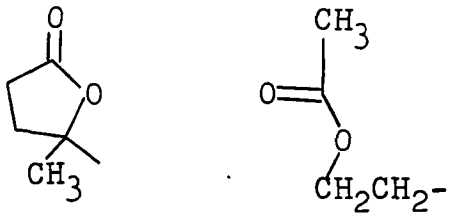
Attempted chromic acid oxidation of (XIX) to the

∝-hydroxy ketone by using a stoichiometric amount of chromium trioxide gave a compound whose infrared spectrum suggested the hemiketal structure (XXI).



(XXI)

Oxidation of (XX) with peroxytrifluoroacetic acid by the procedure of Emmons (30) led in good yield to the acetate (XXII). Its N.M.R. spectrum had a methyl singlet at 2.06 p.p.m., which is characteristic of an acetate methyl group.

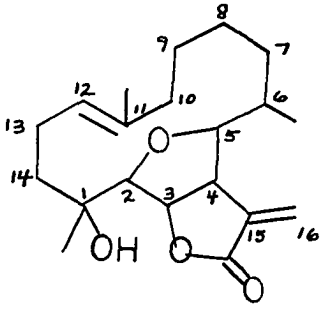


(XXII)

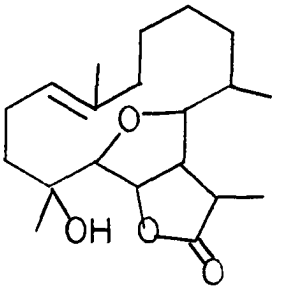
The protons on the carbon bonded to the acetate group appeared at 4.08 p.p.m. as a triplet. This suggested the presence of a methylene group adjacent to the carbon bonded to the acetate group.

Based on the analytical results and the determination of the functional groups mentioned above, it is concluded that mammosin contains a monocarbocyclic ring system. The structure (Ia) is proposed for mammosin on the basis of the chemical transformations outlined above and the following interpretation of its N.M.R. spectrum, shown in Figure 3, as

well as the spectrum of dihydromammosin (VIIIa), shown in Figure 4. The use of nuclear magnetic double resonance (N.M.D.R.) spectroscopy aided substantially in arriving at the structure (Ia).



(Ia)



(VIIIa)

The double doublet at 4.43 p.p.m. in the N.M.R. spectrum of mammosin is attributed to the proton at C-3 and arises from the spin coupling of the C-3 proton with the C-2 and C-4 protons. This was demonstrated, as shown in Table 1, by collapse of the double doublet to a doublet on irradiation at the frequency of the C-2 proton, which is at 2.85 p.p.m. Likewise, by decoupling the C-2 proton from the C-3 proton on double irradiation at the C-3 proton frequency, the doublet at 2.85 p.p.m. collapsed to a singlet. C-1 then has no hydrogen atoms bonded to it since the C-2 proton is spin coupled only to the C-3 proton. The methyl carbinol system then must be at C-1. This conclusion requires no other quaternary centers in the molecule, which are, however, not possible on the basis of a single carbocyclic ring system. The trisubstituted double bond could not be situated adjacent to C-2 to account for this on the basis of the chemical

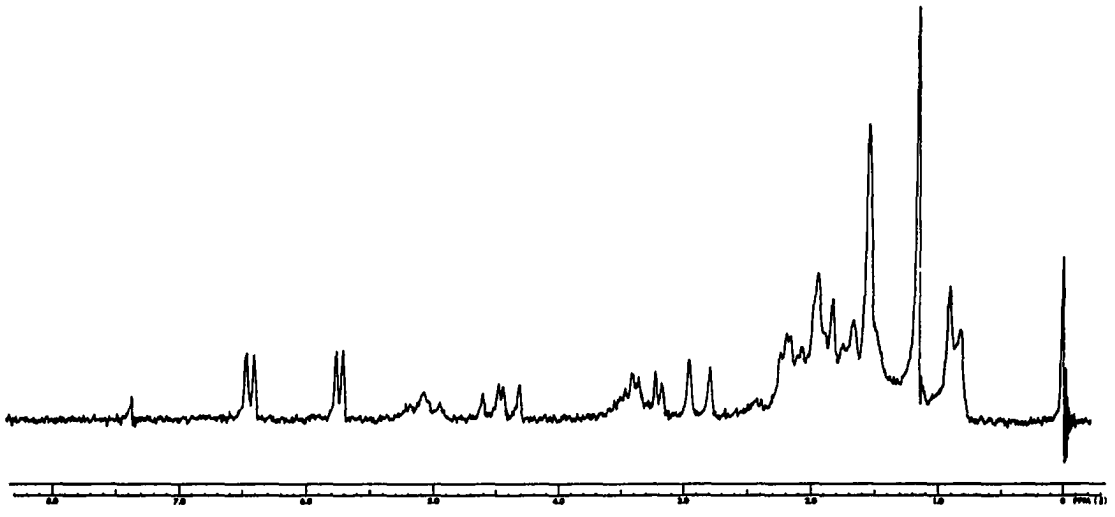


Fig. 3.--N.M.R. Spectrum of Mammosin (Ia)

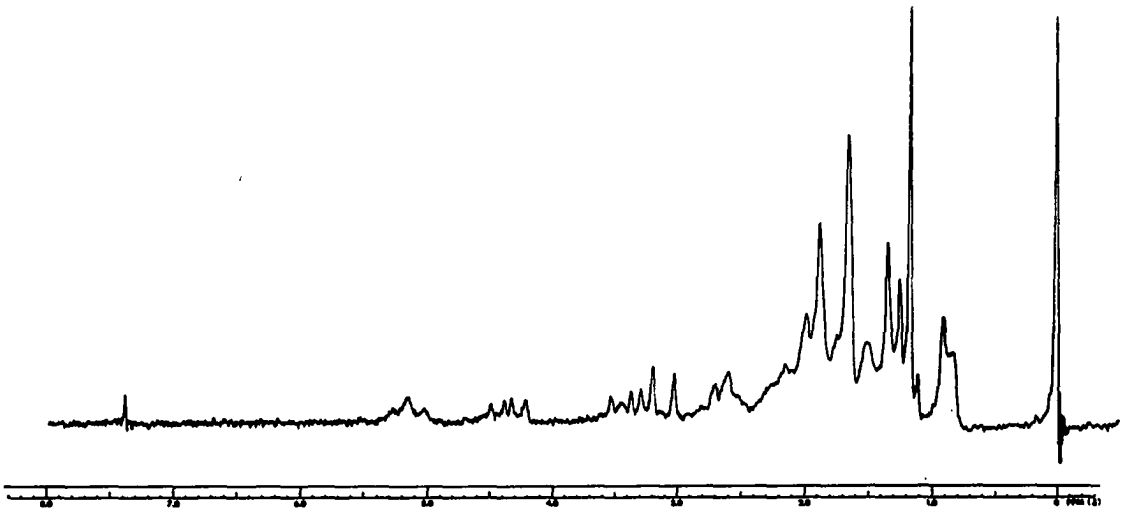
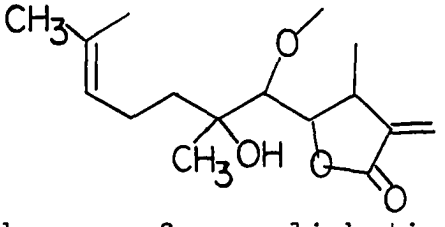


Fig. 4.--N.M.R. Spectrum of Dihydromammosin (VIIIa)

transformations leading to the partial structure (XXII). By then putting together the lactone, ether, and tertiary hydroxyl groups and combining them with the trisubstituted double bond that is beta to the tertiary hydroxyl group, we arrive at the partial structure (XXIII). By combining (XXIII) with the N.M.R. spectrum for a disecondary ether as well as



(XXIII)

the absence of any aliphatic side chains, we can conclude a 14-membered ring structure for mammosin. More information used in arriving at the structure (Ia) was supplied by the following N.M.D.R. results.

The proton at C-4, represented by the multiplet at 3.5 p.p.m., was studied by spin decoupling. The low field doublet at 6.42 p.p.m., attributed to one of the exomethylene protons, collapsed to a singlet on double irradiation at the frequency of the proton at 3.5 p.p.m. Likewise, the doublet at 5.68 p.p.m. also collapsed to a singlet by decoupling with the proton at 3.5 p.p.m. Thus, the allylic C-4 proton is spin coupled to the exomethylene protons as well as the C-3 and C-5 protons. This information readily explains the large multiplicity of this signal. Prior to this observation we had assumed that the pair of exomethylene doublets resulted

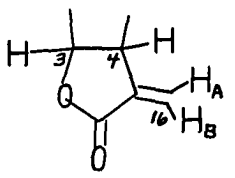
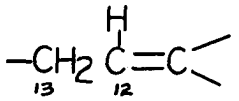
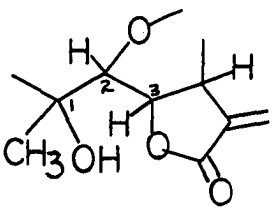
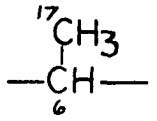
from mutual spin coupling; however, they must not be coupled with each other or their coupling constant is essentially zero. This allylic coupling situation enabled us therefore to locate the C-4 proton as the multiplet at 3.5 p.p.m. This was confirmed by the spectrum of dihydromammosin (VIIIa).

The peak at 3.5 p.p.m. in the spectrum of mammosin is not present in the spectrum of (VIIIa) since the C-4 proton is no longer allylic, but the appearance of two protons at about 2.6 p.p.m. in the spectrum of (VIIIa) suggested that these protons were those at C-4 and C-5. This was proven by decoupling the C-3 and C-4 protons in (VIIIa) by double irradiation at the C-4 proton (2.6 p.p.m.) frequency. The double doublet at 4.32 p.p.m., attributed to the C-3 proton, collapsed to a doublet. Also, by decoupling the protons of the new secondary methyl group at 1.27 p.p.m. with one of the protons at 2.6 p.p.m., the methyl doublet collapsed to a singlet. This then confirmed the suspected position of the C-15 proton as one of the protons at 2.6 p.p.m. These results are summarized in Table 2.

The chemical shift of the C-6 proton in mammosin was located at 1.83 p.p.m. by finding the double irradiation frequency where the C-6 methyl doublet simplified to a singlet. Decoupling this C-6 proton in dihydromammosin (VIIIa) then with the C-5 proton at 3.36 p.p.m. simplified the double doublet to what appeared to be a broad singlet. This broad singlet is probably the result of partial decoupling also

TABLE 1

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

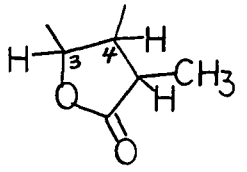
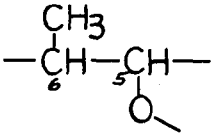
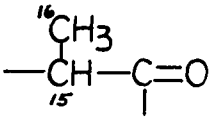
Irradiated	Proton,[a] δ Assignment	Observed	Multiplicity[b] Change
C-4, 3.43		6.42, C-16B	d to s
C-4, 3.43		5.68, C-16A	d to s
C-13, 2.04		5.07, C-12	t to s
C-3, 4.43		2.85, C-2	d to s
C-2, 2.85		4.43, C-3	dd to d
C-6, 1.83		0.85, C-17	d to s

[a] "C-4" refers to the number of the carbon to which the proton(s) is attached.

[b] Abbreviations: s means singlet; d, doublet; t, triplet; dd, double doublet; and m, multiplet.

TABLE 2

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

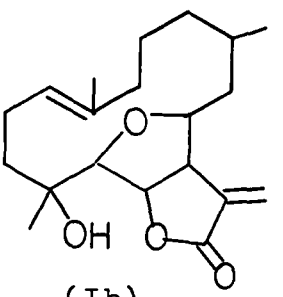
Irradiated	Proton, δ Assignment	Observed	Multiplicity Change
C-4, 2.60		4.32, C-3	dd to d
C-6, 1.83		3.36, C-5	dd to broad s
C-15, 2.60		1.27, C-16	d to s

with the C-4 proton, since it differs by only about 40 c.p.s. from the proton at C-5.

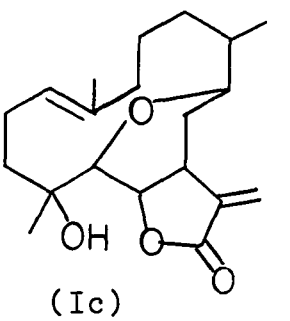
The proposed spin coupling between the proton on C-6 and the proton on C-5 is consistent with the data, but it is not certain since the C-6 proton chemical shift is simply that of ordinary methylene protons. Thus, the observed decoupling could as well have occurred with any of the other protons with a similar chemical shift.

By the biogenetic isoprene rule we prefer to place the secondary methyl group on C-7 as in (Ib); yet the double doublet represented by the C-5 proton permits coupling with only one proton on C-6. The secondary methyl is therefore placed on C-6, assuming that the ether is bonded at C-5.

Otherwise, placing the secondary methyl group at C-7 as in (Ib) would require the coupling constant for the spin coupling between the C-5 ether proton and one of the C-6 methylene protons to be zero. Or, if the ether were bonded at C-6 and the secondary methyl group were at C-7, as in (Ic), the coupling constant between one of the methylene protons at C-5 and the proton at C-6 would have to be zero. Since there is no valid reason for this type of spin coupling to occur in a large ring system, the structure (Ia) is favored. Obviously, further chemical evidence is required to rigorously prove these remaining uncertain features of the molecule.



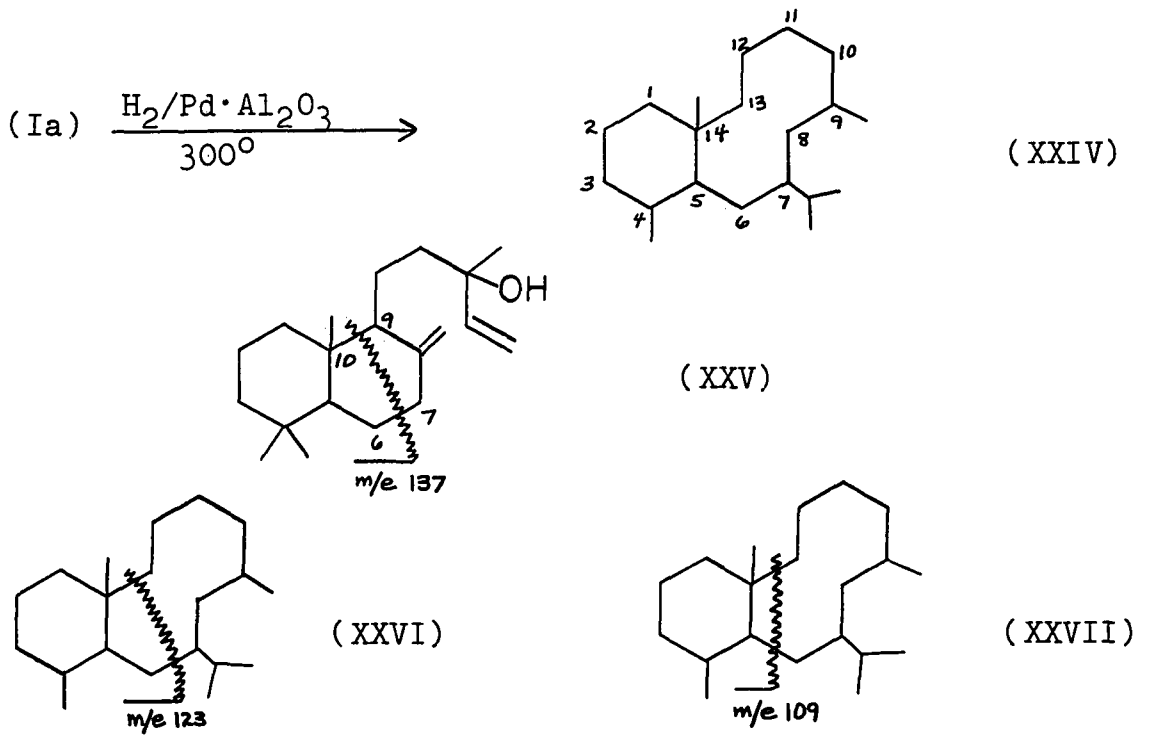
(Ib)



(Ic)

In an attempt to confirm the 14-membered ring structure, mammosin was subjected to a vapor phase hydrogenolysis employing palladium-coated alumina pellets at 300°. A mixture of hydrocarbons was obtained from which a major component was isolated pure by preparative gas chromatography. This hydrocarbon was shown by mass spectrometry to have the formula, $C_{20}H_{38}$. A molecular ion peak was found at m/e 278. Intense peaks at m/e 263 and m/e 235 corresponded to loss of

methyl (mass 15) and isopropyl (mass 43) groups, respectively. From the above formula the hydrocarbon apparently was bicyclic. From the proposed structure (Ia) for mammosin, the hydrocarbon formed on hydrogenolysis could have the bicyclic structure (XXIV) formed by ring closure during cleavage of the ether ring. From the fairly general fragmentation pattern for bicyclic and tricyclic diterpenes such as manool



(XXV), where cleavage of the 6-7 and 9-10 bonds and associated loss of a hydrogen atom from the charge-retaining ring A fragment (31) occurs, we could expect similar behavior from the hydrocarbon (XXIV). This is a reasonable fragmentation pattern for this structure since the ring A fragments would be expected to give the most significant and intense peaks. Besides the two fragments already mentioned, the mass spectrum

exhibited significant peaks at m/e 123 and m/e 109 which could reasonably arise from fragmentation in the manner shown for (XXVI) and (XXVII).

In a portion of our structural studies, we were concerned with the possible cleavage of the ether ring and with various oxidative cleavages of the lactone group. In most cases such a complex mixture of products was obtained that it was difficult to obtain materials sufficiently pure to allow any structural interpretation; and in some cases the isolated products appeared to be more complicated than the starting materials. For instance, in attempting a cleavage of the ether by reaction with acetic anhydride and boron trifluoride etherate at -20° for three hours, mammosin acetate was obtained contaminated with another compound, $C_{24}H_{34}O_6$. This compound was prepared in good yield by extending the reaction time to seven hours. Its infrared spectrum showed γ-lactone, ester, and ketone carbonyl absorptions. On hydrolysis with aqueous sodium hydroxide, a ketone, $C_{20}H_{30}O_4$, was obtained. It was further shown to be a methyl ketone by the methyl singlet at 2.05 p.p.m. in its N.M.R. spectrum.

An analogous compound was obtained from isomammosin under the same reaction conditions, but dihydroisomammosin gave only its acetate ester. The compound derived from isomammosin on catalytic hydrogenation absorbed one mole of hydrogen and gave a compound, $C_{24}H_{36}O_6$, whose infrared spectrum showed γ-lactone, acetate, and ketone carbonyl absorptions.

The formation of these ketone products with mammosin and isomammosin, but not with dihydroisomammosin, leads to the conclusion that the trisubstituted double bond probably was acylated to a methyl ketone which underwent rearrangement. Because of the probable occurrence of these acylation and rearrangement reactions with these materials, they probably no longer contained the original skeleton; therefore, no further work was performed with them.

In earlier work (2), oxidation of dihydroisomammosin (II) with permanganate led to a ketone, $C_{17}H_{28}O_4$. In attempting a milder oxidation of (II) with permanganate, two isomeric γ-lactones, $C_{19}H_{32}O_4$, were isolated. However, they were obtained in rather low yields by laborious chromatography of a complex mixture.

Oxidation of mammosin with chromic acid at room temperature gave a mixture which was shown by thin-layer chromatography to be very complex. On column chromatography, the material first eluted was a crystalline ketone, $C_{20}H_{28}O_6$, whose N.M.R. spectrum indicates reaction at the trisubstituted double bond; yet, we were unable to propose a reasonable structure for this compound that is consistant with the elemental and spectral data. Material later eluted failed to yield any useful products.

Ozonolysis of dihydroisomammosin (II) had led earlier (2) to a ketone, $C_{16}H_{28}O_4$. In the present work, ozonolysis of (II) under different conditions gave a mixture from which

was isolated a compound, $C_{16}H_{26}O_4$, whose infrared spectrum showed aldehyde and γ-lactone absorptions. The N.M.R. spectrum of this compound indicated the aldehyde group to be on a secondary carbon. The absence of an hydroxyl group suggested lactonization of the tertiary hydroxyl group with the newly formed carboxyl group. The N.M.R. spectrum also displayed two protons at about 4.1 p.p.m., presumed to be ether protons. Since these protons are at lower field in this compound than in (II), it is reasonable to conclude that they are deshielded by the aldehyde or lactone groups. This conclusion demonstrates the probability of the proximity of the ether and lactone groups, although no reasonable structure can be proposed for this compound. Assuming that the structure (Ia) for mammosin is correct, this unusual structural situation involving the lactone and ether groups may explain the immense difficulties encountered in these structural studies of mammosin by oxidative methods.

EXPERIMENTAL

All melting points are uncorrected. All commercial solvents were redistilled. Tetrahydrofuran was distilled from lithium aluminum hydride. Diazomethane was prepared from EXR-101 (Du Pont). Adsorbents used in chromatography were Merck (Darmstadt) alumina, active neutral; Florisil (100/200 mesh); and Mallinckrodt's silicic acid (powder).

Thin layer chromatography was performed on 5 x 20 cm. glass plates coated with Merck (Darmstadt) silica gel H. Substances on a developed plate were rendered visible with iodine vapor. Gas chromatography was conducted with a Barber-Colman, Model 20, a Micro-Tek, GC-1600 or an Aerograph Autoprep, A-700. The ultraviolet absorption spectra were obtained from 95% ethanol solutions in 0.1 cm. cells using a Beckman recording spectrometer, Model DK-1. The infrared spectra were run in chloroform or carbon tetrachloride solutions or as potassium bromide pellets. The spectra were recorded on Perkin-Elmer, Model 21 and Beckman IR-8 spectrometers. N.M.R. spectra were taken on a Varian A-60 spectrometer using tetramethylsilane as an internal reference. Samples were run in varying concentrations in deuteriochloroform solutions. The chemical shifts are reported in

δ-values (p.p.m. from TMS) followed by the multiplicity of the signals and corresponding coupling constants. The following symbols are employed to denote multiplicity: s, singlet; d, doublet; t, triplet; dd, double doublet; and m, multiplet.

Analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, Germany. Mass spectra of several samples were kindly provided by Dr. K. Biemann of M.I.T. The vapor phase hydrogenolysis of mammosin was performed by Mr. Paul H. Washecheck. The mass spectrum of the hydrogenolysis product was performed by Dr. C. B. Koons of Jersy Production Co., Tulsa, Oklahoma. The N.M.R. double resonance experiments were kindly performed by Dr. Pat W. Flannigan, Continental Oil Company, Ponca City, Oklahoma.

Mammosin. Mammosin was isolated from the pentane extracts of Eunicea mammosa obtained near Turtle Rock, Bimini, Bahamas (1). The crude pentane extracts were chromatographed on Florisil with benzene giving a colorless solid, which after several recrystallizations from hexane-benzene (3:1), gave an analytical specimen, $C_{20}H_{30}O_4$, m.p. 154-155.5° and $[\alpha]_D^{27.3}$ -89.4° (c=0.75, $CHCl_3$) (2). Analysis for active hydrogen and C-methyl gave results for one active hydrogen and 2.2 C-methyl groups (2). A molecular weight of 334 by mass spectral analysis confirmed the formula $C_{20}H_{30}O_4$. The ultraviolet spectrum displayed strong end absorption which had a shoulder with a maximum probably between 200 and 220 mμ. The

infrared spectrum displayed significant bands at 3623 and 3497 cm^{-1} (hydroxyl), 1765 cm^{-1} (γ-lactone), and 1664 cm^{-1} (double bond). The N.M.R. spectrum in dimethyl sulfoxide showed a sharp singlet at 4.34 indicative of a tertiary hydroxyl group (3). The N.M.R. spectrum in deuteriochloroform showed methyl groups at 1.52 s, 1.15 s, and 0.85 d (J=7 c.p.s.). Olefinic protons appeared at 6.40 d (J=3 c.p.s.), 5.70 d (J=3 c.p.s.), and 5.07 t. The proton on the carbon bonded to the lactone oxygen appeared at 4.43 dd (J=10 c.p.s. and 10 c.p.s.). Two ether protons appeared at 3.25 dd (J=10.5 and 10.5 c.p.s.) and 2.85 d (j=10 c.p.s.). The proton allylic to the exomethylene group appeared as a multiplet at 3.5 p.p.m.

Hydrogenation of Mammosin. A solution of 3.0 g. of mammosin in 50 ml. of absolute ethanol was hydrogenated at atmospheric pressure and room temperature over 0.70 g. of prehydrogenated platinum oxide. The initial uptake of hydrogen was rapid and 0.95 mole of hydrogen per mole of mammosin was taken up in about two hours. After a 24-hr. period hydrogen uptake corresponded to 1.05 moles of hydrogen per mole of mammosin. Filtration of the catalyst and evaporation of the solvent left 3.1 g. of crude dihydroisomammosin which was shown by gas chromatography to consist of a mixture. Chromatography of 1 g. of this mixture on 50 g. of Florisil with benzene (250-ml. fractions) provided the following materials: 0.2 g. (fractions 12-16), m.p. 190-195°; 0.2 g. (fractions

17-21), m.p. 158-190°; and 0.6 g. (fractions 22-26), m.p. 145-152°. After fractions 12-16 were recrystallized several times from hexane, an analytical sample, m.p. 195°, was obtained.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59; mol. wt., 336; one active hydrogen, 0.30. *Found:* C, 71.59; H, 9.42; mol. wt. (mass spectrum), 336; active hydrogen, 0.41.

The ultraviolet spectrum had an absorption peak at 226 mμ (ε 16,500). The infrared spectrum ($CHCl_3$) showed bands at 3450 cm^{-1} (hydroxyl), 1740 cm^{-1} (α,β-unsaturated γ-lactone), and 1680 cm^{-1} (double bond). The N.M.R. spectrum showed methyl signals at 1.85 s, 1.25 s, 0.83 d (J=6 c.p.s.), and 1.00 d (J=6 c.p.s.). The signal at 1.85 is characteristic of a vinyl methyl group adjacent to a carbonyl group. Protons on the carbons bearing the ether oxygen were centered around 3.15 m. The proton on the carbon bearing the lactone oxygen appeared at 4.55 d (J=9 c.p.s.).

Fractions 22-26 were recrystallized from aqueous ethanol and gave needles, m.p. 168-172°. This sample probably contained two diastereomeric dihydroisomammosins, one of which, presumed to be the 195° compound. The infrared spectrum was identical with that of the pure isomer (m.p. 195°). The mother liquor from the above recrystallization afforded a second crop, which, after several recrystallizations from hexane, melted at 109-110°. Despite this rather narrow melting range, this material was not pure. It contained

tetrahydromammosin (see ozonolysis of dihydroisomammosin mixture below), which was identified by thin layer chromatography on silica gel H (benzene-ethyl acetate, 7:3), and presumably the diastereoisomer of the 195° compound. N.M.R. analysis of this material revealed part of a secondary methyl adjacent to a carbonyl, which is consistent with the structure of the tetrahydro derivative. The ultraviolet spectrum, λ max. 226 mμ, had a lower extinction coefficient relative to that of the 195° isomer, indicating the presence of U.V. absorbing material (diastereoisomeric 195° dihydroisomammosin) and a non-absorbing (U.V.) compound (tetrahydromammosin). Finally, the elemental analysis of the 109° mixture fell between values calculated for dihydroiso- and tetrahydromammosin.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59; O, 19.02. Calcd. for $C_{20}H_{34}O_4$: C, 70.94; H, 10.12; O, 18.91. Found: C, 71.27; H, 9.97; O, 19.00.

Catalytic Isomerization of Mammosin to Isomammosin. Gas chromatography on a Micro-Tek, GC-1600 was used to distinguish mammosin from isomammosin in the following experiments. A 10' x 1/16" stainless steel column packed with 5% SE-30 on 120 mesh Gas Chrom Z was employed. The column was maintained at 270°, while the inlet and detector were at 305°. The helium pressure was maintained at 90 psi. with the attenuator at 1 x 64 using sample sizes of 0.1 μl. Mammosin had a retention time of 2.8 min. while isomammosin had a retention time of 3.6 min.

A mixture of 3.8 mg. of platinum oxide in 10 ml. of absolute ethanol was hydrogenated at atmospheric pressure. The system was then evacuated with a water aspirator to remove the hydrogen atmosphere, and a solution of 100 mg. of mammosin in 10 ml. of ethanol was added to the hydrogenated catalyst. With the system evacuated, to prevent the catalyst from being exposed to air, the mixture was stirred for 12 hrs. The catalyst was then filtered and the filtrate evaporated, leaving a colorless crystalline solid, which after recrystallization from hexane gave 98 mg. of isomammosin, m.p. 126-128°. Gas chromatography showed an almost quantitative (98%) conversion to isomammosin.

Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04; O, 19.14. Found: C, 71.91; H, 9.19; O, 19.09.

The ultraviolet spectrum of the product had an absorption maximum at 227 mμ (ε 25,800). The infrared spectrum ($CHCl_3$) exhibited bands at 3610 and 3472 cm^{-1} (hydroxyl), 1745 cm^{-1} (α, β-unsaturated γ-lactone), and 1681 cm^{-1} (double bond). The N.M.R. spectrum showed methyl groups at 1.85 s, 1.56 s, 1.21 s, and 0.94 d (J=6 c.p.s.).

Migration of the double bond of the terminal methylene conjugated with the lactone in mammosin produced the new vinyl methyl group at 1.85. The original olefinic proton at 5.07 t in mammosin was still present, while the proton on the carbon bearing the lactone oxygen was at 4.52 d (J=10 c.p.s.).

In an experiment using 5% platinum-charcoal, the catalyst, previously exposed to hydrogen, was stirred for 2 hrs. with an ethanolic solution of mammosin in the evacuated system. Gas chromatographic analysis of the product showed that the ratio of isomammosin to mammosin was about 3:1.

When platinum-charcoal was used without being exposed to hydrogen, no double bond migration occurred. Likewise, when the catalyst, either platinum oxide or platinum-charcoal, was subjected first to hydrogen and then to the atmosphere, no isomammosin was formed.

The most effective and convenient procedure used to prepare isomammosin involved replacing the hydrogen atmosphere by nitrogen after hydrogenating platinum oxide. The ethanolic solution of mammosin was then carefully added to the catalyst without letting air enter the system. By stirring the mixture under nitrogen for 12 hrs., isomammosin was produced quantitatively (gas chromatographic analysis showed a single peak).

Ozonolysis of Mammosin. Isolation of Formaldehyde. An ozone stream was passed through a solution of 100 mg. of mammosin in 30 ml. of acetic acid at 10° for 15 min. (excess ozone). The resulting solution was steam distilled into an aqueous solution of dimedone, affording 15 mg. (20%) of formaldehyde dimedone derivative, m.p. 188-189°, undepressed on admixture of an authentic sample.

Reaction of Mammosin with Diazomethane. Preparation of the Pyrazoline Derivative. A solution of 100 mg. of mammosin in 5 ml. of ether was mixed with 40 ml. of an ethereal solution of excess diazomethane and allowed to stand for three days at -20°. The excess diazomethane and ether were then removed on a rotary evaporator without heating. Trituration of the residue with warm hexane gave 90 mg. of the crude product, m.p. 117-120°, which after recrystallization from ethyl acetate-hexane solution gave needles, m.p. 121-122° (gas evolution).

Anal. Calcd. for $C_{21}H_{32}N_2O_4$: C, 66.99; H, 8.57; N, 7.44. Found: C, 66.95; H, 8.48; N, 7.64.

The infrared spectrum ($CHCl_3$) had bands at 3597 and 3484 cm^{-1} (hydroxyl) and 1773 cm^{-1} (γ-lactone). The double bond absorption at 1667 cm^{-1} in mammosin was absent.

Treatment of Mammosin with Hydrogen Bromide. Preparation of Mammosin Dihydrobromide. A solution of 2.00 g. (5.98 mmoles) of mammosin in 200 ml. of dry benzene was cooled to 10°. Dry hydrogen bromide was added over a period of one-half hour, until the solution was saturated. The mixture was then poured into 100 ml. of ice water and the benzene phase separated, washed with water, and dried over sodium sulfate. Distillation of the benzene left 2.45 g. of pale-yellow crystalline material. The color was removed by filtering a solution of the material in 100 ml. of ether through a column containing 4 g. of Florisil. Mammosin dihydrobromide crystallized as colorless needles upon concentration of the

filtrate. Recrystallization from benzene-hexane solution gave 2.3 g. (77%) of needles, m.p. 211°.

Anal. Calcd. for $C_{20}H_{32}Br_2O_4$: C, 48.42; H, 6.50; Br, 32.20; mol. wt., 496; one active hydrogen, 0.20. Found: C, 48.30; H, 6.37; Br, 31.95; mol. wt. (mass spectrum), 496; active hydrogen, 0.16.

The infrared spectrum (KBr) exhibited bands at 3448 cm^{-1} (hydroxyl) and 1780 cm^{-1} (γ-lactone). The N.M.R. spectrum showed no olefinic protons.

The mother liquor from the above recrystallization afforded a second crop, which was a mixture of the dihydrobromide derivative and the more soluble monohydrobromide derivative, which had been obtained earlier (2). N.M.R. analysis of this mixture showed part of a new tertiary methyl at 1.50 s, present in the dihydrobromide derivative, and part of a vinyl methyl at 1.61 s, present in the monohydrobromide derivative.

Dehydrobromination of Mammosin Dihydrobromide. A solution of 0.40 g. of mammosin dihydrobromide in 20 ml. of dry pyridine was refluxed for 14 hrs. The solution was cooled, diluted with 50 ml. of ether and 100 ml. of water, the ether phase separated, washed with dilute hydrochloric acid, and then dried over sodium sulfate. Removal of the solvent left a colorless semi-solid, which upon chromatography on 12 g. of Florisil with benzene, gave 50 mg. of colorless needles, m.p. 145-147°. Recrystallization from benzene-hexane (3:4)

afforded 40 mg. of needles, m.p. 147-149°. Admixture of this material with mammosin gave a mixed melting point of 148-150°. The infrared spectrum of the product was identical with that of mammosin. Likewise, gas chromatography using a Barber-Colman, Model 20, gave a retention time (9.5 min.) identical with that of mammosin. A 6 ft. x 6 mm. ID U-shaped glass column which was packed with 5% XE-60 on 120/150 mesh Gas Chrom Z was used at a temperature of 225°. The flash heater was at 260° and the detector was at 300°. A peak having a retention time of 11.3 min. was also present to the extent of about 5%. The compound represented by this peak was not further investigated.

Quantitative Epoxidation of Mammosin with Monoperphthalic Acid. To 0.778 g. (2.33 mmoles) of mammosin in 50 ml. of ether chilled to 0° was added 8.0 ml. of 1.164 N monoperphthalic acid in ether. The mixture was diluted to 100 ml. and the solution kept at 0°. The reaction course was followed iodometrically by taking 10 ml. aliquots and adding them to a mixture of 5 ml. of acetic acid, 10 ml. of 10% potassium iodide solution, and 20 ml. of water. Titration of the aliquots and corresponding blanks with 0.100 N sodium thiosulfate gave the following data for the number of moles of monoperphthalic acid consumed per mole of mammosin: 1 min. — 0.795 mole; 15 min. — 0.805 mole; 45 min. — 0.825 mole; 120 min. — 0.835 mole; 300 min. — 0.995 mole.

Preparation of Mammosin Oxide. To 1.0 g. (2.99 mmoles)

of mammosin in 10 ml. of chloroform was added with stirring 2.36 g. (12 mmoles) of m-chloroperbenzoic acid (87.3%) in 50 ml. of chloroform. Stirring was continued at room temperature for 2 hrs. The excess m-chloroperbenzoic acid was destroyed by washing the chloroform solution with 10% sodium sulfite until a test with starch-iodide paper was negative. The organic layer was then washed with several 50 ml. portions of 10% sodium bicarbonate, dried over sodium sulfate, and finally concentrated. Chromatography of the residue on 30 g. of Florisil with benzene-ether (19:1) removed, at first, traces of unreacted mammosin and m-chloroperbenzoic acid. Further elution with benzene-ether (9:1) gave crude crystalline mammosin oxide. This material was recrystallized from benzene-hexane (3:4), giving 0.60 g. (57%) of colorless crystals, m.p. 186-188°. Later chromatographic fractions afforded impure material that could not be purified by recrystallization. Admixture with mammosin oxide prepared earlier (2) gave an undepressed m.m.p.

The ultraviolet spectrum showed a maximum at 206 mμ (ϵ 10,300). The infrared spectrum (KBr) showed significant bands at 3509 cm^{-1} (hydroxyl), 1786 cm^{-1} (γ-lactone) and 1675 cm^{-1} (double bond). The N.M.R. spectrum showed doublets at 6.4 d (J=3 c.p.s.) and 5.7 d (J=3 c.p.s.) indicating that the exomethylene group conjugated with the γ-lactone was still present. The double doublet (4.55 dd), indicative of the proton on the carbon bearing the lactone oxygen, was also

still present. Protons on carbons bearing the ether oxygen appeared as complex signals from 2.98-3.42. However, methyl group signals appeared at 1.25 s, 1.19 s and 0.92 d (J=6 c.p.s.), the second one replacing the vinyl methyl signal of mammosin.

Reaction of Mammosin with Hydrogen Chloride. Preparation of Mammosin Hydrochloride. To 50 ml. of glacial acetic acid saturated with dry hydrogen chloride at 25° was added 1.0 g. (2.99 mmoles) of mammosin. The mixture was allowed to stand at room temperature for about 36 hrs. and then poured into 150 ml. of water which precipitated a brown crystalline product. After standing several hours, the product was filtered and washed with water. Treatment with Norit, followed by crystallizations from aqueous ethanol gave 0.64 g. (58%) of mammosin hydrochloride, m.p. 205-206°. The test for an isolated double bond with tetranitromethane was positive.

Anal. Calcd. for $C_{20}H_{31}ClO_4$: C, 64.77; H, 8.42; mol. wt., 370. Found: C, 64.42; H, 8.29; mol. wt. (mass spectrum), 370.

The infrared spectrum ($CHCl_3$) exhibited bands at 3570 cm^{-1} (hydroxyl) and 1785 cm^{-1} (γ-lactone). The N.M.R. spectrum showed an olefinic proton at 5.2 t. Methyl groups appeared at 1.62 s, 1.14 s, and 0.91 d (J=6.5 c.p.s.). The proton on the carbon bearing the lactone oxygen appeared at 4.40 dd (J=10 c.p.s. and 10 c.p.s.). The absence of the downfield olefinic protons indicated that hydrogen chloride

added to the conjugated exomethylene group.

Attempted Reaction of Dihydroisomammosin with Hydrogen Bromide. To 100 mg. of dihydroisomammosin, m.p. 195°, in 10 ml. of dry benzene was added excess dry hydrogen bromide and the solution allowed to stand for 4 hrs., then poured into 30 ml. of ice water. The benzene phase was separated, washed with dilute sodium bicarbonate and water, and finally dried over sodium sulfate. Distillation of the benzene left a yellow crystalline residue, which after recrystallization from benzene-hexane was identified as starting material.

Reduction of Mammosin with Sodium in n-Butyl Alcohol. Preparation of Dihydromammosin. To a refluxing and stirring solution of 1.0 g. of mammosin in 60 ml. of n-butyl alcohol under nitrogen was added as rapidly as possible 2.0 g. of sodium. After one hour all of the sodium had dissolved, whereupon the solution was cooled, diluted with 150 ml. of water, and concentrated on the rotary evaporator to remove most of the butyl alcohol. The resulting aqueous solution was extracted with ether to remove neutral material, acidified, the precipitated product extracted with ether, and the dried ether extracts evaporated. Recrystallization of the residual solid from benzene-hexane gave 550 mg. of dihydromammosin, m.p. 156-157°. The mother liquor from the recrystallization afforded an additional 300 mg. of the product in crude condition.

Anal. Calcd. for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59; O,

19.02. Found: C, 71.34; H, 9.50; O, 19.16 (calcd.).

The ultraviolet spectrum showed only end absorption. The infrared spectrum ($CHCl_3$) exhibited bands at 3605 and 3500 cm^{-1} (hydroxyl) and 1770 cm^{-1} (γ-lactone). The N.M.R. spectrum showed methyl signals at 1.62 s, 1.27 d (J=6 c.p.s.), 1.12 s, and 0.85 d (J=5 c.p.s.). An olefinic proton at 5.1 t was also present. The proton on the carbon bearing the lactone oxygen appeared at 4.32 dd (J=10 c.p.s. and 10 c.p.s.). Protons on carbons bearing the ether oxygen appeared from 3.0 to 3.5.

Reduction of Dihydromammosin with Lithium Aluminum Hydride. To 0.70 g. of dihydromammosin in 100 ml. of dry tetrahydrofuran was added 1.5 g. of lithium aluminum hydride. After refluxing for 4 hrs., the mixture was cooled and 2 ml. of water was added. The precipitated solids were filtered, washed with tetrahydrofuran, and the solvent removed on the rotary evaporator. One recrystallization of the crystalline residue from ethyl acetate afforded 0.4 g. of dihydromammosin diol, m.p. 193-194°.

Anal. Calcd. for $C_{20}H_{36}O_4$: C, 70.54; H, 10.66; three active hydrogens, 0.89. Found: C, 70.55; H, 10.55; active hydrogen, 0.90.

The infrared spectrum (KBr) exhibited a very strong band at 3310 cm^{-1} (hydroxyl) and showed the absence of carbonyl bands.

Preparation of the Trityl Ether of Dihydromammosin Diol. A mixture of 100 mg. (0.299 mmole) of dihydromammosin diol and 83.4 mg. (0.30 mmole) of trityl chloride in 1 ml. of pyridine was heated for one minute at steam bath temperature. The solution was then left to stand at room temperature for two days, during which pyridine hydrochloride deposited. The mixture was diluted with 60 ml. of water and extracted with three 20-ml. portions of chloroform. The chloroform extracts were washed with two small portions of dilute hydrochloric acid, with water, and dried over sodium sulfate. Removal of the solvent left a colorless solid, which after two recrystallizations from hexane produced 110 mg. of the trityl ether, m.p. 139-141°.

Anal. Calcd. for $C_{39}H_{50}O_4$: C, 80.33; H, 8.65; two active hydrogens, 0.34. Found: C, 79.55; H, 8.75; active hydrogen, 0.36.

The infrared spectrum showed bands at 3605 and 3400 cm^{-1} (hydroxyl) and 1490, 1480, 695, and 670 cm^{-1} (aromatic). The N.M.R. spectrum displayed aromatic protons centered at 7.3 c. The olefinic proton of the trisubstituted double bond appeared at 5.2 t. Protons on the carbons containing the ether oxygens appeared as a complex band between 3.0 and 3.9. Methyl groups appeared at 1.65 s, 1.05 s, 0.95 d (J=7 c.p.s.), and 0.71 d (J=5 c.p.s.).

Chromic Acid Oxidation of the Trityl Ether of Dihydromammosin Diol. To 100 mg. of the trityl ether in 2 ml. of

pyridine was added a mixture of 100 mg. of chromium trioxide in 1 ml. of pyridine. The mixture was allowed to stand at room temperature overnight, then diluted with about 5 ml. of methanol. After this solution had stood for 2 hrs., the solvents were removed on the rotary evaporator at room temperature. After extraction of the residue with ether, the extracts were washed with dilute sodium bicarbonate and dried over sodium sulfate. Removal of the ether followed by thin layer chromatography (benzene-acetone, 8:2) of the product on silica gel H, showed a major product (R_f=0.83) with some starting material (R_f=0.59). Traces of other products were also observed. Chromatography on silicic acid with benzene-ethyl acetate (8:1) produced crystalline material, which showed infrared bands at 3600 and 3460 cm^{-1} (hydroxyl) and 1717 cm^{-1} (ketone). The N.M.R. spectrum displayed aromatic protons of the trityl group at 7.3 m. The olefinic proton appeared at 5.18 t. Methyl groups appeared at 1.62 s, 1.10 s, 0.82 d (J-6.5 c.p.s.), and 0.76 d (J=6.5 c.p.s.).

Reduction of Mammosin with Lithium Aluminum Hydride. To 1.0 g. of mammosin in 60 ml. of tetrahydrofuran was added 2.5 g. of lithium aluminum hydride. After stirring at room temperature over a 24-hr. period, 3 ml. of water was added. The precipitated solids were filtered, washed with tetrahydrofuran, and the solvent removed on the rotary evaporator. All attempts to crystallize the resulting gummy solid failed. Sublimation under reduced pressure gave a colorless solid,

m.p. 62-66°. Attempts to recrystallize this material also failed. Infrared analysis showed the absence of carbonyl bands. A tetranitromethane test was positive.

Hydrogenation of 0.2 g. of the above sublimed material over 0.1 g. of prereduced platinum oxide required 15 ml. of hydrogen, which corresponded to 0.96 double bond. The gummy material obtained could not be induced to crystallize. A tetranitromethane test was negative.

Reduction of Mammosin with Sodium Borohydride. To 100 mg. (0.3 mmole) of mammosin in 50 ml. of isopropyl alcohol was added 16.3 mg. (0.3 mmole) of sodium borohydride. After stirring for 20 min. at room temperature, 100 ml. of dilute hydrochloric acid and 0.1 g. of mannitol were added. The solution was then extracted with ether and the extracts dried over sodium sulfate. Distillation of the ether gave a colorless powder. An infrared spectrum showed no lactone carbonyl band.

When 100 mg. of mammosin was treated with 2.8 mg. (0.07 mmole) of sodium borohydride, the infrared spectrum of the product showed a weak band corresponding to lactone carbonyl. None of the products in these experiments could be crystallized and were assumed to be mixtures of epimers.

There are several examples of double bond reduction with sodium borohydride (20, 21) but the experiments above indicate that both the double bond and carbonyl functions were reduced by sodium borohydride.

Hydroboration-Oxidation of Isomammosin Followed by Chromic Acid Oxidation of the Resulting Alcohol. To 25 ml. of a 0.6 M solution of diborane (15 mmoles) in tetrahydrofuran at 0° and under a nitrogen atmosphere was added dropwise 4.20 g. of 2-methyl-2-butene (60 mmoles). The cold solution was stirred for two hours. Isomammosin, 1.0 g. (3 mmoles) in 30 ml. of dry tetrahydrofuran was added dropwise to the cold bis-(3-methyl-2-butyl) borane, and the solution was then allowed to warm to room temperature with continued stirring for an additional 5 hrs. The organoborane was oxidized by adding 15 ml. of 1% aqueous sodium hydroxide and 10 ml. of 30% hydrogen peroxide, followed by stirring at 0° for 1 hr. The solution was allowed to come to room temperature and stirred for an additional 2 hrs. before extraction with ether to give a yellow, semicrystalline solid. Infrared bands were observed at 3600 and 3420 cm^{-1} (hydroxyl), 1750 cm^{-1} (α,β-unsaturated γ-lactone), and 1680 cm^{-1} (double bond). This crude product was dissolved in 10 ml. of pyridine, added to a mixture of 1.0 g. of chromium trioxide in 15 ml. of pyridine, and stirred overnight at room temperature. Dilution with water and an attempted extraction with ether gave an emulsion which was filtered through a short column containing Florisil. The filtrates were concentrated to an oily residue which was chromatographed on silicic acid with benzene-acetone (8:2) to give colorless needles, m.p. 162-165°, which after recrystallization from ethyl

acetate-hexane produced an analytical sample, m.p. 163-164°.

Anal. Calcd. for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63; O, 22.83. Found: C, 68.67; H, 8.46; O, 23.03.

Attempted Dehydration of Isomammosin Ketone. The ketone (50 mg.) in 5 ml. of methanol was treated with 2 ml. of 1% aqueous potassium hydroxide. The solution was allowed to stand at room temperature for 2 hrs., acidified with hydrochloric acid, diluted with water, and extracted with ether. The ether extracts were dried over sodium sulfate and the ether distilled. The melting point of the residual crystalline material on admixture with the starting ketone showed no depression.

Attempted Mild Dehydration (24) of Mammosin, Isomammosin, and Dihydromammosin. A mixture of 100 mg. of mammosin, 0.20 g. potassium hydrogen sulfate, and 10 ml. of anhydrous, peroxide-free dioxane was refluxed for 3 hrs. The solution was cooled and decanted from the potassium hydrogen sulfate. The solvent was removed on the rotary evaporator and the residue (98 mg.) was crystallized by trituration with hexane. Recrystallization from hexane gave needles, m.p. 149-150°, identified as mammosin (m.m.p. and I.R.). Similarly, when isomammosin and dihydromammosin were subjected to the same conditions, both failed to dehydrate.

Reaction of Mammosin with Acetic Anhydride and Boron Trifluoride Etherate. To 1.0 g. of mammosin in 10 ml. of dry ether and 30 ml. of acetic anhydride cooled to -20° was added

7 ml. of boron trifluoride etherate. The mixture was kept at -18 to -20° for 3 hrs., then poured into a cold solution of 10 ml. of pyridine and 200 ml. of water in a separatory funnel. The solution was shaken for several minutes and extracted with two 100-ml. portions of chloroform; the chloroform extracts were washed with five 100-ml. portions of water, then twice with dilute hydrochloric acid, once with dilute sodium bicarbonate, and finally with water. The chloroform solution was dried over sodium sulfate and the solvent removed. The light-yellow residue was triturated with cold hexane and recrystallized from hexane-benzene to give needles, m.p. 163-165°. Several more recrystallizations gave an analytical sample, m.p. 167-168°.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.88; H, 8.19; O, 22.94. Found: C, 68.88; H, 8.05; O, 22.85.

The infrared spectrum ($CHCl_3$) had bands at 1777 cm^{-1} (γ-lactone), 1730 and 1240 cm^{-1} (acetate), 1709 cm^{-1} (ketone), and 1667 cm^{-1} (double bond). The N.M.R. spectrum showed methyl signals at 2.03 s (two methyl groups), 1.46 s, 1.39 s, and 0.96 d (J=7 c.p.s.). The exomethylene protons were at 6.40 d (J=3 c.p.s.) and 5.64 d (J=3 c.p.s.). There was also an olefinic proton at 5.64. The proton on the carbon bearing the lactone oxygen appeared at 4.64 dd (J=10 c.p.s. and 10 c.p.s.). Centered at 3.4 m were also four other protons.

The mother liquor from the recrystallization of the

above residue afforded a second crop, m.p. 152-155°. Further recrystallization from hexane gave needles, m.p. 155-157°, which proved to be the acetate of mammosin.

Anal. Calcd. for $C_{22}H_{32}O_5$: C, 70.18; H, 8.57; O, 21.25. Found: C, 70.43; H, 8.36; O, 20.83.

The infrared spectrum showed bands at 1767 cm^{-1} (γ-lactone), 1730 and 1250 cm^{-1} (acetate), and 1662 cm^{-1} (double bond). The N.M.R. spectrum had methyl groups at 2.00 s, 1.51 s, 1.45 s, and 0.85 d (J=5.5 c.p.s.). The exo-methylene protons were at 6.40 d (J=3.5 c.p.s.) and 5.62 d (J=3.5 c.p.s.). Another olefinic proton was at 5.05 t. Other signals appeared at 4.44 dd (J=10 c.p.s. and 10 c.p.s.), 3.4 m, and 2.87 d (J=10 c.p.s.).

Preparation of Mammosin Acetate. To a solution of 1.0 g. of mammosin in 30 ml. of acetic anhydride and 10 ml. of dry ether at -20° was added 5 ml. of boron trifluoride etherate. The mixture was allowed to stand at -20° for 1.5 hrs. The mixture was then poured into a cold solution of 10 ml. of pyridine and 200 ml. of water, shaken, and then extracted with chloroform. The chloroform extracts were washed well with water, twice with dilute hydrochloric acid, once with dilute sodium bicarbonate and finally with water. After drying the extracts over sodium sulfate, the chloroform was removed, and the residual crystalline material recrystallized from hexane-benzene to give 0.62 g. of mammosin acetate, m.p. 152-155°. Concentration of the mother liquor gave

a second crop, 0.22 g., m.p. 155-157°, identical with the first crop and also identical (N.M.R. and I.R.) with the mammosin acetate obtained above.

Alkaline Hydrolysis of Mammosin Acetate to Mammosin. A solution prepared from 100 mg. of mammosin acetate in 10 ml. of ethanol and 10 ml. of 10% sodium hydroxide was allowed to stand at room temperature for 6 hrs. Upon dilution with water and acidification with hydrochloric acid, the solution became cloudy. Extraction with ether, drying, and removal of solvent left crystals, m.p. 144-146°. Recrystallization from hexane gave needles, m.p. 147-149°. Admixture with mammosin did not depress the melting point, m.m.p. 148-149°. The infrared spectrum of this material was identical with that of mammosin. Thin layer chromatography on silica gel H (benzene-acetone, 9:1) gave identical R_f values (0.52) for authentic mammosin and mammosin obtained from the acetate (R_f=0.93).

Reaction of Mammosin with Acetic Anhydride and Boron Trifluoride Etherate. Preparation of the Keto Acetate. To 1.0 g. of mammosin in 30 ml. of acetic anhydride and 10 ml. of dry ether at -20° was added 7 ml. of boron trifluoride etherate. The mixture was allowed to stand at -20° for 7 hrs. The work-up was as described in the preceding experiment. A gummy residue was obtained and was crystallized by triturating with hexane. Recrystallization of this crude material from hexane-benzene gave 0.52 g. of needles, m.p. 172-173°.

This material was identical with the keto acetate obtained above. A second crop, 0.35 g., was obtained by concentration of the mother liquor.

Treatment of Mammosin Keto Acetate with Aqueous Sodium Hydroxide. A solution containing 100 mg. of mammosin keto acetate in 5 ml. of ethanol and 5 ml. of 10% sodium hydroxide was allowed to stand at room temperature for 2 hrs., then was diluted with water, acidified with hydrochloric acid, and extracted with ether. The ether extracts were dried over sodium sulfate and the ether distilled. Thin layer chromatography on silica gel H (benzene-acetone, 8:2) indicated the presence of some starting material (R_f=0.77) as well as the saponification product (R_f=0.36). Chromatography of the residue on silicic acid with benzene-acetone (19:1) gave fine silky needles, m.p. 111-112°.

Anal. Calcd. for $C_{20}H_{30}O_4$: C, 71.82; H, 9.04; O, 19.14. Found: C, 71.62; H, 8.83; O, 19.32.

The infrared spectrum had bands at 3602 and 3500 cm^{-1} (hydroxyl), 1766 cm^{-1} (γ-lactone), 1705 cm^{-1} (ketone), and 1666 cm^{-1} (double bond). The N.M.R. spectrum showed the exomethylene doublets at 6.46 d (J=3.5 c.p.s.) and 5.71 d (J=3.5 c.p.s.). Another olefinic proton was superimposed under the doublet at 5.71. Methyl groups appeared at 2.05 s, 1.48 s, 1.15 s, and 0.96 d (J=7 c.p.s.). The proton on the carbon attached to the lactone oxygen appeared at 4.65 dd (J=10 c.p.s. and 10 c.p.s.).

Reaction of Isomammosin with Acetic Anhydride and Trifluoride Etherate. Boron trifluoride etherate (0.7 ml.) was added to 100 mg. of isomammosin in 3 ml. of acetic anhydride and 1 ml. of ether at -20°, and was kept at that temperature for 7 hrs. before hydrolysis and isolation as described above. Recrystallization of the crude product from benzene-hexane gave colorless needles, m.p. 179-181°.

Anal. Calcd. for $C_{24}H_{34}O_6$: C, 68.88; H, 8.19; O, 22.94. Found: C, 68.72; H, 8.49; O, 22.94.

Significant infrared bands appeared at 1750 cm^{-1} (α,β-unsaturated γ-lactone), 1740 and 1245 cm^{-1} (acetate), 1705 cm^{-1} (ketone), and 1690 cm^{-1} (double bond). The N.M.R. spectrum showed methyl groups at 2.06 s (two methyl groups), 1.90 s, 1.49 s, and 1.05 d (J=6.5 c.p.s.). The proton on the carbon attached to the lactone oxygen displayed a signal at 4.66 d (J=10 c.p.s.).

Hydrogenation of Isomammosin Keto Acetate. A solution of 0.30 g. of isomammosin keto acetate (obtained above by reaction of acetic anhydride and boron trifluoride ethereate with isomammosin) in 20 ml. of ethyl acetate was hydrogenated for 2 hrs. over 100 mg. of 10% palladium-charcoal. After filtration of the catalyst and removal of the solvent, the resulting crystalline material was recrystallized five times from benzene-hexane, giving needles, m.p. 160-161°.

Anal. Calcd. for $C_{24}H_{36}O_6$: C, 68.54; H, 8.63. Found: C, 68.46; H, 8.74.

Significant infrared bands appeared at 1750 cm^{-1} (α,β-unsaturated γ-lactone), 1730 and 1250 cm^{-1} (acetate), 1705 cm^{-1} (ketone), and 1690 cm^{-1} (conjugated double bond). The N.M.R. spectrum showed methyl groups at 2.10 s, 2.00 s, 1.44 s, 1.07 d (J=7 c.p.s.), and 0.66 d (J=7 c.p.s.). The proton on the carbon bonded to the lactone oxygen appeared at 4.51 d (J=9 c.p.s.). Protons on the carbons bonded to the ether oxygens appeared at 4.07 dd (J=10.5 and 10.5 c.p.s.) and 3.07 d (J=10 c.p.s.).

Reaction of Dihydroisomammosin with Acetic Anhydride and Boron Trifluoride Etherate. To a solution of 110 mg. of dihydroisomammosin, m.p. 195°, dissolved in 3 ml. of acetic anhydride and 1 ml. of dry ether cooled to -20° was added 0.7 ml. of boron trifluoride etherate. The mixture was kept at -20° for 8 hrs. and was then poured into a solution of 1 ml. of pyridine and 50 ml. of water. The product was extracted with chloroform, and the chloroform extracts were washed well with water, twice with dilute hydrochloric acid, once with dilute sodium bicarbonate, and finally with water. After the chloroform extracts had been dried over sodium sulfate and the solvent removed, a slightly yellow crystalline product was obtained. Recrystallization of this crude material from hexane gave crystals, m.p. 189-192°. Two more recrystallizations gave colorless plates, m.p. 196-197°.

Anal. Calcd. for $C_{22}H_{34}O_5$: C, 69.81; H, 9.05; O, 21.14. Found: C, 70.07; H, 9.22; O, 20.93.

The infrared spectrum ($CHCl_3$) showed α,β-unsaturated γ-lactone and acetate carbonyl absorptions overlapped at 1742 cm^{-1}. The conjugated double bond appeared at 1682 cm^{-1}, and the acetate carbon-oxygen stretching frequency at 1254 cm^{-1}. The N.M.R. spectrum showed methyl groups at 2.01 s, 1.89 s, 1.54 s, and 1.00 d (J=6.5 c.p.s.). The proton on the carbon bearing the lactone oxygen appeared at 4.59 d (J=9 c.p.s.). The protons on the carbons bearing the ether oxygen appeared between 3.5 and 2.8.

Ozonolysis of Mammosin. Ozone (about 0.1 g./hr.) was bubbled into a solution of 1.0 g. (3.0 mmoles) of mammosin in 100 ml. of methylene chloride-pyridine (99:1) at -70° (27). The reaction was followed by thin layer chromatography by sampling the ozonolysis solution at 15 min. intervals and watching the disappearance of mammosin. Microscope slides coated with aluminum oxide G were used and developed with hexane-acetone (9:1). After 65 min. all the mammosin had been consumed and the reaction mixture was poured into 50 ml. of dilute hydrochloric acid and the organic layer separated. The organic phase was washed with water and dried over sodium sulfate. Removal of the solvent left a gummy yellow residue. After chromatography on Florisil with benzene-ether (1:1) a glassy material was obtained and could not be induced to crystallize. Gas chromatography on a 5% SE-30 column showed a single peak.

Anal. Calcd. for $C_{19}H_{28}O_6$: C, 64.75; H, 8.01.

Found: C, 64.75; H, 8.17.

The infrared spectrum showed bands at 3500 cm^{-1} (hydroxyl), 1780 cm^{-1} (γ-lactone), 1710 cm^{-1} (ketone) and 1664 cm^{-1} (double bond). The N.M.R. spectrum displayed the exomethylene protons at 6.4 d and 5.7 d, indicating that the trisubstituted double bond was selectively ozonized. A methyl group at 2.1 s indicated the presence of a methyl ketone.

Hydroxylation of Dihydromammosin with Potassium Permanganate. A stirred solution of 1.00 g. (2.98 mmoles) of dihydromammosin in 100 ml. of acetone-water (1:1) was oxidized at room temperature for one hour with 0.706 g. (4.47 mmoles) of potassium permanganate. The manganese dioxide was filtered and washed well with acetone. The combined filtrates were concentrated on the rotary evaporator until the product began to precipitate. After extraction with several portions of methylene chloride and drying the extracts over sodium sulfate, dihydroxydihydromammosin was obtained as colorless crystals. Recrystallization from ethyl acetate gave 0.8 g. of needles, m.p. 214-216°.

Anal. Calcd. for $C_{20}H_{34}O_6$: C, 64.84; H, 9.25. Found: C, 64.63; H, 9.07. (Analyzed with an F & M, Model 180 C,H,N Analyzer).

The infrared spectrum (KBr) exhibited bands at 3410 cm^{-1} (hydroxyl) and 1770 cm^{-1} (γ-lactone).

Oxidation of Dihydroxydihydromammosin with Chromic Acid. To 0.10 g. of dihydroxydihydromammosin in 10 ml. of acetone was added a sufficient quantity of Jones Reagent (32) (267 g. of chromium trioxide and 230 ml. of sulfuric acid diluted to 1 L. with water) to give a persistent orange color. The solution was then poured into water and extracted well with ether. The ether extracts were washed with dilute sodium bicarbonate and then with water. The dried ethereal solution was distilled to give an oil, which chromatographed on Florisil with benzene-ethyl acetate (8:2) gave fine silky needles, m.p. 85-86°.

Anal. Calcd. for $C_{20}H_{30}O_6$: C, 65.55; H, 8.25. Found: C, 65.75; H, 8.31.

The infrared spectrum ($CHCl_3$) exhibited bands at 1774 cm^{-1} (two γ-lactones) and 1712 cm^{-1} (ketone). The N.M.R. spectrum showed methyl groups at 2.18 s, 1.40 s, 1.28 d (J=7 c.p.s.), and 0.94 d (J=7 c.p.s.). The proton on the carbon bearing the lactone oxygen appeared at 4.45 dd and the other protons appeared between 3.0 and 4.0.

Controlled Oxidation of Dihydroxydihydromammosin with Chromic Acid. To 0.1074 g. (0.291 mmoles) of dihydroxydihydromammosin in 10 ml. of acetone was added 19.4 mg. (0.194 mmole) of chromium trioxide dissolved in 1 ml. of water and 1 ml. of concentrated sulfuric acid. After standing at room temperature for 15 min., the solution was diluted with water and extracted with ether. The ether extracts were washed

with water and dried over magnesium sulfate. Removal of the ether left a colorless solid, which upon crystallization from benzene-hexane gave needles, m.p. 111-112°.

The infrared spectrum (KBr) exhibited bands at 3580 and 3490 cm^{-1} (hydroxyl) and 1760 cm^{-1} (γ-lactone).

This product was oxidized with excess chromic acid to the same keto lactone produced on oxidation of dihydroxydihydromammosin with excess chromic acid.

Oxidation of Mammosin Keto Lactone with Peroxytrifluoroacetic Acid (30). A solution of peroxytrifluoroacetic acid was prepared by addition of 0.5 ml. (3.6 mmoles) of trifluoroacetic anhydride to a suspension of 0.1 ml. (3.0 mmoles) of 85% hydrogen peroxide in 2 ml. of cold methylene chloride. This solution was then added dropwise to a stirred suspension of 0.1 g. of anhydrous disodium hydrogen phosphate in a mixture of 0.1 g. (0.272 mmoles) of mammosin keto lactone in 2 ml. of methylene chloride. The solution was then stirred for 30 min. at room temperature and the salts filtered and washed with 10 ml. of methylene chloride. The combined filtrates were washed with sodium bisulfite solution and sodium carbonate solution and then dried over magnesium sulfate. The solvent was removed leaving a colorless oil which crystallized on trituration with warm hexane. Two recrystallizations from benzene-hexane gave 80 mg. of needles, m.p. 132-133°.

Anal. Calcd. for $C_{20}H_{30}O_7$: C, 62.81; H, 7.91; Found: C, 62.79; H, 7.69.

The infrared spectrum ($CHCl_3$) showed significant bands at 1775 cm^{-1} (two γ-lactones), 1730 cm^{-1} (ester), and 1230 cm^{-1} (acetate).

The N.M.R. spectrum showed methyl groups at 0.95 d (J=6.5 c.p.s.), 1.29 d (J=5.5 c.p.s.), 1.38 s, and 2.06 s. Other significant signals appeared at 4.40 dd (J=9 c.p.s. and 10 c.p.s.), 4.08 t, 3.61 t, 3.23 d (J=10 c.p.s.), and 2.55 m.

Oxidation of Dihydroisomammosin with Potassium Permanganate. A solution of 10 g. of potassium permanganate in 100 ml. of acetone-water (1:1) was added dropwise over a period of one hour to a stirred solution of 5.0 g. of dihydroisomammosin (mixture of epimers, m.p. 186-192°) in 200 ml. of acetone-water (4:1) at room temperature. The mixture was stirred for an additional hour, and then carefully acidified with cold 50% sulfuric acid and allowed to stand for one-half hour before filtering. The filtrate was made basic to about pH 11, the neutral products removed by extraction with ether, and the acidic products obtained by acidification of the aqueous phase followed by extraction with ether.

The neutral fraction was chromatographed on Florisil with benzene eluting 50 mg. of lactone A, m.p. 204-205°. Further elution with ether gave lactone B, m.p. 132-133°.

Anal. Calcd. for $C_{19}H_{32}O_4$: C, 70.33; H, 9.94; O, 19.72. Found for lactone A: C, 70.45; H, 10.06; O, 19.54. Found for lactone B: C, 70.50; H, 9.95; O, 19.67.

The infrared spectra of the two lactones were identical

and exhibited significant bands at 3540 cm^{-1} (hydroxyl) and 1760 cm^{-1} (γ-lactone).

The acidic fraction was dissolved in ether and treated with an ethereal solution of excess diazomethane. Chromatography of the resulting product on Florisil with benzene gave an oil which solidified after standing several days. Recrystallization of this crude solid from isopropyl alcohol and finally with aqueous ethanol gave 0.2 g. of colorless needles, m.p. 90-91°.

Anal. Calcd. for $C_{18}H_{30}O_5$: C, 66.23; H, 9.26; O, 24.51. Found: C, 66.56; H, 9.17; O, 24.43.

The infrared spectrum ($CHCl_3$) showed a band at 1765 cm^{-1} characteristic of a γ-lactone.

The infrared spectrum ($CHCl_3$) showed bands at 3600 and 3490 cm^{-1} (hydroxyl), 1750 cm^{-1} (α,β-unsaturated γ-lactone), 1710 cm^{-1} (ketone), and 1685 cm^{-1} (double bond). The N.M.R. spectrum showed methyl signals at 1.87 s, 1.28 s, and 1.0 d (J=6.5 c.p.s.) (two methyl groups). The proton on the carbon bonded to the lactone oxygen was at 4.62 d (J=10 c.p.s.).

Ozonolysis of Dihydroisomammosin. Crude dihydroisomammosin, 2.1 g. (prepared by hydrogenation of mammosin in ethanol over platinum oxide at room temperature and 35 psi.) was dissolved in 60 ml. of methanol and ozonized at -70° until the blue color of excess ozone appeared. The mixture was then steam distilled. Formaldehyde was isolated as the

2,4-dinitrophenylhydrazone from the distillate. The residue was extracted with ether, the extracts dried and distilled giving 2.1 g. of glassy material. Thin layer chromatography on silica gel H with benzene-ethyl acetate (8:2) showed three major compounds. Chromatography of this mixture on 70 g. of silicic acid with benzene-ethyl acetate (88:2) (30-ml. fractions) provided the following materials: 180 mg. (fractions 17-21), 72 mg. (fractions 22-26), 30 mg. (fractions 27-30), 50 mg. (fractions 31-37), 58.5 mg. (fractions 38-44), and 350 mg. (fractions 50-62). Fractions 17-21, 22-26, 27-30, and 31-37 were all mixtures (thin layer chromatography) and non-crystalline. Fractions 38-44 were combined and recrystallized from hexane for analysis. Thin layer chromatography showed a single spot.

Anal. Calcd. for $C_{16}H_{26}O_4$: C, 68.06; H, 9.28; O, 22.66. Found: C, 67.96; H, 9.30; O, 22.67.

The infrared spectrum ($CHCl_3$) showed bands at 2695 cm^{-1} (aldehyde C-H), 1770 cm^{-1} (γ-lactone) and 1735 cm^{-1} (aldehyde). The N.M.R. spectrum showed an aldehyde proton at 9.24 d (J=0.85 c.p.s.). Methyl groups appeared at 1.40 s and 0.90 d (J=7 c.p.s.) (two methyl groups). Two ether protons appeared at about 4.1. Two protons also appeared at 2.77.

Fractions 50-62 were recrystallized from hexane to give an analytical sample, m.p. 109-110°.

Anal. Calcd. for $C_{20}H_{34}O_4$: C, 70.97; H, 10.12; O, 18.91; and one active hydrogen, 0.30. Found: C, 71.17; H,

10.14; O, 19.12; and active hydrogen, 0.31.

The infrared spectrum ($CHCl_3$) exhibited bands at 3605 and 3490 cm^{-1} (hydroxyl) and 1768 cm^{-1} (γ-lactone). The N.M.R. spectrum showed methyl groups at 1.30 d (J=7 c.p.s.), 1.12 s, and two secondary methyl groups near 0.90. The proton on the carbon bonded to the lactone oxygen appeared at 4.55 dd. Two ether protons appeared between 3 and 4.

Oxidation of Mammosin with Chromic Acid. To 0.5 g. of mammosin in 100 ml. of acetone (distilled from potassium permanganate) was added 4 ml. of a solution of 267 g. of chromium trioxide and 230 ml. of sulfuric acid diluted to 1 L. with water (32). The mixture was stirred for 2 hrs. at room temperature, then diluted with 200 ml. of water, extracted with five 50-ml. portions of chloroform, the chloroform extracts washed with water, and dried over sodium sulfate. The solvent was removed on the rotary evaporator leaving a yellow residue. Chromatography of this material on 15 g. of Florisil with ether gave 150 mg. of crystalline material. Several recrystallizations from benzene gave an analytical sample, m.p. 201-203°.

Anal. Calcd. for $C_{20}H_{28}O_6$: C, 65.91; H, 7.74. Found: C, 66.24; H, 7.70.

The infrared spectrum ($CHCl_3$) showed bands at 3600 and 3400 cm^{-1} (hydroxyl), 1769 cm^{-1} (γ-lactone), and 1709 cm^{-1} (ketone). The N.M.R. spectrum showed the exomethylene doublets at 6.50 d (J=3 c.p.s.) and 5.72 d

(J=3 c.p.s.). Methyl groups appeared at 1.42 s, 1.24 s, and 0.98 d (J=6 c.p.s.).

Further elution with ether gave only non-crystalline material. Thin layer chromatography on silica gel H using several solvents and solvent mixtures all indicated a complicated mixture of products.

Attempted Hydrolysis of Mammosin with Sulfuric Acid. To 100 mg. of mammosin in 20 ml. of acetone was added 1 ml. of 9 N sulfuric acid. After standing at room temperature for 8 hrs., 100 ml. of water was added. Extraction with chloroform gave 95 mg. of mammosin, m.p. 149-151°, undepressed on admixture of authentic material.

Vapor Phase Hydrogenolysis of Mammosin. A solution of 0.5 g. of mammosin in 3 ml. of ethanol was slowly (over a 4-hr. period) injected onto a 2 ft. glass column at 300° containing pellets of alumina coated with 0.5% palladium. Hydrogen was used as a carrier gas at a flow rate of 77 ml./min. After all the sample had been injected, the column was flushed first with four 0.25-ml. portions of ethanol, then with six 0.5-ml. portions of hexane. The effluent vapors were condensed in a U-shaped tube cooled by ice-water. The collected liquid was dried over sodium sulfate and the solvents removed. The residual oily material was dissolved in hexane and filtered through a column containing 5 g. of neutral alumina to give 100 mg. of a colorless oil. Gas chromatographic analysis of this oil indicated a complex mixture, but

showed a major compound. Preparative gas chromatography on an Aerograph Auto-Prep, Model A-700 furnished 50 mg. (loss due to aerosol formation) of the major component, which was collected about 15 min. after injection. A 20 ft. x 3/8 in. 30% SE-30 column was employed at 255°.

Mass spectral analysis revealed the formula $C_{20}H_{38}$ by a peak at m/e 278, representing the molecular ion. Other significant peaks were at m/e: 263, 235, 123, and 109.

SUMMARY

Mammosin, a diterpene lactone, is isolated from the pentane extracts of the gorgonian, Eunicea mammosa Lamouroux. The mass spectrum showed a molecular ion peak at m/e 334 and confirmed the formula $C_{20}H_{30}O_4$. Mammosin contains an α-methylenic γ-lactone grouping, an isolated trisubstituted double bond, a tertiary hydroxyl group as a methyl carbinol system, and an inert ether group. Based on the analytical results and the determination of the functional groups, it is concluded that mammosin contains a monocarbocyclic ring system. The evidence in the N.M.R. spectrum for a disecondary ether ring as well as the nature of the splitting patterns for the ether protons and the absence of aliphatic side chains all lead to a 14-membered ring structure. The structure (Ia) is proposed for mammosin on the basis of its chemical transformations and interpretations of its N.M.R. spectrum as well as the spectra of its transformation products. N.M.R. decoupling studies aided substantially in arriving at the structure (Ia).

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