STUDIES IN PHYTOSTEROL BIOSYNTHESIS: THE SYNTHESIS OF POSSIBLE POLYENE INTERMEDIATES

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MARIO FRYBERG

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NAME: MARIO FRYBERG

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Dr. A. M. Unrau
Research Supervisor

Dr. A. C. Oehlschlager
Committee Member

Dr. A. G. Sherwood
Committee Member

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ABSTRACT

The previously unknown steroids 3β-acetoxy-24-methylene Δ5, 22, 24(28)-cholestatriene, 3β-acetoxy-Δ5, 22, 24(28)-stigmastatriene, 3β-acetoxy-24-methyl-Δ5, 22, 24-cholestatriene and 3β-acetoxy-Δ5, 7, 22, 24-ergostatetraene have been synthesized. The naturally occurring Δ5,7, 22, 24(28)-ergostatetraene-3β-ol has been prepared for the first time starting from stigmasterol and has been proven to be identical with the steroid isolated from Saccharomyces cerevisiae. The synthetic sequence developed involved a new and highly selective method for specific bromination of the 5, 6 double bond of stigmasterol acetate. The sequence is readily adaptable to the preparation of several more variations of sterols with unsaturated side chains.

During these syntheses several new intermediates have been prepared and characterized. Infrared, ultraviolet and nuclear magnetic resonance spectra of the new compounds are discussed. The effect of new functional groups on the chemical shift of the steroidal angular methyl groups in the n.m.r. has been determined.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Considerable work has recently been reported on problems concerning the biosynthesis of phytosterols. Special interests and efforts have been directed to the transformation of the side chain of sterols in fungi and higher plants. Several reaction schemes have been proposed but an exact sequence of events cannot yet be established. Available results seem to point to the possibility of two parallel pathways. The first route could involve a steroid with a fully saturated side chain as a key intermediate. The second could proceed via several metabolic intermediates of varying degrees of unsaturation. Which of the two is the major one may well depend on the conditions under which a system has been investigated. Of interest in this connection are steroidal compounds with side chains possessing conjugated double bonds. Only one such compound has, up to this time, been isolated and characterized. Several analogous compounds could however be postulated as possible sequential intermediates. (See Literature Review)

To establish the involvement of a compound as a precursor in a biosynthetic sequence it is necessary that 1, the precursor is detected in the organism either by

isolation or trapping experiments and 2, that feeding experiments show positive results. Many postulated intermediates may however be present in insufficient quantities in order to be isolated or even detected without prior knowledge of their physical and chemical properties. It is the aim of the present investigation to synthesize, with and without ^{14}C-label, and characterize several of the possible intermediates. These appropriately ^{14}C-labelled compounds could then be used in later investigations to further elucidate the dynamic transformations of phytosterols in plants and fungi.

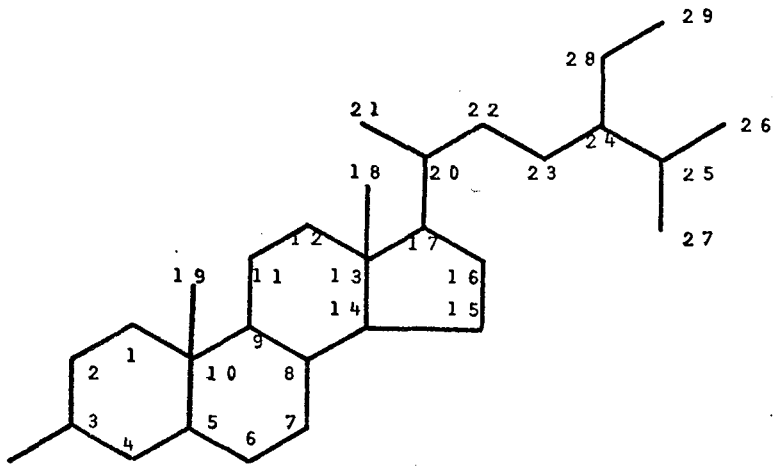
LITERATURE REVIEW

The key roles of mevalonic acid and squalene in the metabolic sequence leading to sterols are firmly established. Work published up to 1965 has been reviewed by Clayton (1) and by Goad (2). Incorporation of 2-^{14}C mevalonate into squalene and into the major phytosterols has been shown more recently by several workers, e.g. in peas and maize leaves by Goad et al, (3), in Euphorbia peplus by Baisted (4), in Larix decidua leaves (5) and Pisum sativum leaves (6) by Goad et al. Cyclisation of 2, 3-epoxy squalene to cycloartenol in Nicotina tabacum has been shown by Eppenberger et al (7). Incorporation of ^{14}C - cycloartenol and/or ^{14}C - lanosterol into biosynthetic "end products" has recently been demonstrated by Hall et al (8) in Ochromonas malhamensis, Hewlins et al (9) in cultures of Nicotina tabacum and by Akhtar et al (10) and Marimoto et al (11) in yeast.

Transformation of the side chain of these precursors to the side chain of the final steroids involves firstly the introduction of an alkyl- or alkylene- group at C-24 and secondly, the formation of double bonds at C-22, C-23, C-24(28) or C-25. (For numbering system see Figure 1). The origin of the extra alkyl group at C-24 has been well established in numerous experiments. The pertinent literature has been extensively reviewed by Lederer (12). This extra alkyl group (at C-24) in phytosterols has been shown to arise from methionine (13). In ergosterol, the extra

FIGURE 1

NUMBERING SYSTEM OF STEROIDS



carbon is derived from a single methylation at C-24. In C-29-sterols the two extra carbons are added via a double transmethylation at C-24. Castle et al (14) and Tomita et al (15) proposed the methylation mechanism shown in Figure 2. Figures 3 and 4, in turn, show the numerous possibilities for the stabilization of the formed carbonium ion and the subsequent transformations leading to the end products. Numerous 24-methylene compounds have been isolated. (For references see Ourisson et al (16)) While some appear to be end products, e.g. eburicoic acid or ergosta-5,7,24(28) trien-3β-ol (17), others seem to be biogenetic intermediates.

It has been demonstrated by Akhtar et al (18C) and Barton et al, 1966 (19) that 24-methylenedihydrolanosterol is incorporated by yeast into ergosterol. 4α — Methyl — 24-methylene — 24,25-dihydrozymosterol and obtusifoliol, a sterol which has never been detected in yeast, are transformed into ergosterol by yeast as shown by Barton et al (19, 20).

Lederer et al (21) and Jaureguiberry et al (22) have shown that ergosterol synthesized by Neurospora crassa in the presence of (CD_3)-methionine contains only two deuterium atoms. This suggests that a C-24-methylene derivative is a precursor of C-24 methylsterols. Several publications however seem to provide evidence against the involvement of a 24-methylene compound as a precursor of ergosterol. 24-Methyldihydrolanosterol is converted to ergosterol in good

FIGURE 2

METHYLATION SCHEME ACCORDING TO
TOMITA et al (15)

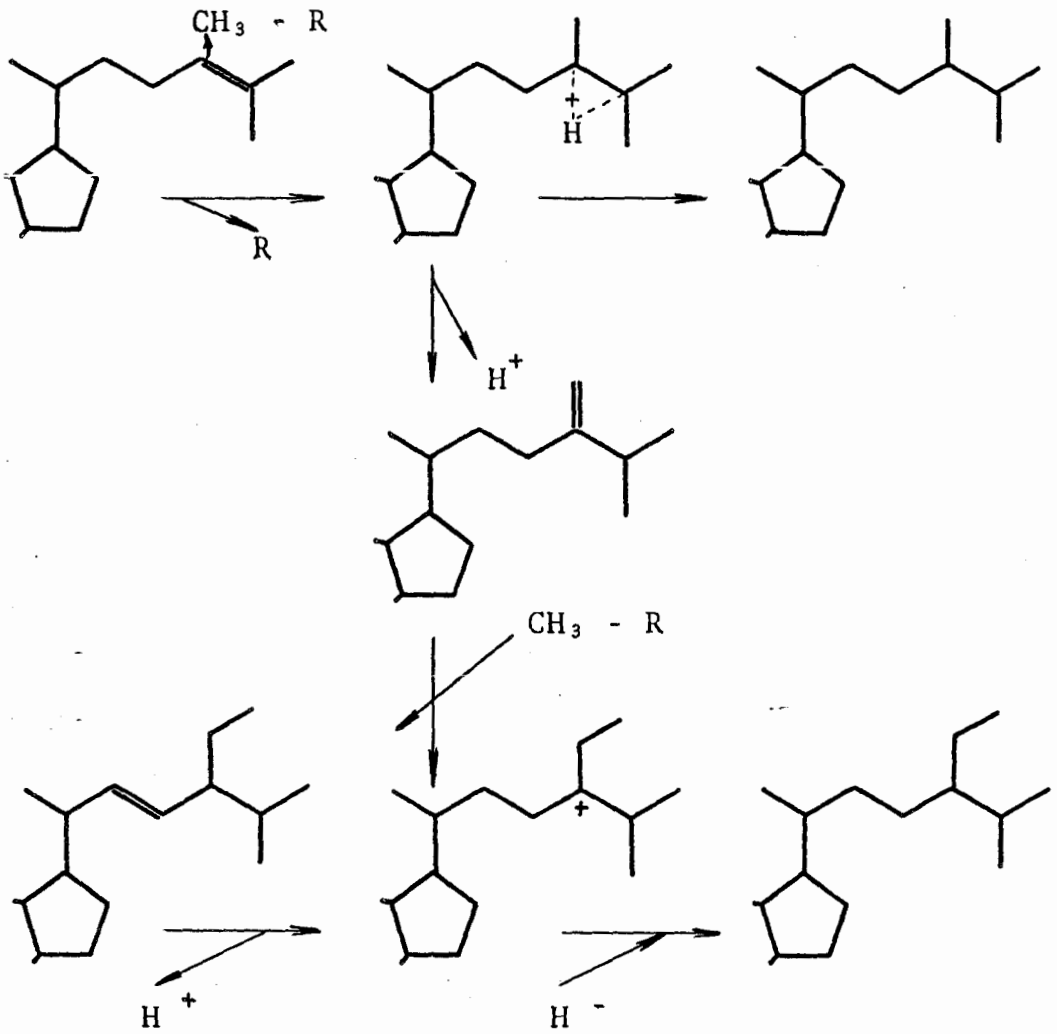


FIGURE 3

POSSIBILITIES FOR THE CARBONIUM ION STABILIZATION IN FUNGI

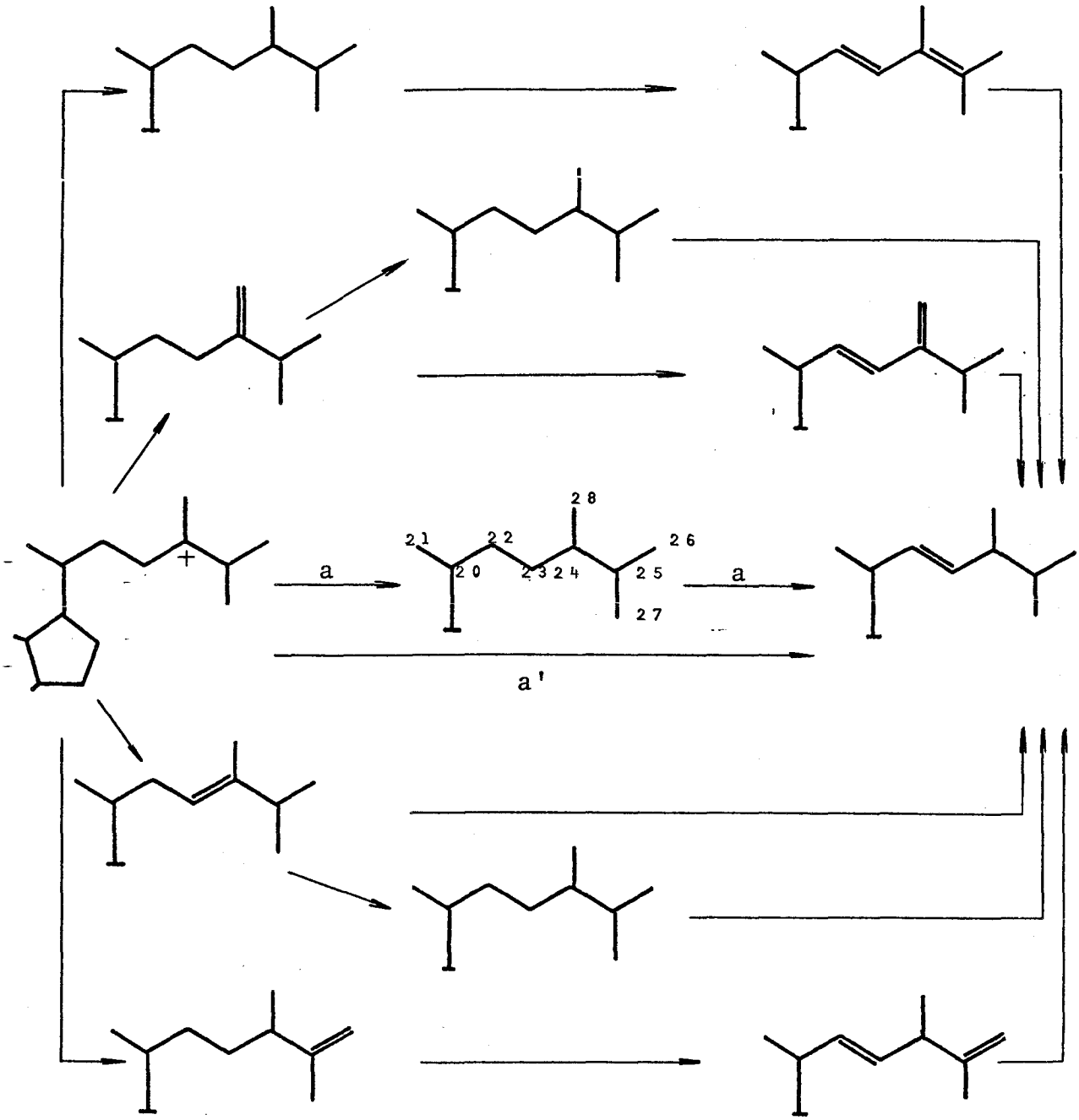
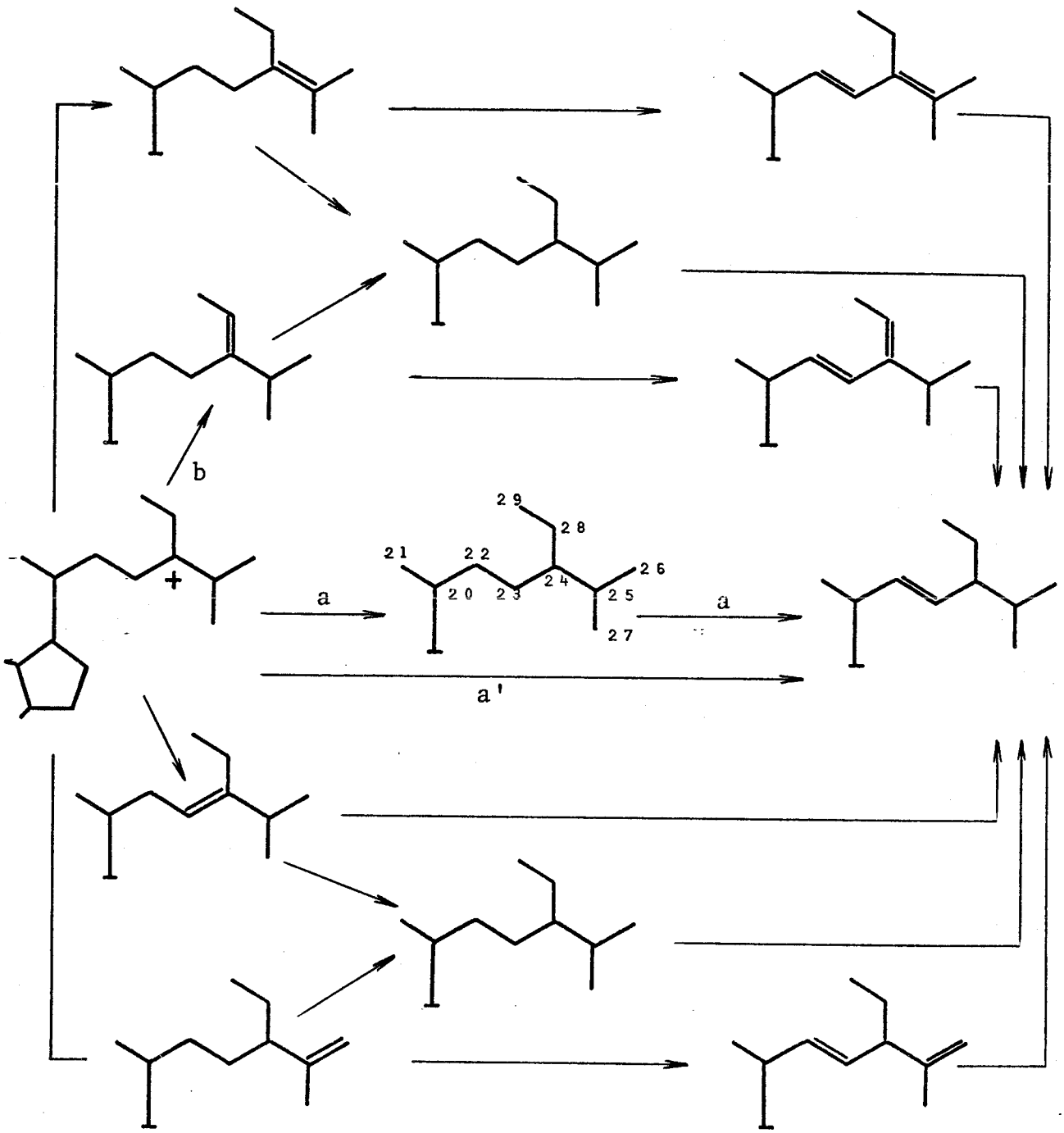


FIGURE 4

POSSIBILITIES FOR THE CARBONIUM ION STABILIZATION IN PLANTS



yield without prior conversion into a 24-methylene derivative (10). This result was deduced from the fact that ^{3}H at C-24 was not lost. Tomita et al (15) reported that Δ7-ergostenol isolated from Chlorella vulgaris, grown in the presence of (CD_3)- methionine contained three deuterium atoms at C-28. This result indicates that a C-24 methylene derivative cannot be involved.

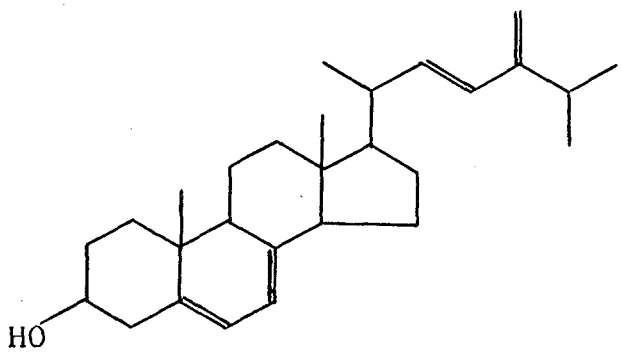
Experimental results reported by Barton's group (20) seem to exclude 24-methylene-lanosterol as a precursor of ergosterol in Saccharomyces cerevisiae. After work up of a cell free yeast system which had been incubated for 14 hours in the presence of a mixture of 1,24,25,30-$^{3}H_4$ — 2,3-epoxysqualene and inactive 24,25-dihydrolanosterol, 13.4% of the radio activity was found in lanosterol while 77% of the active squalene was recovered. No activity was found in 24,25— dihydro-24-methylene lanosterol despite the fact that cell free systems will metabolize sterols beyond the lanosterol stage as shown by Bloch et al (23). The above results seem to indicate that a fully saturated side chain is an appropriate precursor, probably along pathway a in Figure 3. Pathway a' appears to be excluded since no ^{3}H shift from C-23 to C-24 could be detected by Akhtar et al (10).

The same discrepancy which exists in the single methylation seems also to exist in the double methylation. Smith et al (24) found four deuterium atoms in poriferasterol isolated from Ochromonas malhamensis which had been grown in the presence of (CD_3) – methionine. This observation indicates that

fucosterol could be the precursor for C-24 ethylsterols. The same result was found for a number of 24-ethyl sterols in several plant systems by Goad et al (25). However, Lenfant et al (26, 27) have reported the isolation of Δ22-stigmasterol containing five deuterium atoms from Dictiostelium discoideum. Further, Tomita (15) has isolated chondrillasterol and Δ7-chondrillasterol from Chlorella vulgaris, grown in presence of (CD_3)-methionine, also containing five deuterium atoms. This means that the C-24 ethyl group is synthesized by a mechanism which does not involve a C-24 ethylidene group. This observation, in turn, rules out pathway b (see Figure 4) in favour of the more direct pathways a or a' involving a fully saturated side chain.

Eblouz et al (28a) have been able to show that sitosterol (saturated side chain) is transformed into Δ22-stigmasterol in Dictiostelium discoideum. The same authors (28b) showed that the reverse transformation, however, was not possible.

A compound of the type illustrated below has only been isolated in one instance. Breivik et al (29) reported in 1954 the isolation of this new yeast sterol to which they assigned structure illustrated.



The structure assignment was based on spectroscopic evidence and on biosynthetic considerations. This sterol is reported to be a minor sterol under usual conditions. Special growth conditions, unspecified by the authors, were reported to produce the new sterol as a major product by several yeast strains. The same authors published a method (30) for the quantitative determination of ergosterol and ergostatetraeneol in 15 different yeast strains. The method was based on the u.v. absorption, typical for the two compounds.

Petzold et al (31) developed an improved method for the isolation of 24(28)-dehydroergosterol from yeast. They confirmed Breivik's structure assignment by NMR and chemical transformations. Maugenet et al (32, 33) report, however, that they were unable to detect 24(28)-dehydroergosterol in an investigation involving 13 species of yeast, while Marimoto et al (11) did not find the ergostatetraeneol in the yeast Candida utilis.

The transformation of ergostatetraeneol into ergosterol has not yet been unambiguously demonstrated. Katsuki et al (23) however, isolated an unsaturated derivative of ergosterol (named E_{S2} by the authors) from cell free yeast extracts to which they assigned tentatively the structure of ergostatetraeneol as isolated by Breivik et al (29). Intact yeast cells were found to convert E_{S2} aerobically to ergosterol.

DISCUSSION AND RESULTS

1. Synthesis

Studies on the biosynthesis of phytosterols in fungi and higher plants have not as yet been able to demonstrate the importance of steroids possessing conjugated unsaturation in the side chain as biosynthetic intermediates. In particular, the role which such steroids play in the sequence of modifications occurring in the side chain has not yet been fully investigated. With the exception of $\Delta 5,7,22,24(28)$-ergostatetraene-3β-ol in yeast (29) no such compounds have in fact been isolated. In order to study the possible intermediacy of this type of steroid in the biosynthesis of ergosterol in yeast or of the major phytosterols in plants, it became necessary to develop a synthetic procedure for the preparation of steroids with such doubly unsaturated side chains.

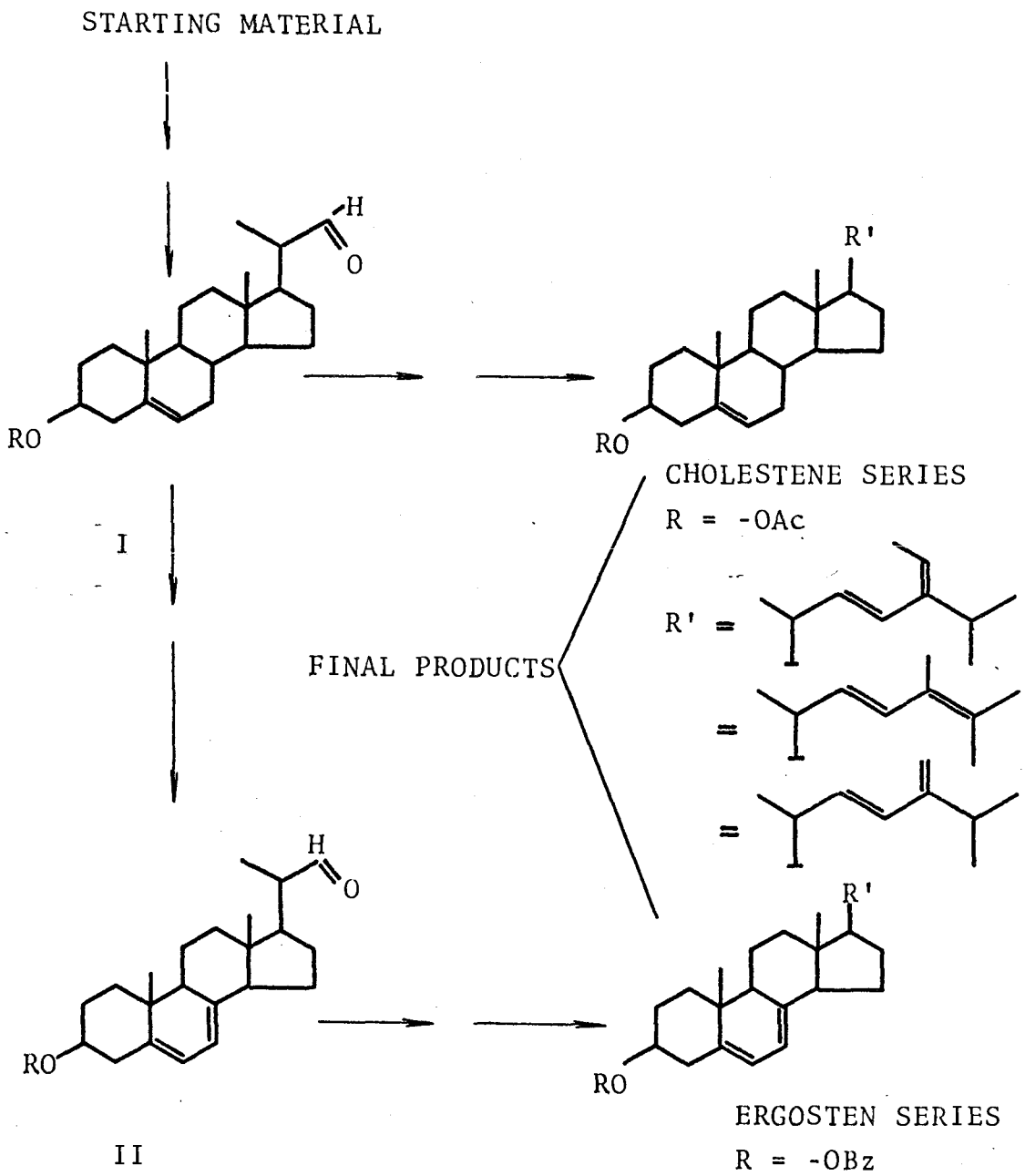
The proposed synthesis for these compounds follows the route shown in Figure 5, page 13 . It involves the preparation of two key intermediates, aldehyde I and II, and their transformation to final products via Wittig reactions.

The immediate goals of this work were therefore:

1) to develop an efficient synthetic sequence for the conversion of a readily available sterol into aldehyde I and II

FIGURE 5

GENERAL OUTLINE OF THE PROPOSED SYNTHESIS FOR STEROIDS POSSESSING DOUBLY UNSATURATED SIDE CHAINS



2) to introduce the unsaturated side chains via Wittig reaction in such a way as to allow for easy incorporation of ^{14}C-label, and

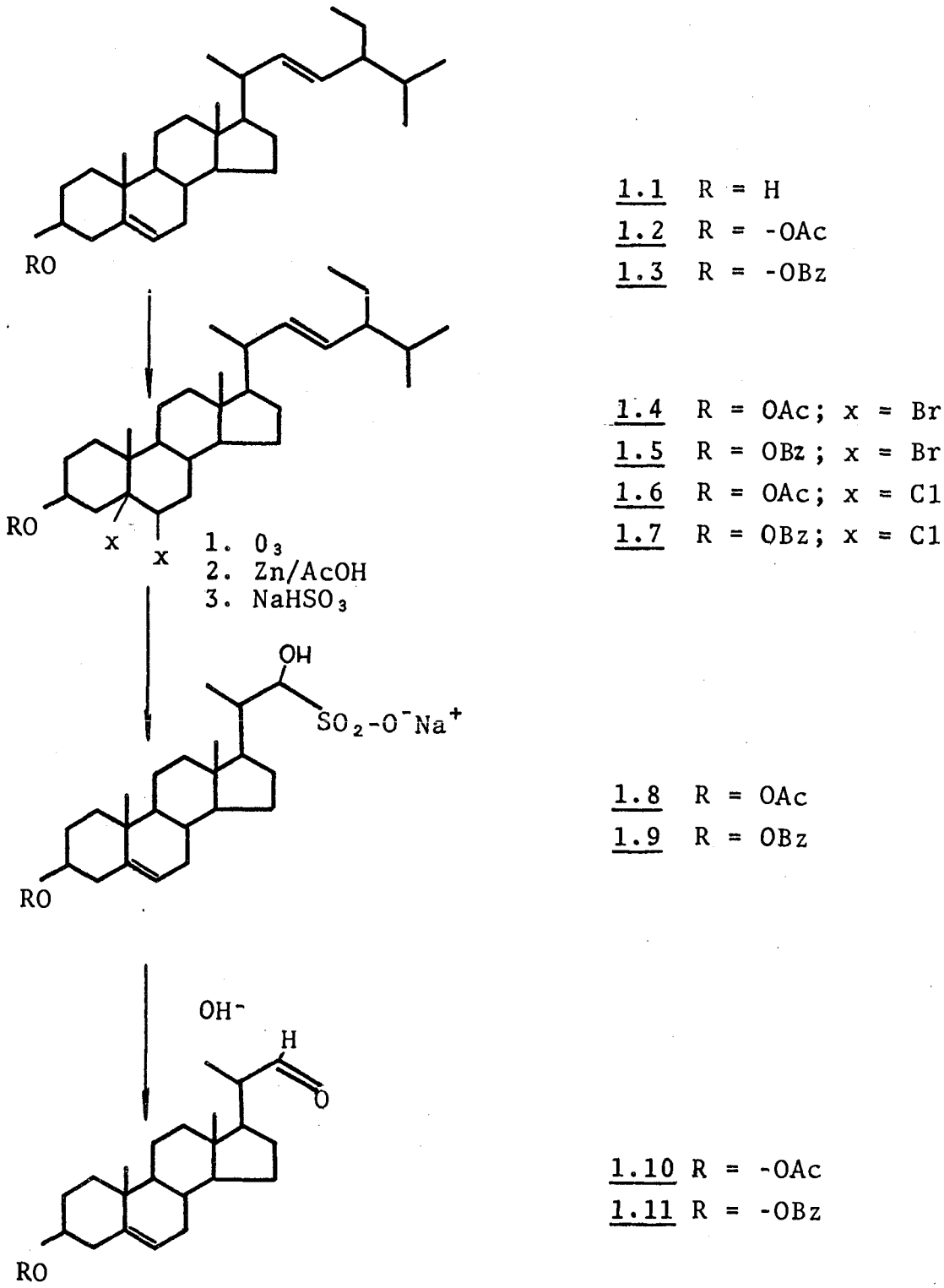
3) to prove the identity of the synthetic and of the naturally occurring and isolated Δ5,7,22,24(28)-ergostatetraene-3β-ol by an unambiguous synthesis.

Stigmasterol was found to be the most convenient starting material. After esterification of the C-3 alcohol the 5,6 double bond could be protected and the side chain cleaved by ozonolysis to give aldehyde I. This aldehyde could be transformed directly to the steroids of the cholesten series or it could be transformed to aldehyde II by protecting the aldehyde function and by introducing the double bond at C-7. Subsequent removal of the aldehyde protecting group followed by appropriate Wittig reaction would give the sterols of the ergosten series.

The synthesis of 3β-acetoxy-22, 24-bisnor-Δ5-cholen-22-al was accomplished by the route shown in Figure 6, page 15. This aldehyde had been obtained previously by Heyl et al (34) as its semicarbazone derivative and later in pure form by Centolella et al (35). The present synthesis follows essentially the sequence used by Centolella et al with the exception that bromination of stigmasterol acetate was performed using iodobenzene dibromide. Thus

FIGURE 6

OUTLINE OF THE SYNTHESIS OF
3β-ACETOXY-Δ5-BISNORCHOLEN-
22-AL (ALDEHYDE I)



1.1 R = H
1.2 R = -OAc
1.3 R = -OBz

1.4 R = OAc; x = Br
1.5 R = OBz; x = Br
1.6 R = OAc; x = Cl
1.7 R = OBz; x = Cl

1.8 R = OAc
1.9 R = OBz

1.10 R = -OAc
1.11 R = -OBz

bromination of stigmasterol ester followed by ozonolysis of the 22,23 double bond and purification of the aldehyde via the bisulfite adduct gave generally low yields (20-28%) of aldehyde in accordance with the published literature. The fact that a considerable amount of unchanged stigmasterol ester could be recovered suggested that the 22,23 double bond had been protected from ozonolysis, e.g. the bromination of the 5,6 double bond was not very selective. This was also partly confirmed by the fact that high yields of aldehyde or the corresponding acid were obtained on ozonolysis when the 5,6 - double bond was protected by means other than direct bromination (Br_2) e.g. by hydrochlorination (36), by formation of i-stigmasteryl-methyl ether, by the formation of the 5,6 dichloro compound (37) or by transforming stigmasterol into the corresponding Δ4,22-stigmastadiene-3-one (38).

The specificity of the bromination of stigmasterol acetate with bromine was therefore investigated. Figure 7, page 17 shows the region δ3.5-6 in the NMR spectrum of a mixture of bromosterols obtained after bromination according to the method of Fernholz (39). Figure 8, page 18 shows the same region of the NMR spectra of stigmasterol and of pure 5,6 dibromostigmasterol obtained according to the method described below. Comparison of the spectra allow the unambiguous assignment of the peak at δ4.48 to the protons

FIGURE 7

N.M.R. SPECTRUM (δ4-5.5) OF 3β-ACETOXY-5,6 DIBROMOSTIGMASTEROL + 3β-ACETOXY-22, 23 DIBROMOSTIGMASTEROL IN $CDCl_3$ (SWEEP WIDTH 250 Hz)

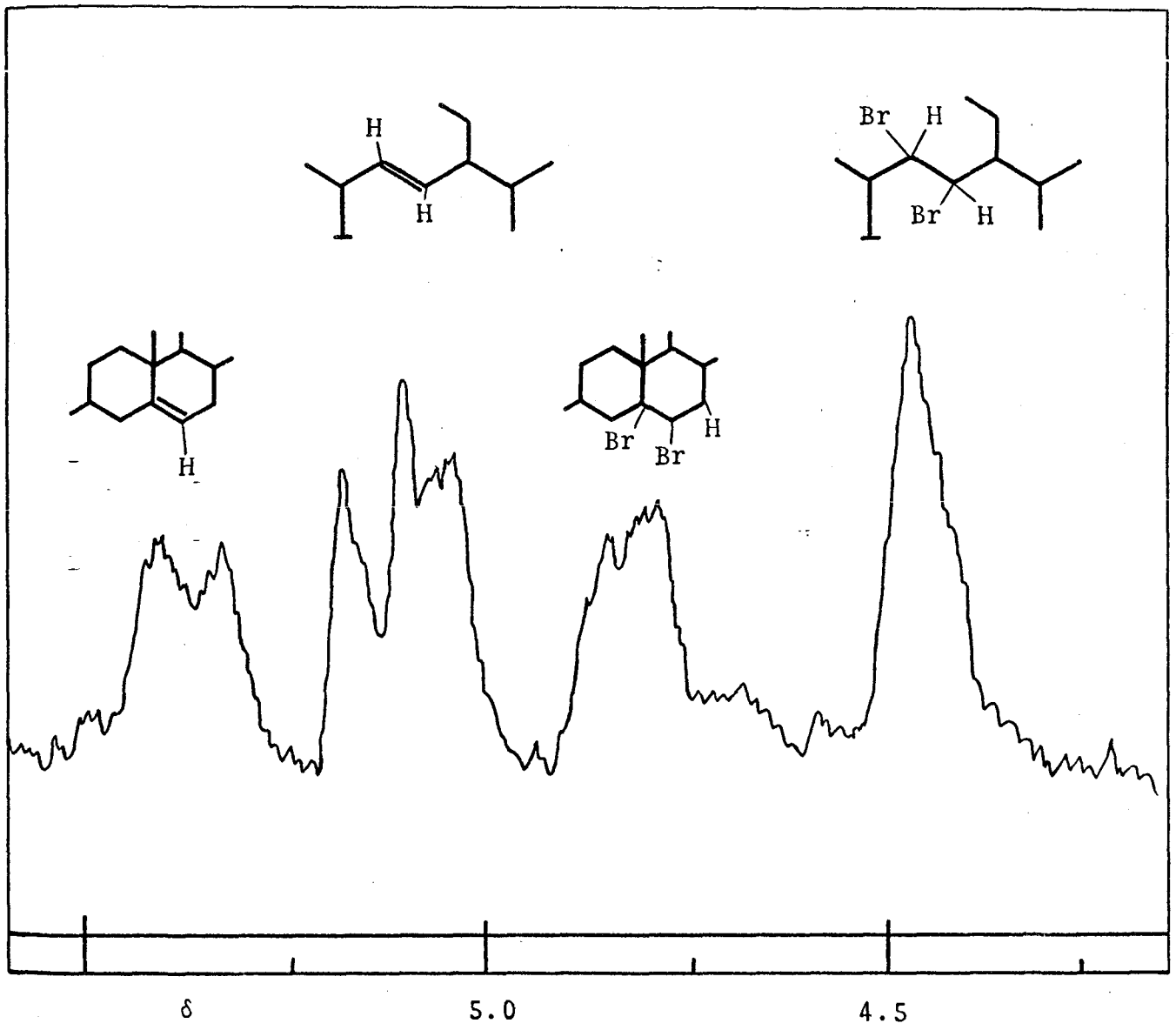
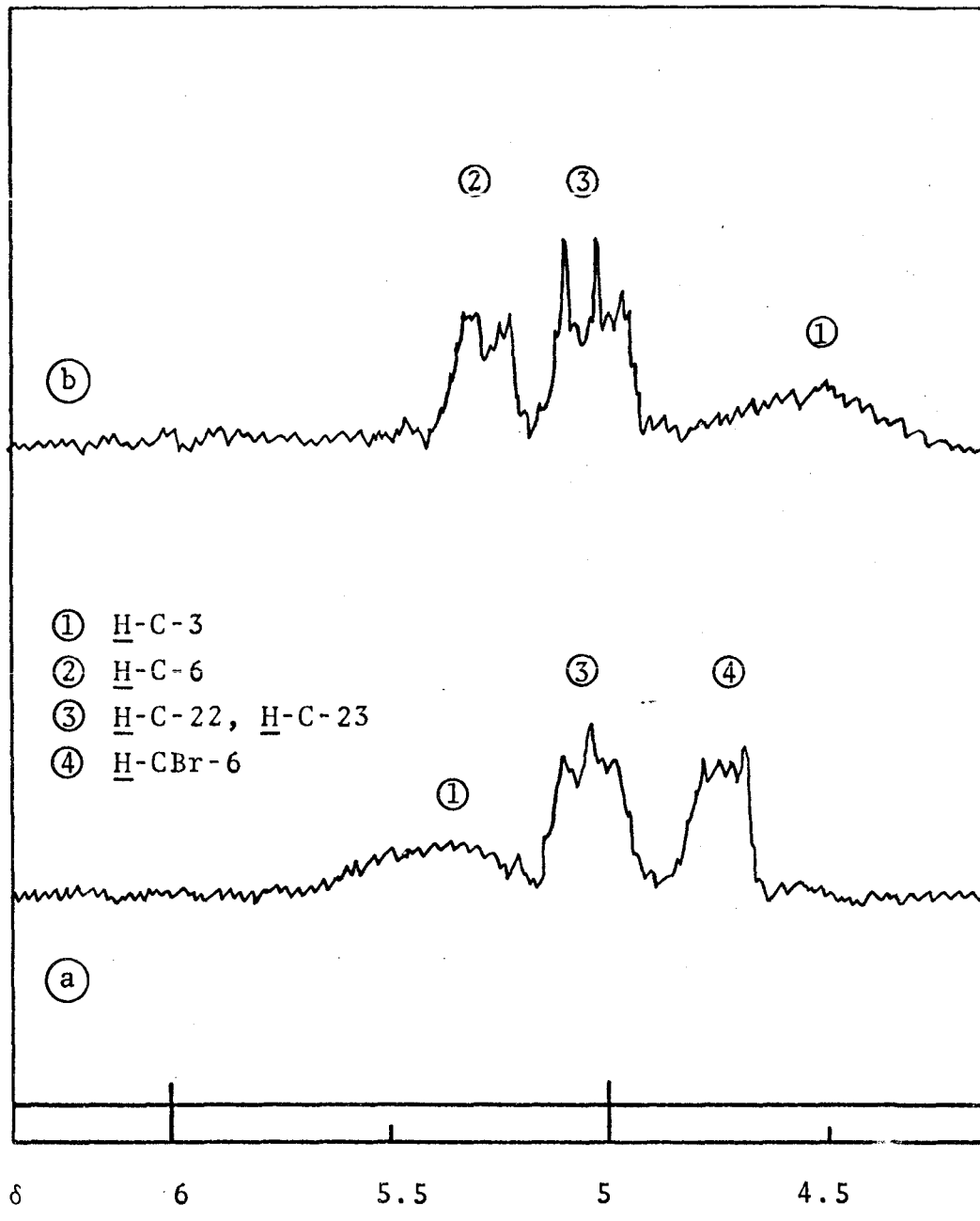


FIGURE 8

a) N.M.R. SPECTRUM 1δ4-5.5 OF 3β-ACETOXY-5, 6 DIBROMOSTIGMASTEROL

b) 3β-ACETOXY-STIGMASTEROL

(BOTH COMPOUNDS TAKEN IN $CDCl_3$ 500 Hz SWEEP WIDTH)



at C-22 and C-23 of the brominated side chain. Several brominations were carried out and the respective peak areas evaluated. It was found that 5,6-dibromostigmasterol was formed in yields of 60-68%. These results, obtained from the NMR, were confirmed by quantitative measurements using i.r.

Smith et al (37) have shown that stigmasterol esters can be chlorinated in the 5,6 position specifically, using iodobenzene dichloride as chlorinating agent, and that subsequent ozonolysis gives 5-bisnorcholenic acid in high yields. Ozonolysis was therefore carried out on 5,6 dichlorostigmasterol acetate, obtained by the method of Smith et al. The yields of aldehyde, however, were low. This is essentially due to the fact that the reaction mixture after ozonolysis had to be heated with zinc and acetic acid in order to achieve dechlorination. Since the corresponding dibromo compound can be dehalogenated at room temperature, a new approach was sought utilizing bromination to protect the 5,6 double bond. A search of the literature revealed no evidence for the use of iodobenzene dibromide as brominating agent. Iodobenzene dibromide itself had been described by Thiele et al (40) as an oil at room temperature, crystallizing at -45°. These properties were confirmed. The compound gives off bromine rapidly at room temperature but is quite stable even at 0°. It is best prepared in a solution of pure hexane and added to the reaction mixture as the hexane solution. Bromination of stigmasterol acetate using

iodobenzene dibromide gives a high yield of the 5,6 dibromide. The product is of high purity and can be used without further purification for the ozonolysis. No detectable amounts of 22,23-dibromide could be found. The evidence for the high selectivity of the reaction comes from the NMR spectra of the crude reaction product. Protons at C-22 and C-23 in 22,23-dibromostigmasterol resonate at δ4.48 (see Figure 7, page 17). No such absorption could be detected in the crude bromination product using iodobenzene dibromide (see Figure 8a, page 18). Furthermore, halogenation of the 22,23 double bond would be expected to shift C-18 and C-19 methyl resonance to lower field (see Table I, page 35). No such absorptions as observed for the C-18 and C-19 methyl resonance of 22,23-dibromostigmasterol acetate obtained by bromination with molecular bromine could be detected in the present case. The shift of the C-3 acetate group in the 5,6- and the 22,23-dibromide differ by 1.5-2 Hz and two sharp singlets could be seen in the mixture derived from bromination according to the procedure of Fernholz. A single peak was observed for these protons in the spectrum of the product formed in above reaction.

Yields of aldehyde 1.10 as its bisulfite adduct were consistantly between 75% and 85%. In addition to the aldehyde, the following products were isolated after several ozonolyses:

1-2% 3β-acetoxy-Δ5-bisnorcholenic acid as its sodium salt,

4-5% stigmasterol acetate,

Ca. 5% unidentified neutral, non ketonic products.

The aldehyde obtained from the decomposition of the bisulfite adduct was of sufficient purity to be used for the subsequent reactions. The aldehyde can either be used directly for the preparation of the sterols in the cholesten series (see Figure 9, page 22) or it can be transformed into 3β-benzoxy-bisnorergosten-22-al 1.16 (see Figure 10, page 23).

The conversion of aldehyde 1.10 to aldehyde 1.16 involved protection of the aldehyde function by acetal formation prior to introduction of the Δ7-double bond. Thus treating the compound with ethylene glycol and BF_3-etherate in glacial acetic acid by the method of Fieser et al (41) gave high yields of acetal. This method is generally used for the protection of ketones only. In the present case it is, however, superior to the usual method for aldehyde protection which involves prolonged refluxing of the aldehyde in benzene in the presence of PTS and ethylene glycol. It is essential to cool the solution rapidly in ice after addition of BF_3-etherate and to filter off the product immediately. Prolonged standing of the product in the mother liquor can reduce the yield to as low as 10-20%. When ethane dithiol was used under the same conditions, the corresponding thioacetal was also formed in high yield.

FIGURE 9

PREPARATION OF STEROIDS IN THE CHOLESTENE SERIES

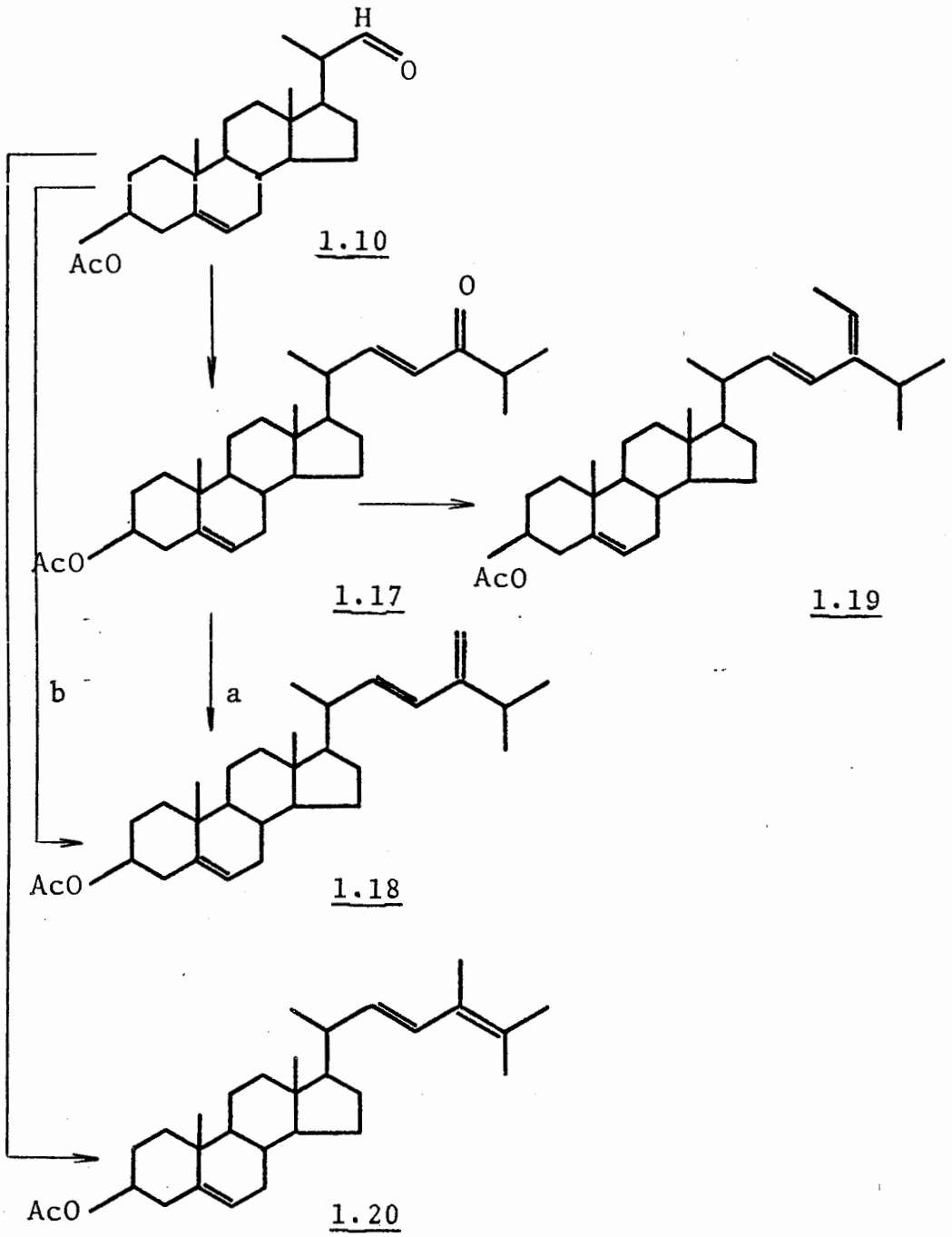
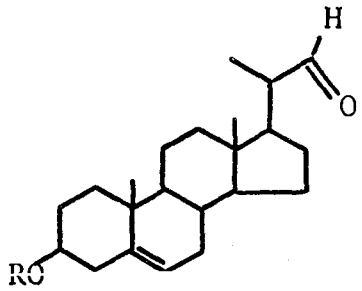


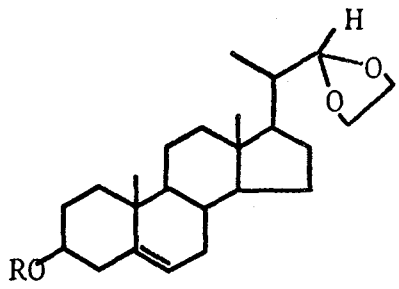
FIGURE 10

TRANSFORMATION OF 3β-ACETOXY-Δ5-BISNORCHOLEN-22-AL to 3β-BENZOXY-Δ5, 7-ERGOSTEN-22-AL

ALDEHYDE II

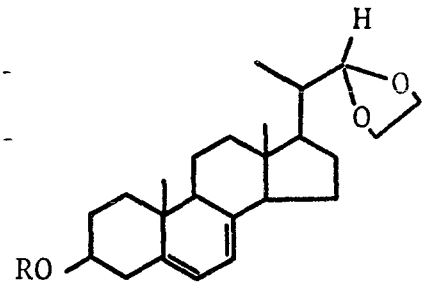


1.10 R = -OAc



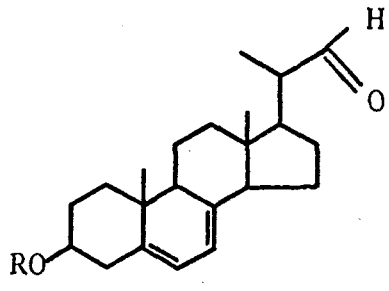
1.12 R = -OAc

1.13 R = -OBz



1.14 R = -OBz

1.15 R = -OAc



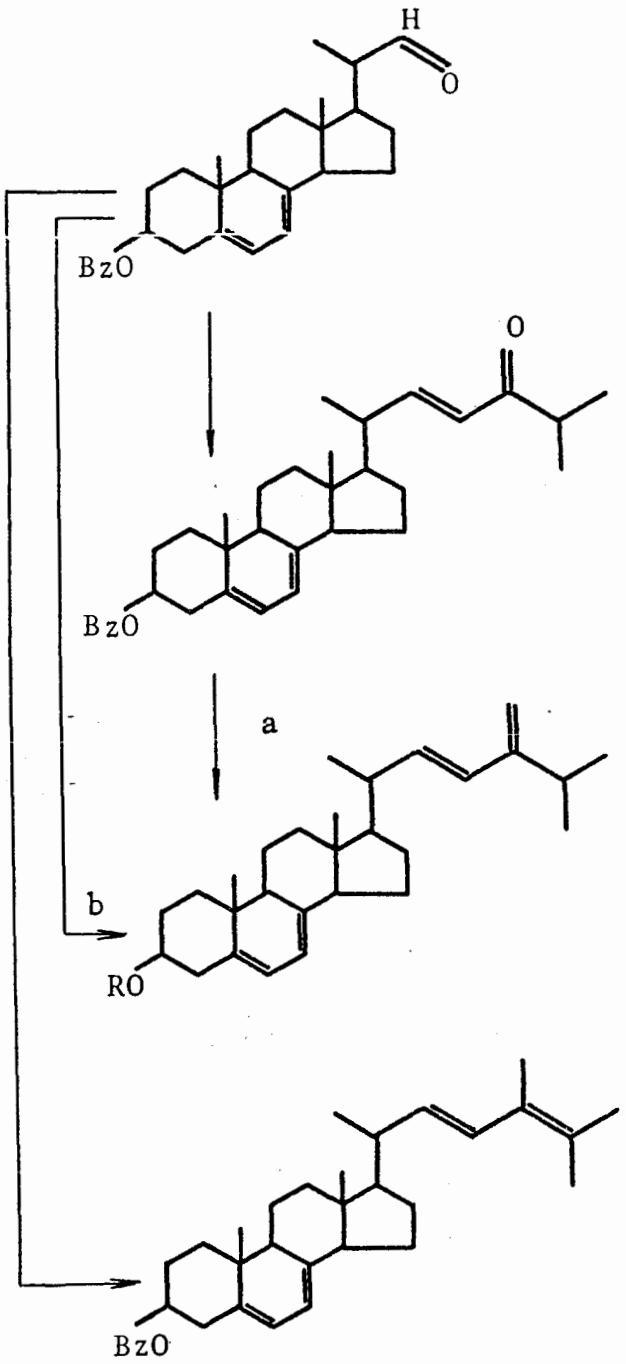
1.16 R = -OBz

The Δ7-double bond was introduced according to the slightly modified method described by Hunziken et al (42) e.g. bromination of 3β-benzoxy-Δ5-bisnor-22,22-ethylene dioxy-cholen with NBS followed by dehydrohalogenation with trimethyl phosphite to give the Δ5, 7-diene. The reaction can be performed on either the C-3 acetate or C-3 benzoate, however, when the acetate was used, it was found that up to 30% of the Δ4, 6-diene was formed. This was evident from the u.v. absorption maxima at 232, 238 and 248 mμ. Evidence for minor quantities of the Δ4, 6 isomer could also be obtained from the NMR spectrum of the crude reaction product by looking at the C-18 and C-19 methyl resonances (see Table I, page 35). Since no detectable amount of the Δ4, 6 isomer was found when the C-3 benzoate was subjected to the bromination/dehydrobromination sequence the acetate was hydrolyzed and the C-3 hydroxyl benzoylated before introduction of the Δ7 double bond.

Hydrolysis of the acetal, in the usual manner, by refluxing in H_2SO_4-water-alcohol gave a poor yield of the free aldehyde. A considerable amount of the isolated product seemed to consist of the 22-methyl hemiacetal, (strong hydroxyl absorption in the infra-red and methoxy peak at δ3.5 in the NMR). High yields of free aldehyde were obtained when the reaction was carried out in THF-water-H_2SO_4. This aldehyde was then further reacted as shown in Figure 11, page 25.

FIGURE 11

PREPARATION OF STEROIDS IN
THE ERGOSTENE SERIES



1.16

1.21

1.22 R = -OBz

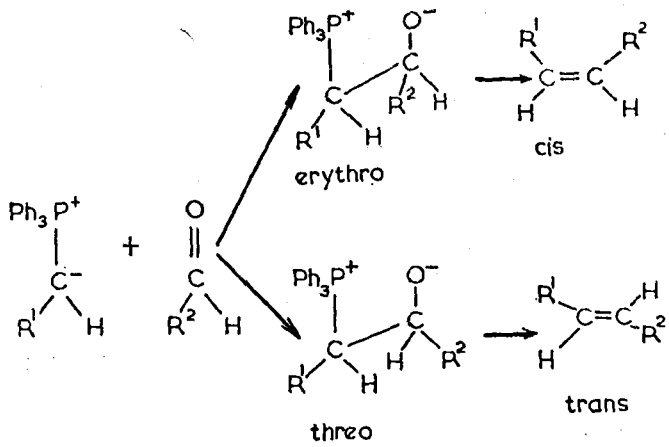
1.23 R = H

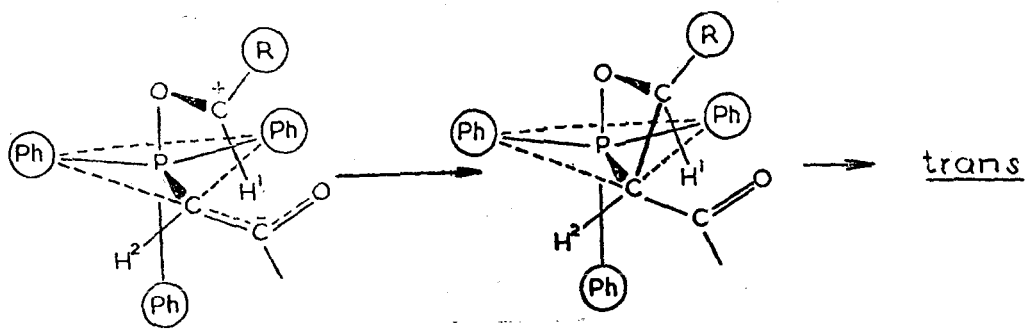
1.24

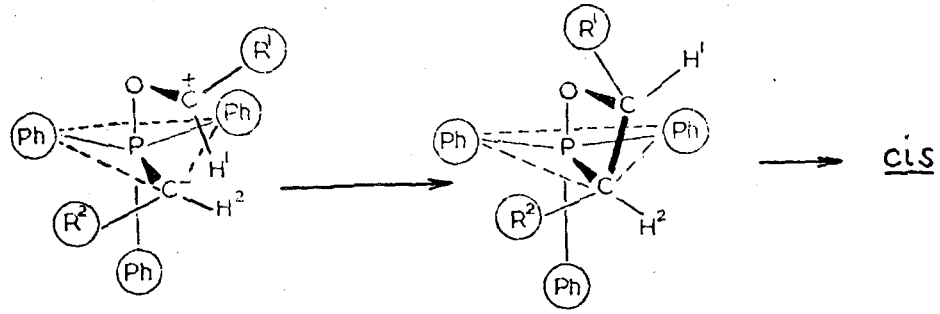
The unsaturated side chains were introduced into the steroidal aldehyde via the Wittig reaction. (For reviews see (43, 44)). The method involved formation of an ylide in a suitable solvent and subsequent reaction of this ylide with the carbonyl compound. It has been reported (45, 46) that resonance stabilized ylides e.g. 3.16 react with carbonyl compounds to give trans olefines nearly exclusively while unstable ylides yield mixtures of the two possible isomers. In such mixtures the cis isomer usually predominates.

Whether a cis or a trans olefin is formed depends mainly on the form and the rate of formation of the postulated intermediate betaine. This intermediate can be either in erythro or threo form. Erythro betaine leads to cis olefin while the threo form results in a trans olefin . Betaine formation is reversible. Decay of this four centered intermediate is therefore directly related to the final cis-trans ratio. The threo form is of lower energy and therefore more stable. If collapse of the intermediate betaine to final product is slow the erythro form collapses faster and cis olefin will be the major product. Schneider (47) suggests an explanation for the different behavior of the two types of ylides. His explanation is based on a triganol-bipyramidal structure (see Figure 12, page 27) of the intermediate betaine formed upon reaction of the carbonyl and the ylide. Coordination of the carbonyl-compound with the bipyramidal ylide tends to favor the

FIGURE 12







TRIGONAL-BIPYRAMIDAL STRUCTURE OF THE WITTIG REACTION INTERMEDIATE ACCORDING TO SCHNEIDER (47)

erythro form due to less steric interaction. The electron-rich, stabilized ylide however tends to stabilize the intermediate in the threo form, regardless of the greater steric interaction. Schneider's model, proposed for ylids stablized by carbonyl groups, can also be applied to double-bond-stabilized ylids. Based on these considerations one would expect the products obtained via Wittig reactions as carried out in this work to have the required trans structure.

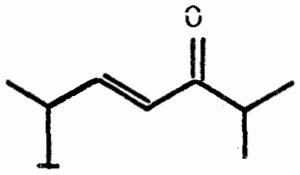
Several of the compounds were synthesized by two different pathways as shown in Figure 10, page 23 and Figure 11, page 25. The correct structure and stereochemistry of the synthesized compounds were finally asserted by:

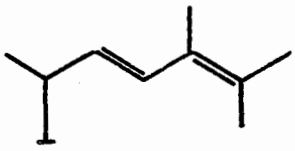
- Ultraviolet Absorption
- Infra-red Absorption
- Nuclear Magnetic Resonance and
- Mass Spectrometry.

2. Spectra

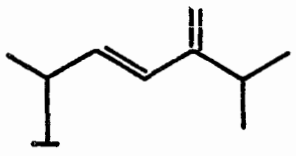
Ultraviolet Absorption

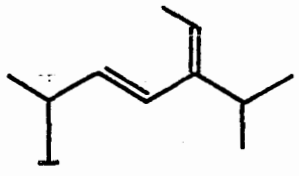
The u.v. absorptions for the double bonds in the ring system agree with the calculated (48) values. The calculated values for the conjugation in the side chain, however, are not in good agreement with the measured values:





Calc.	227	234	mμ
Measured	222	240	mμ





Calc.	224	229	mμ
Measured	230	234	mμ

It has been reported by Koch (49) that the intensity of the major u.v. absorption bands, due to conjugated double bonds, are diminished in the case of cis compounds compared to their trans forms. In the case of compound 1.20 where the two isomers have been isolated this has been verified. (ε_{trans} = 28,300, ε_{cis} = 14,500). The

u.v. absorptions do confirm the presence of conjugation in the side chain, however, it reveals little about specific structural features.

Infrared Absorption

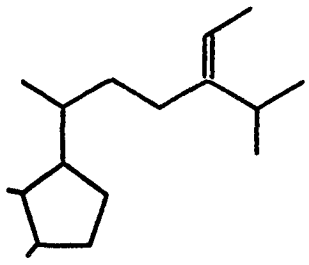
Trans disubstituted ethylenes give rise to medium to strong bands at 990-965 cm^{-1}. Rasmussen et al (50) have shown that this absorption band appears only with trans double bonds. Stigmasterol has 2 strong bands at 975 and 965 cm^{-1} which can be related to the 22,23 double bond. Identical bands are also found in the ergosterol spectrum. Effects of conjugation are reported to be small (51).

All the synthesized compounds have medium to strong absorptions at 975 and 965 cm^{-1}. The 975 cm^{-1} band is not always well resolved and appears as a shoulder on the stronger 965 cm^{-1} band. In the case of 5,22-cis-24 erogstatriene, 1.20, these bands are missing entirely. Two new bonds appear in this case at 745 and 690 cm^{-1}. Bands in this region have been assigned to cis double bonds by several authors (52).

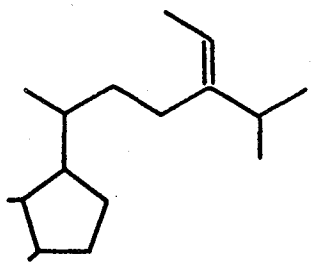
The presence of a terminal methylene group in compounds 1.18 and 1.22 is well documented by the presence of a medium intensity band at 890 cm^{-1}. This assignment is confirmed by the presence of the same absorption in the naturally occurring ergostatetraene isolated from yeast.

Compound 1.19 can have two possible isomers at C-28 e.g. trans - trans or trans - cis. The designation cis and trans is not entirely unambiguous. The Chemical Abstracts nomenclature, put forward by Blackwod et al (53)

therefore designates the two isomers as E and Z



E



Z

A differentiation between the two isomers based on i.r. absorption alone is not possible and one has to rely on the NMR spectra for a definite structure assignment.

NMR Spectroscopy

The steroechemistry at C-28 in compound 1.19 has been assigned to the Z form on the following grounds. Frost et al (54) have isolated 7,24(28) stigmastadiene-3βol and found it to be identical with isofucosterol. In the former compound, H-C-25 resonates at δ2.82. Goodwin et al (55) compared fucosterol and isofucosterol and found H-C-25 resonance to be δ2.2 and δ2.8 respectively. Beates et al (56) correlated several model compounds and 24-ethylidine sterols and found that in all of the examined compounds the heptet, due to the isopropyl methine proton, occurred at either δ2.8 or δ2.2. They assigned δ2.2 to the E- and δ2.8 to the Z- configuration. Compound 1.19 NMR shows a septet at δ2.84 for H-C-25 and must therefore be assigned Z- configuration. The stereochemistry of the 22,23 double bond was established by the resonance in the olefinic region. All the compounds showed the expected doublet (J = 16 ± 1 Hz) for the trans proton at C-23 and the quartet (J = 16 ± 1, 8) for the proton at C-22 trans to H-C-23.

NMR - Signals of the Angular Methyl Protons

Shoolery et al (57) have shown that the resonance frequency of angular methyl protons is highly dependent on the nature and orientation of functional groups in the steroid skeleton. He and later Zürcher (58) and Cohen et al (59) were able to show that the frequency shifts caused by the individual functional groups are approximately additive. Enough data have been compiled and tabulated to allow the calculation of the position of the angular methyl groups in most steroids. This not only permits checking the structure assignment of the steroids synthesized but it also allows the detection of small amounts of by-products contaminating the major products of these syntheses. The method was found to be useful in determing the amount of 22,23-cis-isomer in the crude reaction product from the Wittig reaction. 1-2 Hz down field from the resonance of the C-18 protons a smaller peak is observed. This peak is not present after purification of the major trans product and must be due to the 22, 23 cis isomer. This peak disappeared after purification of the compound. No resonance for olefinic cis protons could be detected in the crude reaction product.

The close agreement of the calculated and the measured values can be seen from Table I. The published values used for the calculation were taken exclusively from the publications mentioned at the beginning. The values in Table II, page 38 were obtained from the newly synthesized steroids.

TABLE I

CHEMICAL SHIFTS OF ANGULAR METHYL GROUPS

NAME	H-C-19			H-C-18			
	Measured	Calc	Δ	Measured	Calc	Δ	
1. Ergosterol benzoate	58.8	58.5	0.3	38.0	38.5	0.5	1)
2. Stigmasterol acetate	61.0			42.0			2)
3. Stigmasterol benzoate	63.2	63.0	0.2	42.0	42.0	0	3)
4. 5,6-dibromostigmasterol acetate (5αBr, 6βBr)	83.0	86.2	3.2	44.0	43.2	0.8	4)
5. 5,6-dichlorostigmasterol benzoate	83.5	83.0	0.5	43.0	43.0	0	
6. 22,23-dibromostigmasterol acetate	61.5	62.0	0.5	46.0	45.0	1.0	5)
7. 3β-acetoxy-bisnor-22,22-ethylene dioxy cholene 1.12	60.0			41.5			6)
8. 3β-benzoxy-bisnor-22,22-ethylene-dioxy cholene 1.13	62.5	62.0	0.5	41.5	41.5	0	
9. 3β-acetoxy-bisnor-22,22-ethylene-dioxy-Δ5,7-ergosten 1.15	56.5	56.5	0	37.5	37.5	0	
10. 3β-acetoxy-bisnor-22,22-ethylene-dioxy-Δ4,6-ergostene	61.0	61.5	0.5	41.5	40.9	0.6	7)
11. 3β-benzoxy-bisnor-22,22-ethylene-dioxy-Δ5,7-ergostene 1.14	59.0	56.5	2.5	38.0	37.5	0.5	
12. 3β-acetoxy-bisnor-Δ5-cholesten-22-al 1.10	62.5			44.0			8)
13. 3β-benzoxy-bisnor-5,7-ergosten-22-al 1.16	59.0	59.0	0	40.5	40.5	0.5	

TABLE I (continued)

NAME	H-C-19			H-C-18			
	Measured	Calc.	Δ	Measured	Calc.	Δ	
14. 3β-acetoxy -Δ5,22-cholestadiene-24-one 1.17	62.5			44.0			9)
15. 3β-benzoxy-Δ5,7, 22-ergostatriene-24-one 1.21	59.4	59.0	0.4	40.0	40.0	0	
16. 3β-acetoxy-24-methyl -Δ5,22,24-cholestatriene 1.20	61.0	62.0	1.0	42.6	42.0	0.6	10)
17. 3β-benzoxy-Δ5,7, 22,24-ergostatetraene 1.24	59.5	58.5	1.0	39.0	38.0	1.0	
18. Δ5,7,22,24(28) ergostatetraene-3βOH 1.23	57.0			39.0			11)
19. 3β benzoxy-Δ5,7,22, 24(28) ergostatetraene 1.22	58.2	58.0	0.2	39.0	38.5	0.5	
20. 3β-acetoxy-24-methylene-Δ5,22,24 (28) cholestatriene 1.18	61.5	61.5	0	42.6	42.5	0.1	
21. 3β-acetoxy-Δ5,22, 24(28) Z-stigmastatriene 1.19	60.5	61.5	1.0	42.0	42.5	0.5	12)

FOOTNOTES

1) Calculated by using the value 3.0 / 0 for C-3 benzoate

2) No value for 17β-$C_{10}H_{19}$ is available; by difference the value for 17β $C_{10}H_{19}$ is -1.5 / -2.0.

3) Calculated by using values obtained from 1 and 2

4) The bad fit for H-C-19 is probably due to the value for the C-3-acetate-5-Br which seems to be different for just C-3-acetate.

5) Calculated using the value for $C_9H_{17}Br_2$/-0.5/+3.0/ given by C. F. Hammer et al STEROIDS 637 (1955)

6) Deducted the value of -2.5/-2.5 for $C_5H_9O_2$ from this measurement. This value is used in all calculations having this group at C-17.

7) No value was available for $\Delta 4,6$. Values are calculated by the unjustified procedure of adding the values for $\Delta 4$ and $\Delta 6$.

8) Deducted value of 0/0 for 17β-C_3H_5O; used for the calculation of No. 13.

9) Deducted value of 0/0 for 17β-$C_8H_{17}O$; used for the calculation of No. 15.

10) The value -0.5/-2.0, reported for 17β-C_9H_{17} has been used for calculation of No. 16 and 17.

11) N.m.r. of the natural compound. From this spectrum the values -1.0/-1.5 were deducted for 17β-C_9H_{15} and used for the calculation of No. 19 and 20.

12) Calculated value obtained by using the values for 17β-C_9H_{15}.

TABLE II

EFFECT OF SOME NEW SUBSTITUENTS ON THE CHEMICAL SHIFT OF C-18 AND C-19 PROTONS

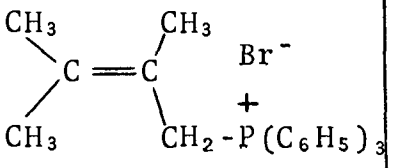
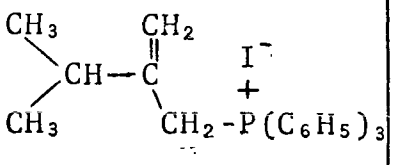
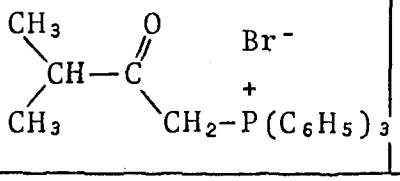
Functional Group	C-19 H Hz	C-18 H Hz
3β - O_2C - C_6H_5 (Δ5 or Δ5,7)	3.0	0
17β - $C_{10}H_{19}$	-1.5	-2.0
17β - $C_5H_9O_2$	-2.5	-2.5
17β - C_3H_5O	0	0
17β - $C_8H_{17}O$	0	0
17 - $C_{10}H_{15}$	-1.0	-1.5

3. Preparation of Alkylhalides and Triphenylphosphonium Salts

Triphenylphosphonium salts are generally prepared by treating the corresponding halide in a suitable solvent, mostly benzene, with triphenylphosphine. The reaction rate increases along the sequence RCl, RBr, RI. The rate depends, however, also on the nature of the alkyl part of the halide. Methyl halides, alkyl halides and halides, activated by the presence of an α- carbonyl group, react most rapidly. The salt of methyliodide precipitated in 92% yield after reaction at room temperature for two hours whereas the salt of ethyliodide was formed only after refluxing. The allylic bromide 1-bromo-2,3 dimethyl-2-butene 3.3 formed the corresponding phosphonium salt spontaneously on mixing with triphenylphosphine. The isomeric bromide 2-methylene-3-methyl-1-bromo butane 3.9, although allylic, did not react in refluxing benzene at normal pressure. Only after heating the reaction mixture at 80° under pressure was a quantitative yield of phosphonium salt obtained. The product was a mixture of two isomeric salts 3.4 and 3.12. Heating of this mixture in $CHCl_3$ under pressure did not alter the ratio of the two isomers. When the corresponding iodo halide 3.10 was treated at room temperature with triphenylphosphine the expected salt 3.12 was formed within one hour in good yield and no isomeric salt was formed.

TABLE III

PREPARED TRIPHENYPHOSPHONIUM SALTS

NO.	NAME	FORMULA	M.P.
3.1	Methyltriphenyl-Phosphonium Iodide	$CH_3-\overset{+}{P}(C_6H_5)_3\ I^-$	184-184.5°
3.2	Ethyltripheny-Phosphonium Iodide	$CH_3-CH_2-\overset{+}{P}(C_6H_5)_3\ I^-$	164-165°
3.4	2,3-dimethy-1-butene-tri-phenyl-phosphonium bromide		164-166°
3.12	2-methylene-3-methyl-1-triphenylphosphonium iodide		184-187°
3.15	Isopropylmethyltri-phenylphosphonium bromide		235-237°

The synthesis of 1-iodo-2-methylene-3-methylbutane 3.10 was accomplished by the reaction sequence shown in Figure 13, page 42 .

The ketoylide 3.16 was prepared by the sequence shown in Figure 14, page 43.

FIGURE 13

PREPARATION OF 1-IODO-2-METHYLENE-3-METHYLBUTANE

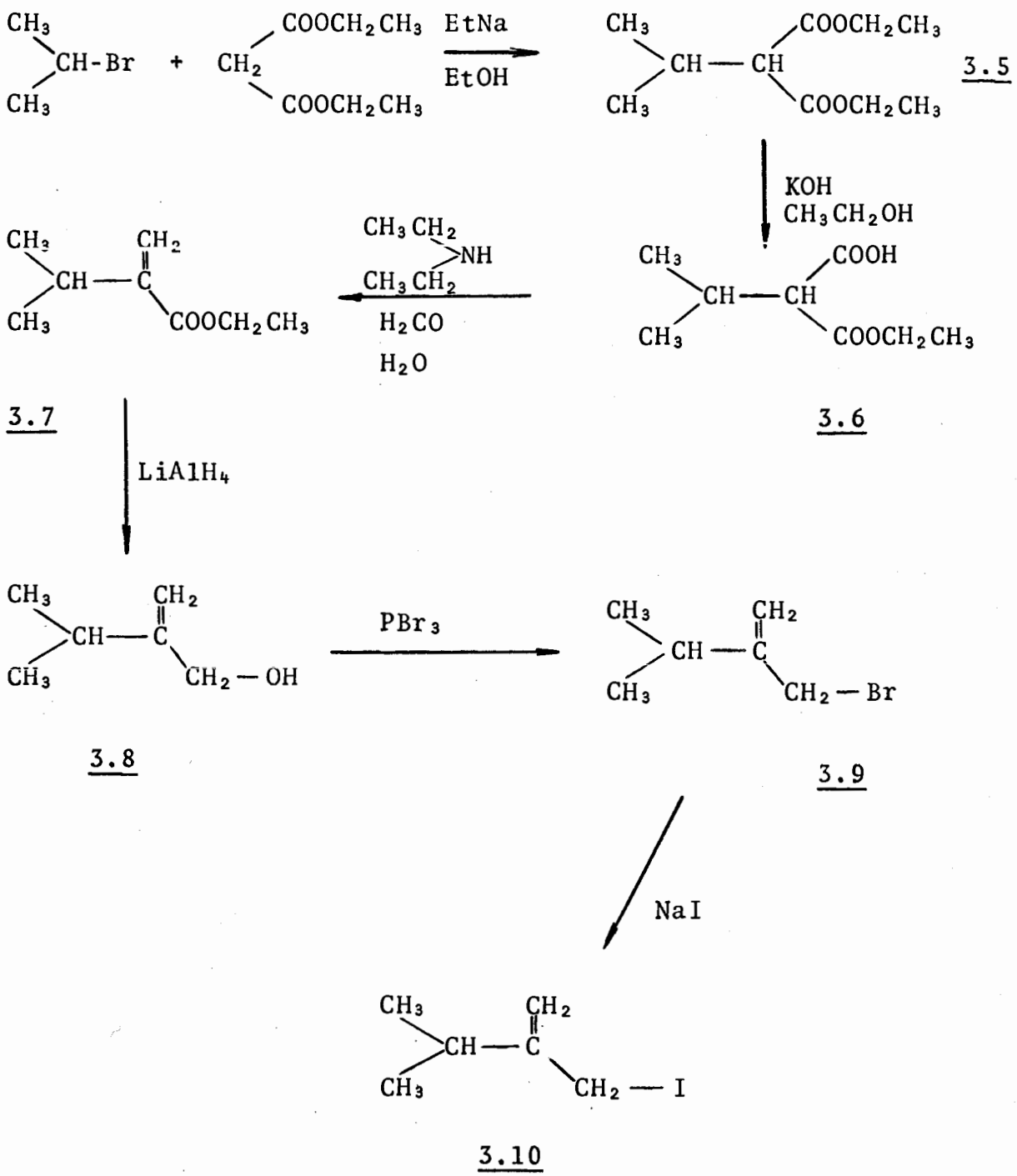
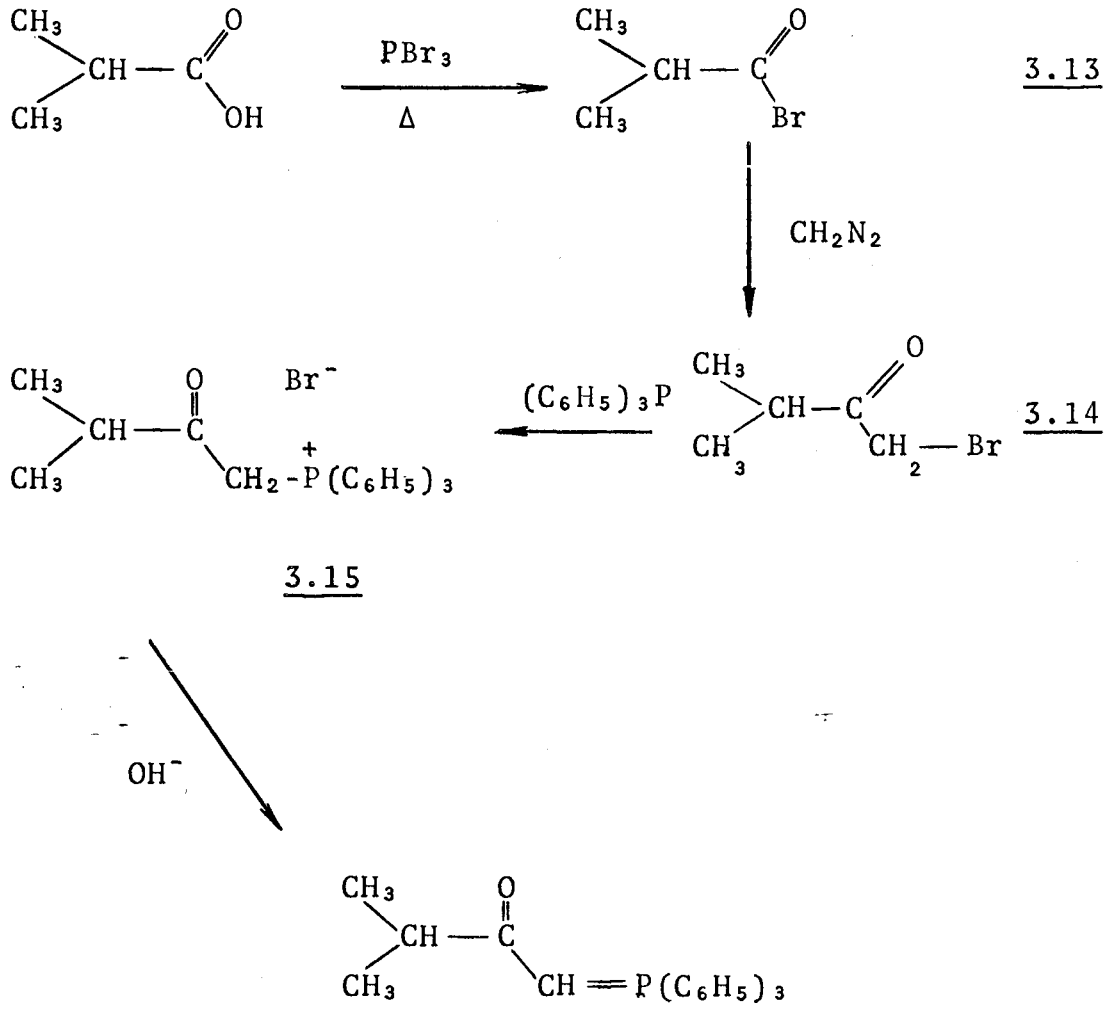


FIGURE 14

PREPARATION OF TRIPHENYLPHOSPINE - ISOPROPYLMETHYLENE



EXPERIMENTAL

Instruments and Materials

Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Spectra were obtained on the following instruments: Perkin-Elmer 457 (i.r.), Unicam SP 800 (u.v.), Varian A 56/60 (n.m.r.) and Hitachi-Perkin-Elmer RMU-7 double focusing mass spectrometer. N.m.r. results are reported as δ using TMS as internal standard (δ = 0). Inlet voltage for m.s. was 80 eV if not specifically mentioned otherwise. Stigmasterol was generously supplied free of charge by the Upjohn Company, Kalamazoo, Michigan. Yeast was donated by the Carling Breweries Limited in Vancouver.

3β-acetoxy-24-ethyl, Δ-5, 22-cholestadiene 1.2 (stigmasterol acetate

To a filtered solution of 50 g (0.12 mmole) stigmasterol 1.1 in 500 ml of pyridine 60 ml acetic anhydride were added. The mixture was left standing at room temperature for 15 hours. The solution was then heated to dissolve the precipitate which had formed and poured onto ice. The solid which separated was filtered off and washed on the filter with 5% HCl and again with water until the washwater was neutral. The solid was dried under vacuum in a desiccator and recrystallized from ethanol to give 50.5 g (91.5%) of stigmasterolacetate. No starting material could be detected by TLC analysis (Silica gel, benzene: hexane 1:1). m.p. 143-144°. (lit. (60) m.p. 144-144.6°). ν_{max} (nujol): 1732 (C = O acetate), 975, 965 (C - 22, C - 23 trans double bond), 888, 846, 818 and 805 cm^{-1} (4 bands associated with C-5, C-6 double bond). N.m.r. (in $CDCl_3$):δ 0.7 (CH -C-18, s)

1.02 (CH_3-C-19,s), 1.98 (CH_3COO-,s) 4.22 - 4.80 (H-C-3), 5.05 (H-C-22, H-C-23, broad m), 5.3 (H-C-6, broad m).

Stigmasterol benzoate 1.3

To 15 g (0.036 mole) of stigmasterol 1.1, dissolved in 150 ml of pyridine, 10 g (0.072 mole) of benzoyl chloride were added. The reaction mixture was treated and worked up as described for the acetate. m.p. 158-159°. (lit. (60) m.p. 160°) ν_{max} (nujol): 1715 (C = O), 975, 965 (C-22, C-23 trans double bond), 845 830 and 808 cm^{-1} (C-5, C-6 double bond). N.m.r. (in $CDCl_3$): δ0.7 (CH_3-C-18, s), 1.06 (CH_3- C-19), 4.65-4.96 (H-C-3, broad m), 5.05 (H-C-22, H-C-23, broad m), 5.3 (H-C-6, broad m), 7.65 (arom. H).

5,6 Dibromostigmasteryl acetate 1.4

1. Preparation of iodobenzene dibromide: To 7 g of iodobenzene (34 mmole) in 10 ml of dry hexane 4.8 g. of bromine (33 mmole) was added. The solution was mixed at r.t. and cooled to below -5°. 2. Bromination: A solution of 15 g of stigmasteryl acetate (33 mmole) in 900 ml of dry hexane was cooled to below -5°. The solution was stirred vigorously with a magnetic stirrer and the reagent prepared as above was added at such a rate as to maintain the solution pale yellow. The addition took 3-4 hours. The slightly turbid solution was filtered and concentrated under vacuum until the major part of the bromosterol precipitated. The mixture was heated until the solid dissolved again and the solution refrigerated for several hours. The 5,6-dibromo-stigmasteryl acetate which crystallized, was filtered, washed thoroughly with ice cold

methanol and dried under vacuum. Yield 13.5 g, m.p. 130-132°. The filtrate and the washings were concentrated to dryness. The solid was triturated with hot methanol, cooled and filtered. 6.2 g of product was collected, m.p. 124-128°. Recrystallization of the total bromosterol from 95% ethanol gave 18.5 g (91.5%) of product, m.p. 131-135°. (lit (39) m.p. 132-135°). ν_{max} (nujol): 1732 (C = O), 975 and 965 cm^{-1}, (C-22, C-23 double bond). N.m.r. (in $CDCl_3$): δ0.70 (CH_3-C-18, s), 1.44 (CH_3-C-18, s), 2.10 (CH_3COO-, s), 4.77 (H-C-6, broad m), 5.15 - 5.60 (H-C-3, broad m), 5.05 (H-C-22, H-C-23, broad m).

Bromination of stigmasterol with molecular bromine

The bromination was carried out according to Fernholz (39). The crude bromosterol obtained after evaporation of the $CHCl_3$ was used for the n.m.r. and i.r. spectra. For the quantitative determination of the 22-23 double bond by i.r. the following equation was used (38):

$$\% \; C{=}C \; = \; \frac{A_{Br} \times 100}{A_0} \; ,$$

where: A_{Br} = % absorption at 975 cm^{-1} after bromination and A_0 = % absorption at 975 cm^{-1} of stigmasterol acetate.
% absorption was obtained according to the base-line technique (61), using a line going through the point on the spectrum at 1000 cm^{-1} and parallel to the background. N.m.r. for 5,6-dibromosterol (in $CDCl_3$): δ0.70 (CH_3-C-18, s), 1.45 (CH_3-C-19, s), 2.10 (OCH_3, s); for 22,23 dibromosterol: δ0.77 (CH_3-C-18, s), 1.0 (CH_3-C-19, s). The region δ4.0 - 6.0 is reproduced in Figure 7, page 17.

5,6 Dichlorostigmasteryl benzoate 1.7

The chlorination was carried out according to Smith and Wallis (37) and Berg and Wallis (62). 16 g (31 mmole) of stigmasteryl benzoate 1.3 and 8.5 g (31 mmole) of iodobenzene dichloride (63) were dissolved in 300 ml of chloroform. The solution was kept at 40-45° for 30 minutes. The chloroform was then distilled off at normal pressure and the dark oil poured into 150 ml of ethanol. Water was added and the precipitate collected. The product was crystallized twice from ethanol to give 14 g (77.5%) of dichlorosterol. No attempt was made to separate the two isomers (5α, 6α and 5α, 6β) and, therefore, a sharp melting point was not obtained. (m.p. 120-135°). The mixture of the two isomers was used for ozonolysis. ν_{max} (nujol): 1715 (C = O), 975 and 965 cm^{-1} (C-22, C-23 double bond). N.m.r. (in $CDCl_3$): δ0.7 (CH_3-C-18, s), 1.42 (CH_3-C-19, s), 4.18 (HC-6 broad m), 5.05 (HC-22, HC-23, broad m), 5.4 (H-C-3, broad m), 7.65 (arom. H, m).

Ozonolysis of 5,6-Dibromostigmasteryl acetate

A solution of 15 g (0.0244 mole) of 1.4 in 600 ml. of dichloromethane, containing 8 ml of pyridine, was cooled to -70°. A mixture of ozone and oxygen was introduced at a constant flow rate of 2 l/min. (0.5 mmole ozone/min.). The ozone passing through the solution unreacted was monitored by titrating the iodine liberated from a 5% potassium iodide solution against sodium thiosulfate. The reaction was terminated after 1.7 molar equivalents of ozone were absorbed.

Zinc powder (40 g) and glacial acetic acid (60 ml) were added. The solution was warmed to r.t. and stirred vigorously for 3-4 hours. The solution was filtered, washed twice with water then with $NaHCO_3$ solution and finally with 5% NaOH. The salt of 3-acetoxy-5-bisnor-cholenic acid which concentrated at the interface was discarded. The dichloromethane solution was washed with water until neutral. The solvent was removed at reduced pressure, leaving a crystalline residue. This product was dissolved in a small amount of methanol then 200 ml of a saturated $NaHSO_3$ solution was added and the mixture shaken for several hours. The suspension was then added to an equal amount of ether in a separatory funnel and shaken. Most of the aqueous phase was drawn off and discarded. The remainder of the mixture was centrifuged and the ether decanted. The solid was washed several times with water and ether and dried under vacuum in a desiccator to give 9.5 g (81.5%) of the bisulfite addition compound of 3β-acetoxy-Δ5-bisnorcholen-22-al Ozonolysis of stigmasterol acetate dichloride 1.6 was carried out in an analogous manner.

Conversion of bisulfite adduct 1.8 to 3β-acetoxy-bisnor-22, 22-ethylene-dioxycholene 1.12

To 7 g of bisulfite adduct of Δ5-bisnor-cholen-22-al 1.8 suspended in 20 ml of water, 500 ml of ether and 300 ml of 10% Na_2CO_3 solution were added. The mixture was shaken until two clear layers formed (5-10 minutes). The aqueous layer was drawn off and washed twice with ether. The combined ether

solutions were washed with water until neutral and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum at r.t. to give a white, crystalline residue of 3β-acetoxy-Δ5-bisnor cholen-22-al 1.10 m.p. 116-118° (lit. (35) m.p. 116-117°). ν_{max} (nujol): 1724 cm^{-1}, aldehyde C = O. N.m.r. (in $CDCl_3$): δ0.74 (CH_3-C-18, s), 1.03 (CH_3-C-19, s), 2.0 (OCH_3, s), 5.3 (H-C-6, broad m), 9.55 (-CHO, d, J=3Hz). The aldehyde 1.10 was immediately dissolved in a boiling solution of 8 ml of diethylene glycol and 25 ml glacial acetic acid. The solution was cooled to 40° and 4 ml of borontrifluoride-etherate added. Crystallization occurred immediately. The mixture was quickly cooled in ice and filtered. The solid was washed with a few drops of ice cold methanol and dried under vacuum for several hours to give 5 g (82%) of 3β-acetoxy-bisnor-22,22-ethylenedioxycholene 1.12. Recrystallization from ethyl acetate gave a product with m.p. 209-210°. N.m.r. (in $CDCl_3$): δ0.685 (CH_3-C-18, s), 0.92 (CH_3-C-21, d, J=6.5 Hz), 1.0 (CH_3-C-19, s), 2.0 (CH_3COO-, s), 3.85 (4H, ethylenedioxy H, d, J=2Hz), 4.81 (H-C-22, d, J=2 Hz), 5.35 (H-C-6, broad m).

Anal. Calcd: C, 74.96; H, 9.68

Found: C, 74.63; H, 9.56.

3β-Benzoxy-bisnor-22,22-ethylene-dioxycholene 1.13

The acetate 1.12 (5 g) was refluxed for one hour in 300 ml of 5% alcoholic KOH, poured into ice/water and extracted with CH_2Cl_2. The extract was washed with water until neutral, dried over anhydrous $MgSO_4$ and evaporated to dryness. The

residue was taken up in 80 ml of pyridine and 10 ml of benzoyl chloride was added. After standing at r.t. for 12 hours the solution was poured into ice. The formed precipitate was collected, washed free of pyridine with water and recrystallized from ethyl acetate to give 5.2 g (90.5%) of benzoate 1.13, m.p. 178-180°. After three more recrystallizations the analytical sample melted at 182.5-183°. ν_{max} (KBr): 1715, 1600, 1585, 1260, 1250, 1105 1030, 1010, 800 and 710 cm^{-1}. N.m.r. (in $CDCl_3$): δ0.710 (CH_3-C-18, s), 0.925 (CH_3-C-21, d, J=6.5 Hz), 1.08 (CH_3-C-19, s), 3.9 (-O-CH_2—CH_2-O-, d, J=2 Hz), 4.875 (H-C-22, d, J=1.5 Hz), 5.44 (H-C-6, m) 7.7 (arom. H, m)

Anal. Calcd: C, 77.79; H, 8.84 Found: C, 78.04; H, 8.90.

3β- Benzoxybisnor-22,22-ethylenedioxyergostene 1.14

A solution of 4 g (0.084 mole) of bisnor-22,22-ethylene-dioxybenzoate 1.13 in 100 ml of CCl_4 was heated to reflux and 1.65 g (0.093 mole) of NBS added. The mixture was refluxed for 8 minutes then cooled in ice and the solid (995 mg, m.p. 122-126°) filtered and washed with cold petroleum ether. The filtrate was evaporated at r.t. under vacuum and the obtained crystalline residue taken up in 75 ml of xylene. The solution containing the bromosterol was added dropwise to a vigorously boiling solution of 5.2 g (0.042 mole) of trimethylphosphite in 25 ml of xylene. After refluxing for 90 minutes the xylene was distilled off at 75° under vacuum and the crystalline residue was treated with little ethyl acetate in the cold, filtered and washed on the filter with little ice cold petroleum ether. The crude product was then recrystallized from ethyl acetate

to give 2.77 g (69%) of 1.14 m.p. 155-158°. The analytical sample had m.p. 166-167° after three recrystallizations from the same solvent. ν_{max} (nujol): 1715, 1600, 1585, 1270, 1110, 835, 807 and 710 cm^{-1}; λ_{max}^{Hexane} 226 mμ (ε= 15,400), 260 mμ (ε= 9,000), 271 mμ (ε= 13,200), 282 mμ (ε= 13,300) and 293 mμ (ε = 7,200). N.m.r. (in $CDCl_3$): δ0.634 (CH_3-C-18, s), 0.984 (CH_3- C-19, s), 0.966 (CH_3-C-21, d, J= 7.0 Hz), 3.85 (ethyldioxy H, d, J=1.8 Hz), 4.81 (H-C-22, d, J= 2.0 Hz), 5.54 (H-C-6, H-C-7 broad m), 7.84 (arom. H, m).

Anal Calcd: C, 78.12; H, 8.46. Found: C, 78.24; H, 8.53

3β-acetoxy-bisnor-22,22-ethylenedioxy-ergostene 1.15

The reaction was carried out in the same manner as described for the 3β-benzoxy analog. The crude product was a mixture of Δ4, 6-diene (Ca. 30%). λ_{max} 232,238 and 248 mμ; Δ5,7-diene (Ca. 65%) λ_{max} 261, 273, 282 and 295 mμ and Ca 5% of a more highly conjugated compound λ_{max} 307 and 320 mμ. N.m.r. for Δ5,7-diene (in $CDCl_3$): δ0.63 (CH_3-C-18, s), 0.94 (CH_3-C-19, s); for Δ4, 6 diene: δ0.69 (CH_3-C-18, s), 1.03 (CH_3-C-19, s).

3β-Benzoxy-bisnorergostene-22-al 1.16

To a solution of 2.0 g (4.2 mole) acetal 1.14 in 50 ml of THF 5% sulfuric acid was added until precipitate appeared. The solution was clarified by addition of THF. The reaction vessel was flushed for 10 minutes with a stream of N_2, sealed and stirred vigorously at r.t. for 12 hours. The solution was neutralized with Na_2CO_3 and most of the the THF distilled off. The product was extracted with CH_2Cl_2, dried over anhydrous $MgSO_4$ and the solvent evaporated. The crystalline residue was taken up in little cold ether and filtered to give 1.57 g (86.7%) of 1.16

m.p. 179.0-182.5°. ν_{max} (KBr: 2930, 2860, 2710, 1724, 1715, 1600, 1585, 1500, 1312, 1270, 1205, 1110, 1070, 835, 807 and 710 cm^{-1}; λ_{max}^{Hexane} 226 mμ (ε= 15,500), 234 mμ shoulder (ε= 11,100), 260 mμ (ε= 9,100), 270.5 mμ (ε= 13,400), 281 mμ (ε = 13,500) and 293 mμ (ε = 7,300). N.m.r. (in $CDCl_3$): δ0.68 (CH_3-C-18, s), 0.965 (CH_3-C-19, s), 1.1 (CH_3-C-21, d, J=7.0 Hz), 4.9 (H-C-3, broad m), 5.5 (H-C-6, H-C-7, broad m), 7.84 (arom. H, m), 9.6 (-CHO, d, J=3 Hz).

Mass Spec.: Found mol. wt. 432. $C_{29}H_{36}O_3$ required: mol. wt. 432.

General Procedure for the Wittig Reaction with the Nonketonic Phosphonium Salts

Carefully dried and pulverized phosphonium salt was suspended in dry diethyl ether in a 500 ml pressure bottle. The bottle was flushed for 10 minutes with dry nitrogen and capped with a seriological septum. The calculated amount of butyllithium in heptane was introduced with a syringe through the septum. The bottle was then shaken mechanically at r.t. until most of the salt dissolved. The carbonyl compound, dissolved in dry ether or THF, was then added to the ylide solution. The addition was again made with a syringe through the septum. The reaction mixture was shaken at r.t. for 1-2 hours. The septum was replaced by a rubber stopper, fitted with a thermometer. The mixture was heated to 60° and shaken for 10-12 hours. After cooling, moist ether was added until excess reagent was decomposed. The crystalline solid was filtered and the ether solution, containing the product, was dried over anhydrous Na_2SO_4, evaporated to dryness and the

residue taken up in pyridine if reesterification was necessary. Otherwise the product was purified further as described in the individual cases.

3β-acetoxy-Δ5, 22-cholestadiene-24-one 1.17

Bisulfite adduct 1.8 (3.5 g, 7.4 mole) was decomposed as described previously to give 2.1 g (77.5%) of aldehyde 1.10. To a solution of the aldehyde in 150 ml of DMSO 12 g (35 mmole) of triphenylphosphineisopropylmethylene 3.16 was added and the reaction mixture heated at 95° for 65 hours. Then 500 ml of water and 100 ml of 10% H_2SO_4 were added and the product extracted with ether. The ether extract was washed several times with water and dried over anhydrous $MgSO_4$. The crude product was purified on a SiO_2 column, using hexane:ether 95:5 as eluting solvent to give 1.7 g (68.4%) 1.17. Recrystallization from methanol gave m.p. 141.5-142.5°. ν_{max} (KBr): 1732 (acetate C=O), 1695, 1670 (sh), 1625 and 990 cm^{-1} (-C=CH-CO-); λ_{max}^{EtOH} 222 mμ (ε= 19,500). N.m.r. (in $CDCl_3$):δ0.73 (CH_3-C-18, s), 1.1 (CH_3-C-19, s), 1.15 (CH_3-C-21, $2CH_3$-C-25, d, J=7.0 Hz) 2.0 (CH_3COO-, s), 2.8 (H-C-25, m, J=7 Hz), 4.42 - 4.85 (H-C-3), 5.36 (H-C-6, m), 6.08 (H-C-23, d, J=16 Hz), 6.74 (H-C-22, d of d, J= 8.5 and 16 Hz).

Mass Spec: Found 440. $C_{29}H_{44}O_3$ required mol wt. 440

2.4-Dinitrophenylhydrazone derivative of 1.17 This characteristic compound was made in the usual fashion by refluxing ketone 1.17 and ethanolic sulphuric acid solution of 2.4-dinitrophenylhydrazine. The separated product was recrystallized two times from ethyl-acetate-methanol and had m.p. 214.0-214.5°.

3β-acetoxy-24-methylene-Δ5,22,24(28)-cholestatriene 1.18

a) Preparation via ketone 1.17

Methyltriphenylphosphonium iodide 3.1 (1.7 g, 4.2 mmole) in 25 ml of ether was treated with 4.1 mmole butyllithium in heptane. This mixture was reacted with ketone 1.17 (270 mg, 0.6 mmole) in 20 ml of ether. The reaction mixture was shaken for one hour at r.t. and three hours at 50°. After work up and acetylation, the residue was chromatographed on 20 g of SiO_2. The product was eluted with benzene:hexane 1:1 and recrystallized twice from methanol to give 86 mg (32%) of 1.18 m.p. 143.5-144°. Stripping the column with ether gave 65 mg of 1.17. $(\alpha)_D^{20} = -58°$ (C=0.99); ν_{max}(KBr): 3095, 1600, 975 (sh), 965, 890 (-HC=CH-C=CH_2), 1732 cm^{-1} (C=O); λ_{max}^{EtOH} 231 (ε= 21,400), 226 sh (ε ~20,000) and 238 sh (ε ~16,000). N.m.r. (in $CDCl_3$): δ0.71 (CH_3-C-18, s), 1.025 (CH_3-C-19, s), 1.07 (12H, CH_3-C-21, $2CH_3$-C-25, d, J=6.5 Hz), 2.0 (CH_3COO-, s), 4.3-4.7 (H-C-3), 4.79 (=CH_2, broad, s), 5.3 (H-C-6, m), 5.62 (H-C-22, quart., J=15 and 8 Hz), 5.91 (H-C-23, d, J=15 Hz).

Mass Spec: Found: mol. wt. 438. $C_{30}H_{46}O_2$ required mol wt. 438.

b) Preparation from aldehyde 1.10

To a suspension of 6.24 g (0.0132 mole) of 2-methylene-3-methylbutane-1-triphenylphosphonium iodide 3.12 in 30 ml of ether 0.013 mole of butyllithium in heptane was added. After 30 minutes at r.t. 1.0 g (2.64 mmole) of aldehyde 1.10 was added. The reaction mixture was then treated as above to give 510 mg (44%) of 1.18. After two recrystallizations from alcohol the product had m.p. 143.0-144.0°. The i.r., u.v. and n.m.r. spectra obtained were superimposable with those obtained in preparation a).

3β-acetoxyΔ5,22(24(28)Z)stigmastatriene 1.19

Ethyltriphenylphosphonium iodide 3.2 (1.66 g, 3.97 mmole), suspended in 35 ml dry ether, was reacted with 3.85 mmole butyllithium in heptane at r.t. for 1 hour. Ketone 1.17 (350 mg, 0.795 mmole) in 20 ml of dry ether was added and the reaction allowed to proceed at r.t. for 1 hour. The reaction mixture was then shaken at 60° for 10 hours. After cooling, excess reagent was decomposed with moist ether and the reaction worked up in the usual manner. The product 1.19 (C-3-OH) was reacetylated with acetic anhydride in pyridine. The acetate was purified by preparative TLC (SiO_2, benzene). After recrystallization from ethanol 1.19 had m.p. 122-122.5°; $(\alpha)_D^{20}$ (C=1.1) -54°; ν_{max}(KBr): 1732 (C=O), 970, 962, 903, 886, 844, 810, 803 and 795 cm^{-1}; λ_{max}^{EtOH} 234 (ε= 24,000). N.m.r. (in $CDCl_3$): δ0.70 (CH_3-C-18, s), 1.01 (CH_3-C-19, s), 1.0 (CH_3-C-26, C-27, d, J=7Hz); 1.625 (CH_3-C-29, d, J=7 Hz), 2.0 (CH_3COO-, s), 2.82 (H-C-25 sept. J=7 Hz), 4.3 - 4.8 (H-C-3, m), 5.15 - 6.0 (4H, H-C-6, H-C-22, H-C-23 and H-C-28, m).

Mass Spec: Found: mol wt. 452. $C_{31}H_{48}O_2$ required: mol wt. 452

3β-acetoxy-24-methyl-Δ7,22,24 cholestatriene 1.20

To a suspension of 1.6 g (3.8 mmole) of 2,3,-dimethyl-2-butenetriphenyphosphonium bromide 3.4 in 30 ml of dry ether, 3.8 mmole of butyllithium in heptane was added. The mixture was left to react at r.t. for 1 hour then 1.0 g (2.64 mmole) of aldehyde 1.10 was added. After 1 hour, all the ylide had reacted. The mixture was then shaken at 55-60° for 10 hours, cooled, filtered and concentrated. The crystalline product, obtained when chromatographed on prep TLC (SiO_2 benzene), showed two components. The

major product and a less polar minor component were recovered separately. (The starting material and some triene-alcohol remained near the origin.) Both recovered components had identical u.v. absorptions. The major product was trans- 3β-acetoxy-7,8-dihydro-Δ5,22,24-ergostatriene (for structure assignment see discussion). After recrystallization from ethanol, it analyzed as a single component on TLC ($SiO_2/AgNO_3$). The product (735 mg, 63%) had m.p. 110-110.5°; $(\alpha)_D^{20}$ (C = 1.05) -51°; ν_{max}(KBr): 1732 (C=O), 1140, 1045, 965, 810, 840, 855 and 890 cm^{-1}; λ_{max}^{EtOH} 241.5 μm (ε= 28,300). N.m.r. (in $CDCl_3$); δ0.71 (CH -C-18, s), 1.015 (CH_3-C-19, s), 1.05 (CH_3-C-21, d, J=7 Hz), 1.72((CH_3)$_2$ C=, s), 1.76 (CH_3-C-24, s), 1.98 (CH_3COO-, s), 4.3 - 4.8 (H-C-3, m), 5.33 (H-C-22, quart. J=16 and 8 Hz), 5.37 (H-C-6, m), 6.32 (H-C-23, d, J=16 Hz).

Mass Spec: Found mol. wt. 438. $C_{30}H_{46}O_2$ required: mol wt. 438.

3β-benzoxy-Δ5,7-ergostatriene-24-one 1.21

3β-benzoxy-22-bisnor-Δ5,7-ergosten-22-al 1.16 (1.4 g, 3.24 mmole) and 7 g (20 mmole) of triphenylphosphineisopropylmethylene 3.16 in 200 ml of DMSO were treated in the same way as in the preparation of ketone 1.17 to give 0.68 g (42%) of 1.21 m.p. 148-149°. ν_{max} (KBr): 1715 (C=O), 1695 (sh), 1670, 1625, 995, 985 (-C=CH-CO-), 1600, 1585, 835 and 710 cm^{-1}. $\lambda_{max}^{CH_3OH}$ μm 293 (ε= 7,800) 281 (ε= 14,400), 270.5 (ε= 14,600), 261 (ε= 11,800) and 228 (ε= 29,000). N.m.r. (in $CDCl_3$):δ0.67 (CH_3-C-18, s), 0.99 (CH_3-C-19, s), 1.1 ($2CH_3$-C-25, d, J=7 Hz), 1.14 (CH_3-C-21, d, J=7 Hz),2.75 (H-C-25, sept., J=7 Hz), 4.65 - 5.2 (H-C-3), 5.44 (H-C-6, H-C-7, m), 6.12 (H-C-23, d, J=16 Hz), 6.8 (C-22, quart., J=16, 8 Hz), 7.82 (arom. H, m).

Mass Spec: Found mol. wt. 500. $C_{34}H_{44}O_3$ required: mol. wt. 500.

3β-benoxy,Δ5,7,22,24-ergostatetraene 1.24

3β-benzoxy-22-bisnor-Δ5,7-ergosten-22-al 1.16 in THF (1.1 g, 2.55 mmole) was added to a solution of 12.1 mmole of the ylide prepared in the usual way from 2,3-dimethyl-triphenyl-phosphonium bromide 3.4 and treated as in the preparation of 1.20 to give 0.925 g (73%) of 1.24 m.p. 147.5-148°. ν_{max} (KBr): 1716 (C=O), 1600, 1585, 1500, 965, 890, 835 and 710 cm^{-1}; λ_{max}^{EtOH} 234 mμ (ε= 43,500), 271 mμ (ε= 12,100) and 293.5 mμ (ε= 6,200); N.m.r. (in $CDCl_3$): δ0.66 (CH_3-C-18, s), 1.0 (CH_3-C-21, d, J=7 Hz), 1.01 (CH_3-C-19, s), 1.72 ((CH_3)$_2$-C=, s), 1.75 (CH_3-C-24, s), 4.45 - 4.9 (H-C-3), 5.3 (H-C-22 quart., J=8 and 8 Hz), 5.36 (H-C-6, H-C-7, m), 6.25 (H-C-23, d, J=15.5 Hz), 7.7 (arom. H, m).

Mass Spec: Found mol. wt. 498. $C_{35}H_{46}O_2$ required: mol. wt. 498.

3β-benzoxy-Δ5,7,22,24(28)-ergostatetraene 1.22

a) Preparation via ketone 1.21

3β- benzoxy-Δ5,7-ergostatriene-24-one 1.16 (1.0 g, 1.97 mmole) in THF was added to 10 mmole of ylide, prepared in ether from 2.47 g (10 mmole) of methyltriphenylphosphonium iodide 3.1 and 9.8 mmole of butyllithium. The reaction product was rebenzoylated and purified on preparative TLC to give 0.72 g (72%) 1.22 m.p. 148.5-150.0°.

b) Preparation via aldehyde 1.16

3β-benzoxy-22-bisnor -Δ5,7-ergosten-22-al 1.16 (1.1 g, 2.55 mmole) in THF was added to a solution of 12.7 mmole of 2-methylene-

3-methylbutane-1-triphenylphosphonium ylide, prepared from the salt 3.12 and butyllithium. The reaction mixture was treated as in the preparation of 1.18b. The crude product was a mixture of C-3 benzoate and free alcohol. The mixture was treated with benzoyl chloride in pyridine in the usual way and chromatographed on SiO_2 to give 0.69 g (54.5%) of 1.16 m.p. 148-149°. After two recrystallizations from ethyl acetate-methanol the m.p. was 149.0-150.5°. (lit. (29) m.p. 149-151°). ν_{max} (KBr), 1715 (C=O), 1600, 1585, 970, 962, 886, 835, 803, and 710 cm^{-1}; λ_{max}^{EtOH} 229 mμ (ε= 36,900), 271 mμ (ε= 12,200), 281.5 mμ (ε= 12,900) and 293 mμ (ε= 7,000). N.m.r. (in $CDCl_3$): δ0.65 (CH_3-C-18, s), 0.95 (CH_3-C-19, s), 1.06 (CH_3-C-21, $2CH_3$-C-25, d, J=7 Hz), 4.5 - 4.9 (H-C-3), 4.79 ($=CH_2$, m), 5.62 (H-C-22, quart. J=15 and 8 Hz), 5.44 (H-C-6m H-C-7, m), 5.9 (H-C-23, d, J=15 Hz), 7.7 (arom. H, m).

Mass Spec: Found mol. wt. 498. $C_{35}H_{46}O_2$ required: mol. wt. 498.

Isolation of Δ5,7,22,24(28)-ergostatetraene from Saccharomyces cerevisiae

Brewers yeast was digested in alcoholic KOH at reflux temperature for 1 hour. An equal volume of water was added and the lipid fraction extracted with heptane. The extract was washed with water until neutral and dried. After evaporation of the solvent the crude residue was benzoylated in the usual way in pyridine with benzoyl chloride. The major part of the ergosterol benzoate was removed by crystallization. The mother liquor was chromato-

graphed on a SiO_2 column. The fraction, containing most of the tetraene, was further purified by preparative TLC (SiO_2 + 10% $AgNO_3$) and recrystallized from ethyl acetate methanol to give the pure compound, m.p. 149.5-150.5°. (lit. (29) 149-151°). The m.p. was not depressed on mixing with the synthetic compound. The i.r, u.v. and n.m.r. spectra of the natural and the synthetic sterol were essentially identical. Hydrolysis with alcoholic KOH as described above gave the free alcohol 1.23, m.p. 116-116.5° (lit. (31) 116-117°).

Preparation of Phosphonium Salts

Methyltriphenylphosphonium Iodide 3.1

To a solution of 7.2 g (0.027 mole) of triphenylphosphine in 20 ml of benzene 5 g (0.035 mole) of methyliodide was added. A precipitate formed immediately. After stirring at r.t. for 2 hours, the methylphosphonium iodide was filtered, washed with benzene and dried under vacuum for 48 hours. Yield: 10.1 g (92.5%), m.p. 184-184.5°.

Ethyltriphenyl phosphonium Iodide 3.2

To a solution of 9 g (0.034 mole) triphenylphosphine in 30 ml of benzene 6.4 g (0.04 mole) ethyliodide was added. The solution was refluxed for 12 hours and the product collected. The salt was washed on the filter with benzene and ether and dried under vacuum for 48 hours to give 7.8 g (52.4%) of the phosphonium salt, m.p. 164-165° (lit (64) m.p. 163-164.5°)

1-Bromo-2,3-dimethyl-2-butene 3.3

To 8 g (0.09 mole) of tetramethylethylene in 100 ml of CCl_4 14.3 g (0.08 mole) NBS and 0.1 g benzoylperoxide were added and the mixture refluxed for 24 hours. The solution was cooled in ice and filtered (7.5 g, m.p. 122-125°). The solvent was evaporated and the clear, colorless liquid was kept under vacuum (5 mm) for 1 hour and used without further purification for the salt formation.

2,3-dimethyl-2-butene-triphenylphosphonium bromide 3.4

The reaction product was taken up in 100 ml of dry benzene. To this solution 21 g (0.08 mole) triphenylphosphine was added. The precipitate formed immediately, as an oil which crystallized. The mixture was stirred at 30° for 3 hours and filtered. The product was purified by stirring several times with fresh benzene and finally dried under vacuum, m.p. 164-166°.
N.m.r. (in $CDCl_3$): δ1.14 (CH_3-C-2, broad d, J=3 Hz), 1.62 (6H, CH_3-C-3, broad d, J=5.5), 4.54 (2H, $-CH_2$-P, d J=14 Hz), 7.7 (15H, arom. H, m).

Isopropyldiethylmalonate 3.5

To a solution of 14.2 g (6.2 mole) sodium in 350 ml of absolute ethanol 100 g (0.62 mole) of malonic acid diethylester was added. After 2-bromopropane (76 g, 0.62 mole) was added dropwise the reaction mixture was refluxed for 2.5 hours. The ethanol was removed under vacuum and the residue taken up in water. The aqueous phase was discarded and the organic phase distilled under vacuum; b.p. 105-106° at 13 mm. (lit. (65) b.p. 214° at 760 mm) to give 107 g (85.3%) of 3.5. N.m.r. (neat):

δ0.94 (6H, d, J=6.5 Hz), 1.19 (6H, t, J=7 Hz), 2.2 (1 H, d of d, J=6.5 Hz and J=8 Hz), 2.99 (1H, d, J=8 Hz), 4.02 (4H, quart., J=7 Hz).

Isopropylmonoethylmalonate 3.6

To a solution of 23 g (0.49 mole) of KOH in 2 ml of water and 400 ml of methanol 100 g (0.49 mole) of the diester 3.5 was added gradually. The solution was neutral after standing for 15 hours at r.t. Concentrated NaCl solution was added and the unreacted starting material extracted with ether. The ether extract was washed with water until netural and dried over anhydrous Na_2SO_4 . The ether was evaporated. The oily residue was kept under vacuum (ca. 1 mm) for 2 hours and filtered to give 67 g (78%) of 3.6. The product was used without further purification for the next reaction. N.m.r. (neat): δ0.97 and 0.98 (3H each, d, J=6.5 Hz), 1.18 (3H, t, J=7 Hz), 2.33 (m, J=6.5 Hz and J=8 Hz), 3.2 (1H, d, J=8 Hz), 4.01 (2H, quart., J=7 Hz).

2-methylene-3-methyl-butanoic acid-ethylester 3.7

To a solution of 16.8 g (0.115 mole) of diethylamine in 20 ml of water 34.6 g of 40% aqueous formaldehyde solution (0.46 mole formaldehyde) was added. The mixture was kept at r.t. for 15 minutes and 40 g (0.23 mole) of monoester 3.6 added. The solution was refluxed for 15 hours and extracted with ether. The ether extract was washed with dil. HCl, $NaHCO_3$ solution and water until neutral. After drying over anhydrous Na_2SO_4, the

ether was evaporated and the crude product distilled; b.p. 80-81° at 60 mm yielding 26.4 g (81%) of 3.7. ν_{max}(film): 3080, 1610, 895 ($>C = CH_2$) and 1705 (C=O) cm^{-1}. N.m.r. (in $CDCl_3$): δ1.04 ($2CH_3$-C-3, d, J=6.5 Hz), 1.26 (CH_3-CH_2-, t, J=7 Hz), 2.33 ($>CH$-, septet, J=6.5 Hz), 4.18 (-CH_2-O-, quart. J=7 Hz), 5.45, 6.05 ($=CH_2$, 2d, J=1 Hz).

2-Methylene-3-methyl butanol-1 3.8

A mixture of 6.4 g of $LiAlH_4$ and 200 ml of dry ether was refluxed for 30 minutes and the ether sol. of $LiAlH_4$ decanted from undissolved material. To this ether solution 10 g (0.07 mole) of ester 3.7 was added in such a way as to maintain gentle refluxing. After addition was completed the reaction mixture was refluxed vigorously for 30 minutes. The excess $LiAlH_4$ was decomposed by addition of water and 300 ml of 10% H_2SO_4 was added under cooling. The reaction mixture was stirred until two clear layers were obtained. The layers were separated and the aqueous phase extracted three times with ether. The combined ether extracts and the alcohol were dried over anhydrous $MgSO_4$ and the ether evaporated to give 8.5 g of crude product. The crude product was shown by n.m.r. and v.p.c. to be a mixture of 3.8 and about 10% of the fully saturated alcohol. The mixture was distilled to give pure 3.8, b.p. 39° at 4.6 mm in 73% (5.1 g) yield. ν_{max} (film): 3300, 1040 (-OH) and 1645 (>C=CH) cm^{-1}. N.m.r. (in $CDCl_3$): δ1.05 ($(CH_3)_2$-C, d, J=7 Hz), 2.2 (>CH-, sept. J=7 Hz), 4.02 (-CH_2-, s), 4.81 (-OH, s, exchanged in D_2O), 4.84 and 4.98 ($=CH_2$, 2d, J=1 Hz).

1-Bromo-2-methylene-3-methyl-butane 3.9

To 4 g (0.04 mole) of alcohol 3.8, cooled to below 10°, 4.6 g (0.017 mole) of phosphorus tribromide was added gradually so that the temperature did not rise above 10°. After addition was completed the reaction mixture was kept at r.t. for 30 minutes. Saturated $NaHCO_3$ solution was added and the product extracted with ether. The ether extract was washed with water until neutral, dried over Na_2SO_4 and the solvent evaporated under vacuum to give 6.4 g (71%) of a clear liquid. The material darkened rapidly on standing at r.t. Spectra of the crude product showed the presence of the terminal methylene (i.r. 1645 and 895 cm^{-1} and n.m.r. two doublets, J=1 Hz at δ4.9 and δ5.09). The product was used without further purification for further reactions.

Triphenylphosphonium salt of bromide

A solution of 6 g of the crude bromide 3.9 in 200 ml of benzene was added to a solution of 10 g of triphenylphosphine in 50 ml of benzene and refluxed for 10 hours. No precipitate formed. The solution was then heated at 80° under pressure for 24 hours and the precipitated salt collected, washed thoroughly with benzene and dried under vacuum. The n.m.r. showed the product to be a mixture of 46% of the phosphonium salt of 3.9 and 54% of a salt identical with the salt formed from the isomeric bromide 3.3. The salt mixture was redissolved in $CHCl_3$ and heated at 80° under pressure for 12 hours. The ratio of the two isomers did not change.

1-Iodo-2-methylene-3-methylbutane 3.10 and
2-methylene-3-methyl-1-triphenylphosphonium iodide 3.12

A solution of 6 g NaI in 25 ml of acetone was added to a solution of 6 g of the bromoolefin 3.9. A precipitate of NaBr formed immediately. The mixture was kept at r.t. for 40 minutes. Water was added and the compound extracted with ether. The ether extract was washed with water, sodium thiosulfate solution and again with water an dried over anhydrous $MgSO_4$ for 24 hours. Dry benzene was added to the ether solution. The ether and most of the benzene was evaporated under vacuum at 18-20°. The residue was taken up in dry benzene and the solution added to a solution of 10 g of triphenylphosphine in 50 ml of benzene. The salt began to precipitate in fine needles at r.t. after one hour. The mixture was left standing at r.t. for 48 hours, filtered and washed with benzene to give 13.9 g (59%) of phosphonium salt 3.12. After drying under vacuum for several days it melted at 184-187°. ν_{max} (KBr): 3045, 1645, 1585, 1435, 1110, 995, 910 and 865 cm^{-1}. N.m.r. (in $CDCl_3$):δ 0.94 (CH_3-C-, d, J=6.5 Hz), 1.82 (CH- complex m), 4.44 (-CH_2-P, d, J=15 Hz), 4.99 (CH_2=C, quart., J=12 Hz and J=6 Hz), 7.81 (15 arom. H, m).

Isobutyric acid-bromide 3.13

A mixture of 65.5 g (0.74 mole) of isobutyric acid and 100 g (0.37 mole) of phosphorustribromide was heated at 100-120° for 4 hours. The reaction mixture was then distilled at normal pressure and the fraction distilling between 115-118° C collected. (lit. (66) b.p. 116-118° at 760 mm). Yield

90.5 g (81%).

Isopropylbromomethyl ketone 3.14

The bromoketone was prepared according to the slightly modified method of Carch et al (67). Diazomethane was obtained in the usual manner by decanting the etheral solution of the potassium hydroxide decomposition of N-nitrosomethyl urea (prepared according to Arndt et al (68) The CH_2N_2 solution was dried over KOH for 4 hours and cooled in ice. To this ether solution, containing 0.38 mole of diazomethane, 25.5 g (0.17 mole) of bromoacid 3.13 in 50 ml of ether were added dropwise. After 1 hour at 0^o dry hydrogen bromide was passed into the solution until an excess was present. The solution was left at 0^o for 1 hour then washed with H_2O, $NaHCO_3$ sol. and finally with H_2O until neutral and dried over anhydrous $MgSO_4$. The ether was evaporated and the residual oil distilled under vacuum, b.p. 85-86^o at 50 mm (lit (67) b.p. 85-86^o at 50 mm) to give 19.2 g (68.5%) of bromoketone. ν_{max}(film): 1735, 1715 (C=O), 1465, 1260, 1190, 1060, 930, 885, 685, and 620 cm^{-1}. N.m.r. (in $CDCl_3$):δ 1.17 and δ1.14 ($(CH_3)_2C$-, d, J=6.5 Hz), 2.98 (CH-, sept., J= 6.5 Hz), 4.08 (-CH -Br, s).

Isopropylmethyltriphenylphosphonium bromide 3.15

Triphenylphosphine, (25.5 g, 0.07 mole) was dissolved in 80 ml of dry benzene. Isopropylbromomethyl ketone 3.14 (10 g, 0.06 mole) was added and the mixture was left at r.t. for 24 hours then filtered. The solid was taken up in fresh benzene, stirred, filtered and dried under vacuum to give 29.1 g (82%) of phosphonium salt. The product was recrystallized from ethyl acetate - hexane, m.p. 235 - 237^o.

ν_{max} (nujol): 1690 cm^{-1} (C=O). N.m.r. (in $CDCl_3$): δ1.1 ($(CH_3)_2C$, d, J=6.5 Hz), 3.24 (CH-, sept., J=6.5 Hz), 5.85 ($P-CH_2-$, d, J=12 Hz), 7.66 (15 arom. H, m).

Triphenylphosphineisopropylmethylylene 3.16

A solution of 18 g (0.042 mole) of isopropyltriphenylphosphonium bromide 3.3 in 650 ml of water was treated with saturated sodium carbonate solution at r.t. for 12 hours. The solid was collected, washed with water and dried for 12 hours under vacuum. The product was recrystallized from ethyl acetate - hexane to give 13.4 g (91.5%) 3.16, m.p. 170 - 171°. ν_{max} (nujol); 1510 cm^{-1} (C=O) λ_{max}^{EtOH} 266 mμ (ε= 6,700), 273 mμ (ε= 6,500) and 287 mμ (ε= 5,930). N.m.r. (in $CHCl_3$): δ1.18 ($(CH_3)_2C$, d, J=6.5 Hz), 2.53 (CH-, sept., J=6.5 Hz), 4.3 - 5.0 (P=CH-, broad d), 7.5 (15 arom. H, m).

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