ATTEMPTED SYNTHESIS OF

25 - HYDROXYDIHYDROTACHYSTEROL$_3$

BONITA MacDONALD

M.Sc. Thesis
Dept. of Chemistry

McGill University

ABSTRACT

Preliminary studies concerning a general synthesis of 25-hydroxydihydrotachysterol$_3$ from stigmasterol were carried out. In order to differentiate between the two double bonds in stigmasterol, the olefin at C - 5 was protected by means of the i-steroid formation.

The side-chain was then cleaved by ozone, allowing introduction of various other side-chains by a Wittig condensation. Preparation of the "cholesterol-like" side-chain incorporating a 25-hydroxyl function was achieved by a methyl lithium reaction with 3-halo propionyl chloride. Complete details of the Wittig reaction have not yet been successfully worked out.

Acid catalysis restores the double bond at C-5 . Introduction of an olefin at C-9 produces the necessary diene in ring B of the steroid which undergoes ultraviolet irradiation to the triene system of tachysterol.

Details of the U. V. irradiation of the diene were investigated fully on the model compound, ergosterol. Attempts to isolate tachysterol from the photolysis products proved futile. The crude irradiation products were reduced directly to their more stable diene form to yield dihydrotachysterol.

Attempted Synthesis of 25 - Hydroxydihydrotachysterol$_3$

A Thesis

by

Bonita MacDonald

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DEDICATION

To my parents

ACKNOWLEDGEMENTS

Dr. George Just, my research director, for his guidance throughout the course of my studies.

Dr. M.A. Wilson and Mr. Paris Georghiou, for their helpful discussions.

My co-workers, for their encouragement, technical assistance, and warm friendship.

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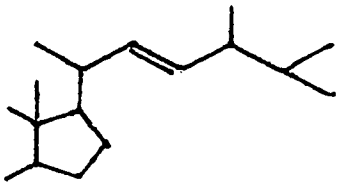
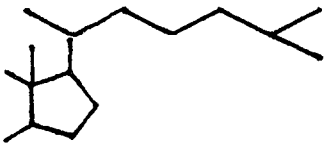
PREFACE

The general term "calciferol" refers to any of a number of anti-rachitic sterols and may be interchangeably used with the term "Vitamin D". All are structurally similar except for the chemical nature of the side-chain at C-17 in the sterol nucleus.

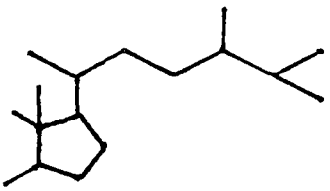
Commonly available forms are:



Vitamin D_2 = ergocalciferol



Vitamin D_3 = cholecalciferol



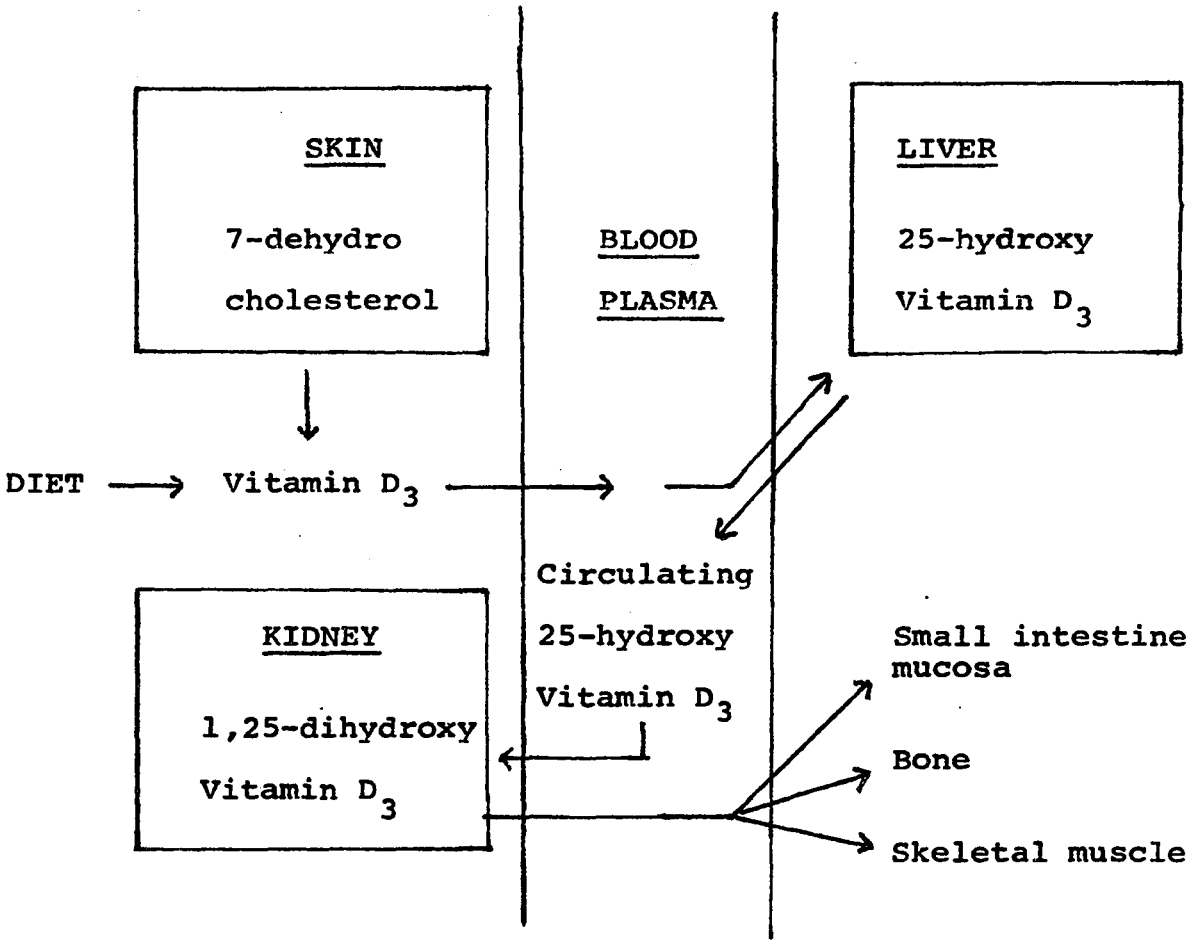
Vitamin D_4 = 22,23-dihydro ergocalciferol

The subscripts 2,3, and 4 are utilized in specifically referring to other related compounds formed during the ultraviolet irradiation of ergosterol (eg., tachysterol$_2$) and similarly of 7-dehydrocholesterol (eg., tachysterol$_3$).

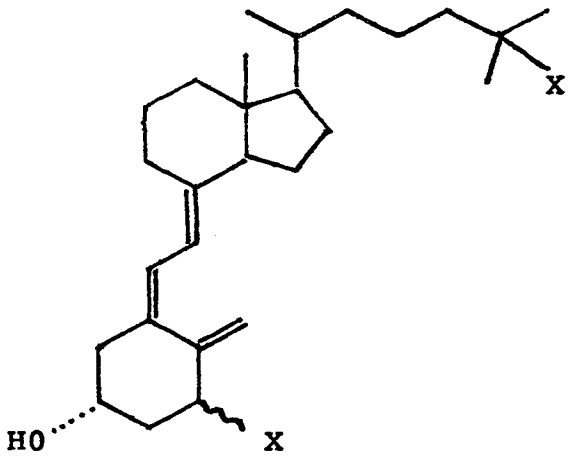
The numbering system for the carbon positions in the steroid is retained for the products derived from it.

INTRODUCTION

The field of Vitamin D has been an exciting one over the past decade as much research is being carried out to elucidate its precise metabolic pathway in the body. The most recent evidence indicates that Vitamin D, perhaps to be reclassified as a steroidal hormone, exhibits its biological activity in a manner summarized in the following scheme[1] :



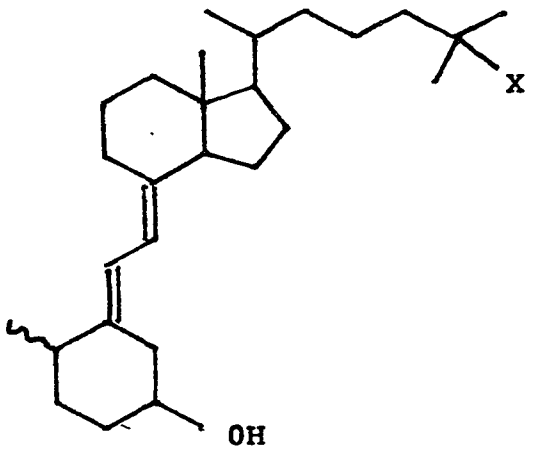
However there are still factors as yet unexplained. One of these is the failure of Vitamin D to alleviate the malabsorbption of calcium in patients with chronic renal failure, whereas dihydrotachysterol (DHT) is biologically active in stimulating intestinal calcium transport.



Vitamin D_3 , X = H

1,25-Dihydroxy

vitamin D_3 , X = OH



DHT_3 , X = H

25-Hydroxy-

DHT_3 , X = OH

Note: See foot-note on page 35 for the configuration of the C-10 methyl

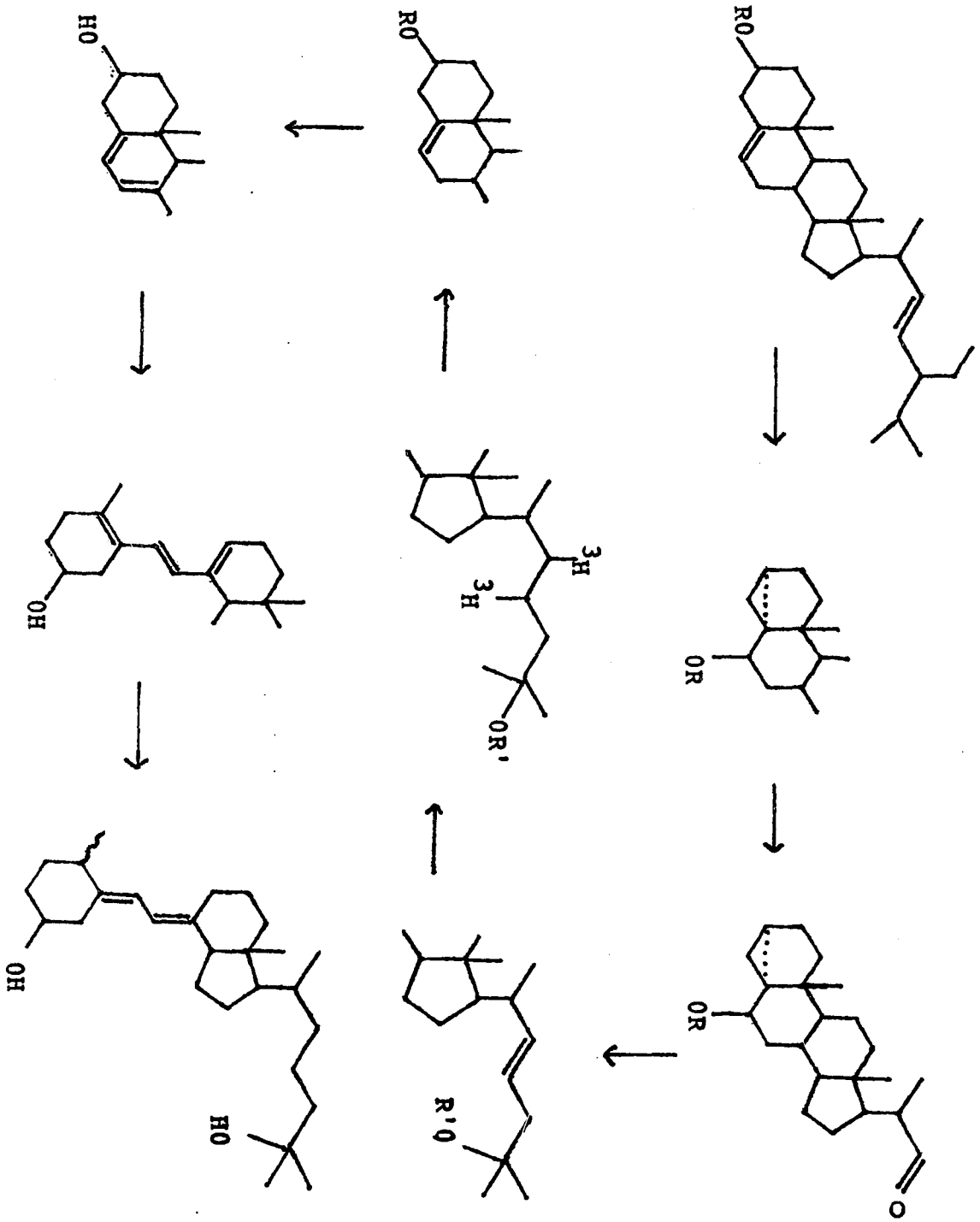
The fact that DHT is already hydroxylated at the steric equivalent of C-1 in 25-hydroxy D_3 may be a possible explanation of the difference in the biological activity of these two compounds in renal failure. Assuming that DHT is metabolized similarly to Vitamin D, it would not require further hydroxylation by the kidney tissue after the initial one in the liver at C-25 [1].

In order to study the metabolic pathway undergone by dihydrotachysterol which possesses slight anti-rachitic activity but is remarkably effective in elevating serum calcium concentrations in conditions of hypoparathyroidism, a chemical synthesis of 25-hydroxydihydrotachysterol allowing modifications in the side-chain is exceedingly important.

There is one reported method for the preparation of 25-hydroxydihydrotachysterol from 3β-acetoxy-26-norcholesten-25-one which however is not a readily available starting material and permits almost no modifications [2]. In addition, methods reported for the chemical synthesis of Vitamin D, its hydroxylated and tritiated analogues, although elegant and highly important in proving their structures are subject to the above limitations [3-10].

Accordingly, this work involves preliminary attempts at a general synthesis of 25-hydroxydihydrotachysterol.

An outline of the proposed scheme of reactions leading to a general synthesis of 25-OH-DHT is indicated in the following diagram :



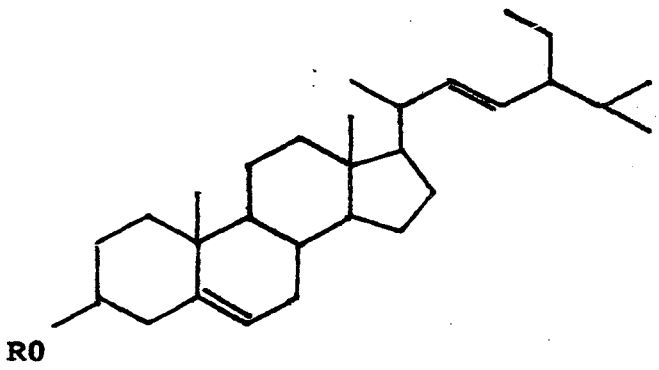
Direct ozonolysis of the side-chain olefin of stigmasterol allows no differentiation between the double bonds. Therefore the i-steroid was utilized to protect the nuclear olefin until the side-chain was cleaved by ozone.

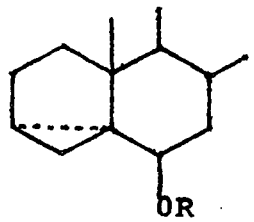
Varying side-chains can be introduced by way of Wittig condensations, allowing incorporation of the 25-hydroxyl function and restoring the 22-olefin. Reduction then with tritium and acid catalyzed rearrangement of the i-steroid to the normal steroid leads directly to tritiated 25-hydroxy-cholesterol.

The bromination - dehydrobromination procedures for introduction of the C-7 olefin have been satisfactorily worked out [2]. Ultraviolet irradiation and succeeding reduction would then yield the desired compound, 25-hydroxy-dihydrotachysterol$_3$.

CHAPTER I

As one approach to synthesize 25-OH-DHT_3 , with tritium incorporation at the C-22,23 positions, stigmasterol (1A) was the chosen starting material.





(1A) : R=H

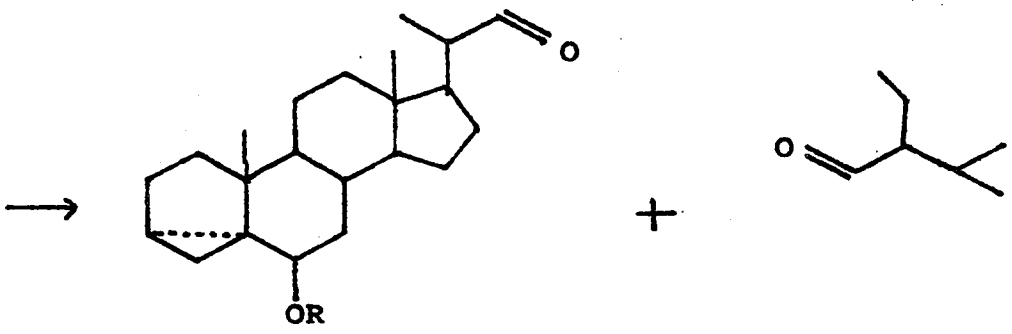
(1B) : R=Ts

(2A) : R=H

(2B) : R=Ac

The olefin at C-5 was protected by solvolysis of the tosylate (1B) in aqueous acetone buffered with sodium carbonate to obtain (2A) as well as a small amount of starting material. The NMR spectrum of the crude solvolysis product shows the olefinic protons essentially disappeared and the appearance of small high-field peaks at 0.2-0.6 ppm indicative of the 3,4,4' cyclopropyl protons. Column chromatography on alumina yielded pure crystalline (2A) in 70% overall yield from (1A).

After acetylation to give (2B) , the material was ozonolyzed in methylene chloride to cleave the side-chain, yielding the 22-aldehyde, (3). Various methods were examined for reducing the ozonide, including hexamethyl-phosphorustriamide (HMPT) and dimethylsulphide. However, the most efficient procedure for obtaining (3) consisted of pouring the methylene chloride solution at -70 °C., immediately after completion of ozone passage, into a 0.5N hydrochloric acid solution accompanied by rapid extraction of the organic material with methylene chloride.

(2) 

(3A) : R=H

(3B) : R=Ac

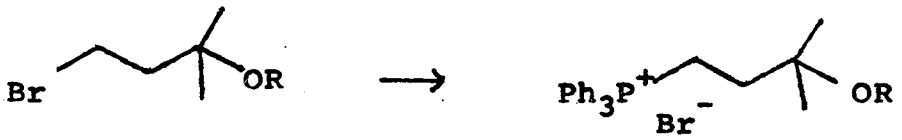
The side-chain aldehyde, 2-ethyl-3-methylbutanal resulting from ozonolysis with the steroidal product could be removed by distillation in vacuo along with the solvent.

The next section of the synthetic scheme where the appropiate side-chain is added to the steroid nucleus by a Wittig condensation depended on the preparation of the

corresponding alkyl phosphonium salt.

PREPARATION OF THE SIDE-CHAIN :

The first attempt envisioned making 2-methyl-4-bromo-2-butanol (4A), protection of the hydroxyl group as its tetrahydropyranyl ether (4B), and reaction with triphenylphosphine to give the phosphonium salt (5).



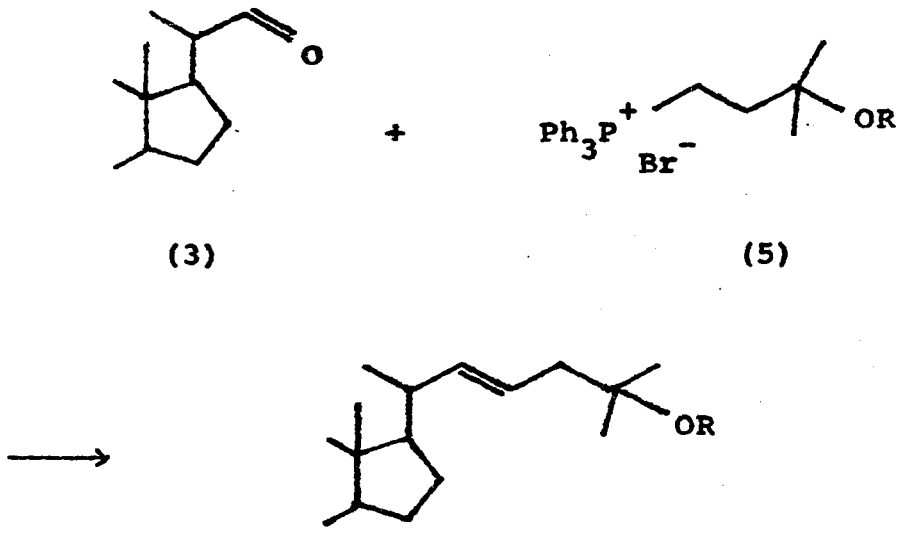
(4A) : R=H

(4B) : R=THP

(5A) : R=H

(5B) : R=THP

A Wittig condensation of compound (5) with the steroid aldehyde (3) would then incorporate the 25-hydroxyl function into the steroid, giving (6), and regenerate the olefinic bond at C-22. This may be further reduced employing tritium to obtain the "cholesterol-like" side-chain in the D_3 series of compounds.



(3) (5)

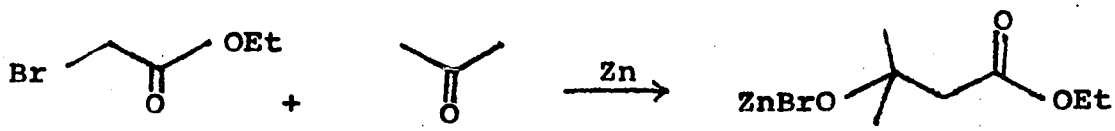
(6A) : R=THP

(6B) : R=H

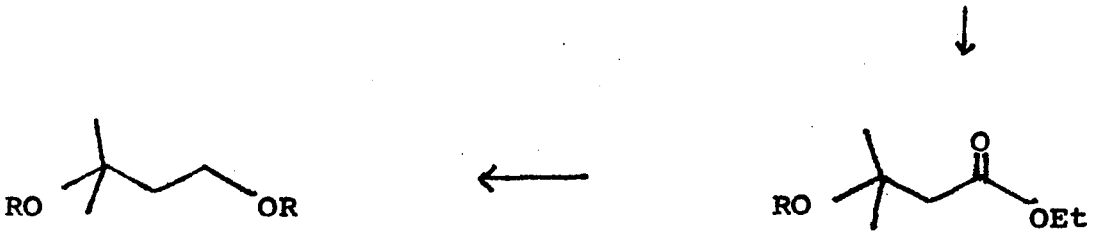
Compound (4A), 2-methyl-4-bromo-2-butanol, proved difficult to prepare from readily available materials without extensive decomposition and varying by-products.

Four general procedures were investigated for the synthesis of (4) :

METHOD I



(7) (8)



(10A) : R=H (9A) : R=H

(10B) : R=Ac (9B) : R=Ac

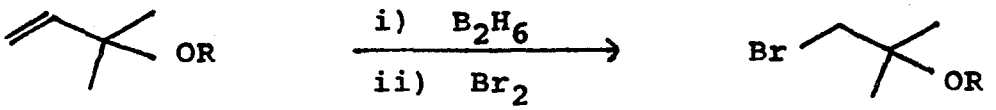
A Reformatsky reaction employing ethyl bromoacetate (7) and acetone yielded the intermediate (8) which on hydrolysis of an aliquot gave (9A). Without work-up of the remaining sample, solid lithium aluminum hydride was added to the reaction flask to effect reduction of the ester. One portion of the reduction mixture was added to acetic anhydride which produced (10B), while the second portion was quenched with ethyl acetate and hydrolysed by dilute sulphuric acid to give (10A). Analysis of the reaction products in both cases by NMR and thin layer chromatography showed very low yields of desired compounds. Because of the numerous side-products resulting from the multi-step procedure, the

reaction was not further investigated.

METHOD II

According to a literature procedure, hydrogen bromide can be added to an olefin in an anti-Markownikov fashion by way of cleaving a boron hydride complex with bromine[12-13].

This approach was attempted with 2-hydroxy-2-methyl-3-butene , compound (11A).



(11A) : R=H

(11B) : R=THP

(11C) : R=Ac

(4A) : R=H

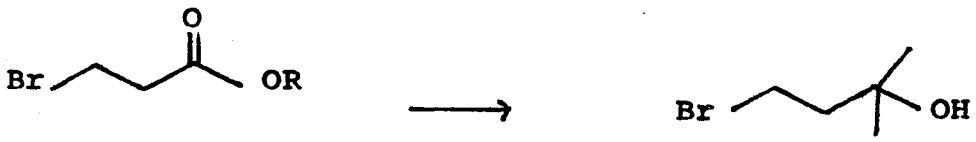
(4B) : R=THP

(4C) : R=Ac

Interference by the hydroxyl group prevented the formation of the desired boron complex. It was found impossible to protect the hydroxyl group by either the tetrahydropyranyl ether or the acetate derivatives without complete polymerization of (11A).

METHOD III

As further investigation into the preparation of the side-chain precursor (4), the methyl ester obtained by treatment of 3-bromopropionic acid with diazomethane was subjected to Grignard reaction conditions with methylmagnesium iodide[14].



(12A) : R=H

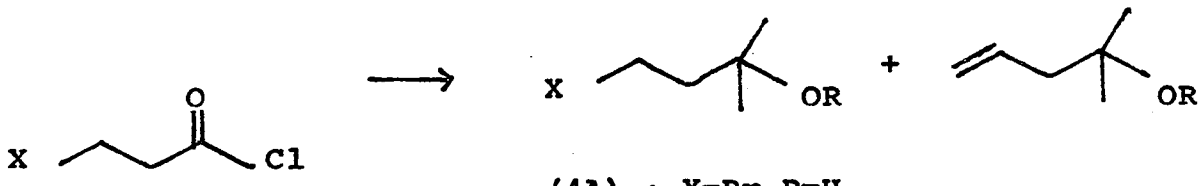
(12B) : R=Me

(4)

Again yields were low, as judged from NMR spectral evidence; in addition, there were many by-products due to magnesium complexation with the bromo function. These were inseparable by chromatography. However, this reaction was later successfully carried out with 3-chloropropionyl chloride by a co-worker in good yield[15].

METHOD IV

The approach yielding most favorable results involved reacting an excess of methyl lithium in ether solution with either 3-bromo- or 3-chloropropionyl chloride, (13A) and (13B), which are available commercially.



(13A) : X=Br

(13B) : X=Cl

(4A) : X=Br,R=H

(4B) : X=Cl,R=H

(4C) : X=Br,R=THP

(4D) : X=Cl,R=THP

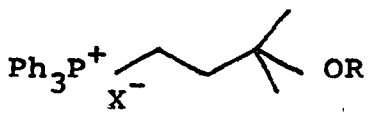
(4')

After quenching with ammonium chloride solution, 4-bromo-2-methyl-2-butanol (4A),and 4-chloro-2-methyl-2-butanol (4B) were obtained respectively; in addition, there occured also the products of elimination (4'), which are separable by distillation under reduced pressure. These compounds polymerize even at room temperature, and overall yields of (4A) and (4B) range between 30-40%.

The tetrahydropyranyl ethers, (4C) and (4D), were prepared without difficulty in the usual way, although the acid catalysis necessary was expected to cause some elimination of the tertiary hydroxyl function.

Fortunately there is no evidence of this occuring when the reaction temperature is kept at -15 to -10 ^{o}C.

The succeeding step of the synthesis requires preparation of the triphenylphosphonium salt (14) but here further complications resulted.



(14A) : X=Cl,R=THP

(14B) : X=Br,R=THP

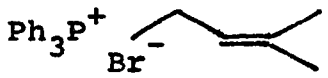
(14C) : X=I ,R=THP

Heating under reflux a benzene solution of 4-chloro-2-methyl-2-butanyl tetrahydropyranyl ether (4D) with triphenylphosphine for 24 hours gave starting material only. A sample of (4D) was therefore treated with sodium iodide in methyl ethyl ketone to give 4-iodo-2-methyl-2-butanyl tetrahydropyranyl ether .

Reaction with triphenylphosphine in refluxing benzene of this iodo compound afforded a tarry product which possibly contained the desired phosphonium salt (14C) on the basis of NMR spectral data. All attempts to purify the product by crystallization yielded only pure triphenyl

phosphine. An NMR spectrum of the mother liquors showed that the tetrahydropyranyl ether protecting group had been lost in the reaction.

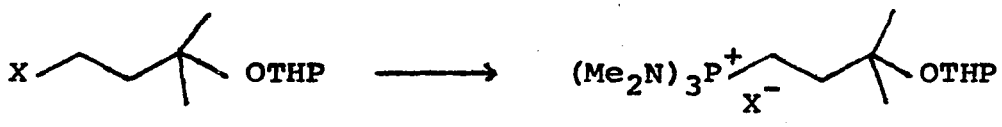
Because the THP ether function exists in two epimeric forms and consequently causes difficulty in crystallization , it was decided to find out whether the phosphonium salt of the unprotected alcohol (4A) could be prepared. Treating (4A) with triphenylphosphine in refluxing acetonitrile gave a white precipitate. Its NMR spectrum and elemental analysis indicated that compound (15) had been formed.



(15)

The mass spectrum of (15) shows only a mass ion peak at 262; that is, triphenylphosphine. Further variation in reaction conditions led to negative results.

Hexamethylphosphorus triamide has been used to replace triphenylphosphine in numerous forms of the Wittig reaction[16] with improved speed of formation of the corresponding phosphonium salt (16).



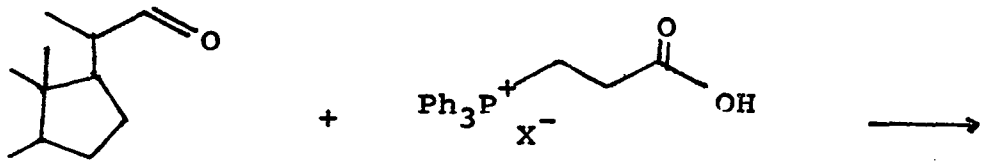
(4C) X=Br

(4D) X=Cl

(16)

Our preliminary attempts to prepare the salts such as (16) were inconclusive. Further work in this area, however, appears to be worthwhile, especially since one of the by-products of the Wittig reaction, which is tris-dimethylaminophosphine oxide, is water soluble and thus readily separable from the steroidal product.

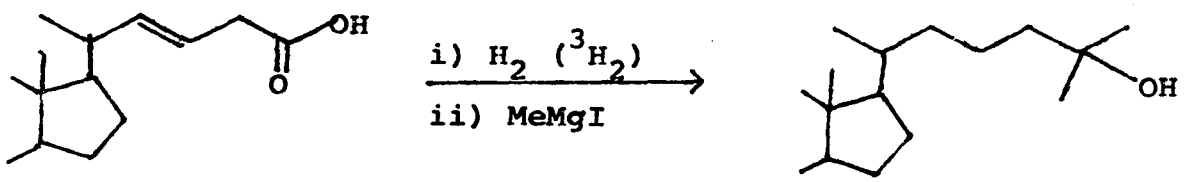
At this point it was decided to change the nature of the side-chain precursor. The proposed scheme was as follows:



(3)

(17A) : X=Br

(17B) : X=Cl

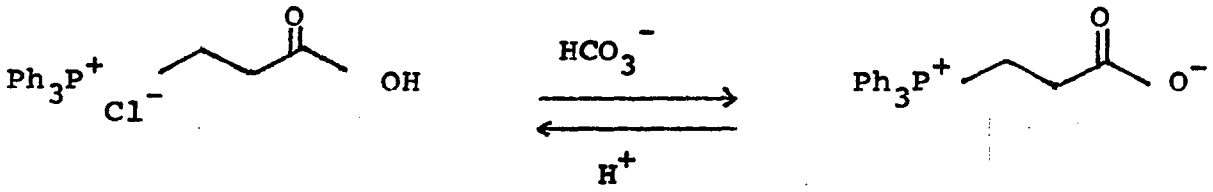


(18)

(19)

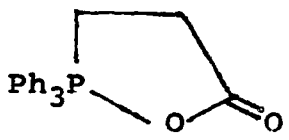
The phosphonium salts , (17A) and (17B), were prepared from the 3-bromo- and 3-chloropropionic acids respectively. The succeeding step employing a modified Wittig reaction involved unsuspected difficulties and led to products other than the compound (18).

It has been reported by Denny and Smith that upon treatment of (20) with base (aqueous sodium bicarbonate) an 87% yield of the phosphobetain (21) was obtained,



(20) (21)

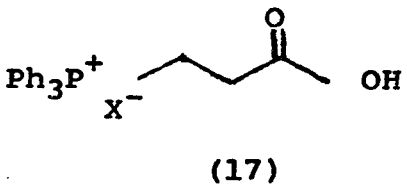
with no evidence of a cyclic phosphorane (22) occuring as a possible alternative, as judged from Infrared and phosphorus Nuclear Magnetic Resonance spectral data.[18]



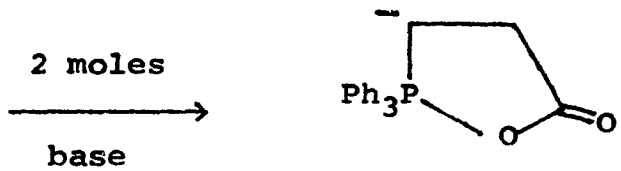
(22)

When the Wittig condensation was attempted with compounds (17) and (3), according to a procedure by E.J. Corey and co-workers[19], using methyl sulphinyl carbanion in dimethyl sulphoxide as base, it was found that no corresponding olefin (18) was produced.

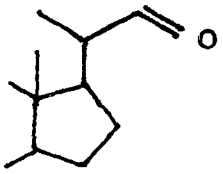
It is proposed that in the strong basic medium required for reaction, the ylid exists in a cyclic form (17') and adds to the steroid aldehyde in such a manner as to produce a cyclic phosphorane (23) :



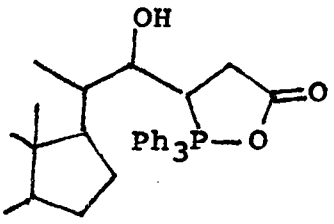
(17)



(17')



(3)



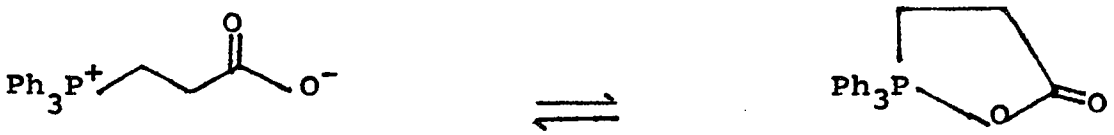
(23)

The NMR spectrum of the crude Wittig product shows disappearance of the aldehyde proton (9.6 ppm from TMS , internal standard), appearance of phosphorus coupled phenyl protons at 7.80 ppm, and two complex multiplets between 3.80 and 3.00 ppm corresponding to the methylene protons. There was no indication of any olefinic protons present. Heating this product in neutral and basic solvents at increasing temperatures over an extended time period up to eighteen hours, caused no change in the spectrum.

Alumina chromatography presumably resulted in decomposition from which was recovered epimerized aldehyde, triphenylphosphine and a small amount of triphenyl-phosphine oxide. These results were supported by analogous data obtained in this laboratory using compound (17) in Wittig condensations with other steroid aldehydes[20].

In addition, H.S. Corey et al report similar difficulty in carrying out the usual Wittig procedures with 3-chloro propionic acid as the ylid precursor[21]. They do not specify what their undesired products were but found, however, that varying the order of addition of reagents during the condensations increased yields of the appropriate olefin from 10% to approximately 60%. This is reasonable if there exists an equilibrium between the

zwitterionic phosphorane (21) and its cyclic form (22) which can be influenced by the amount of base present.



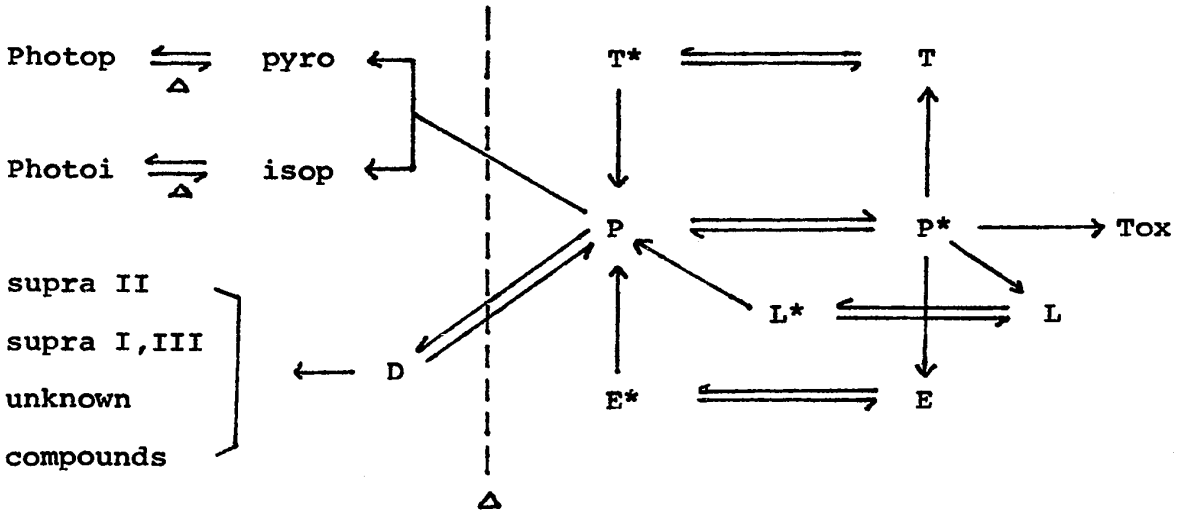
(21) (22)

The Wittig reaction was repeated a number of times using the latter's procedure [21] to determine the proper experimental conditions yielding the desired olefin (18). However, insufficient time prevented further results by the author and work is continuing in this area[20].

CHAPTER II

Photochemical irradiation of the diene in ring B of ergosterol yields tachysterol as one of the major products according to a reaction pathway outlined in Figure I [22].

Figure I : Scheme of Established Isomerizations



D= vitamin D_2

P= pre-vitamin D_2

E= ergosterol

T= $tachysterol_2$

L= lumisterol

Δ= thermal transformation

Tox = toxisterols

Pyro = pyrocalciferol

Isop = isopyrocalciferol

Photop = photopyrocalciferol

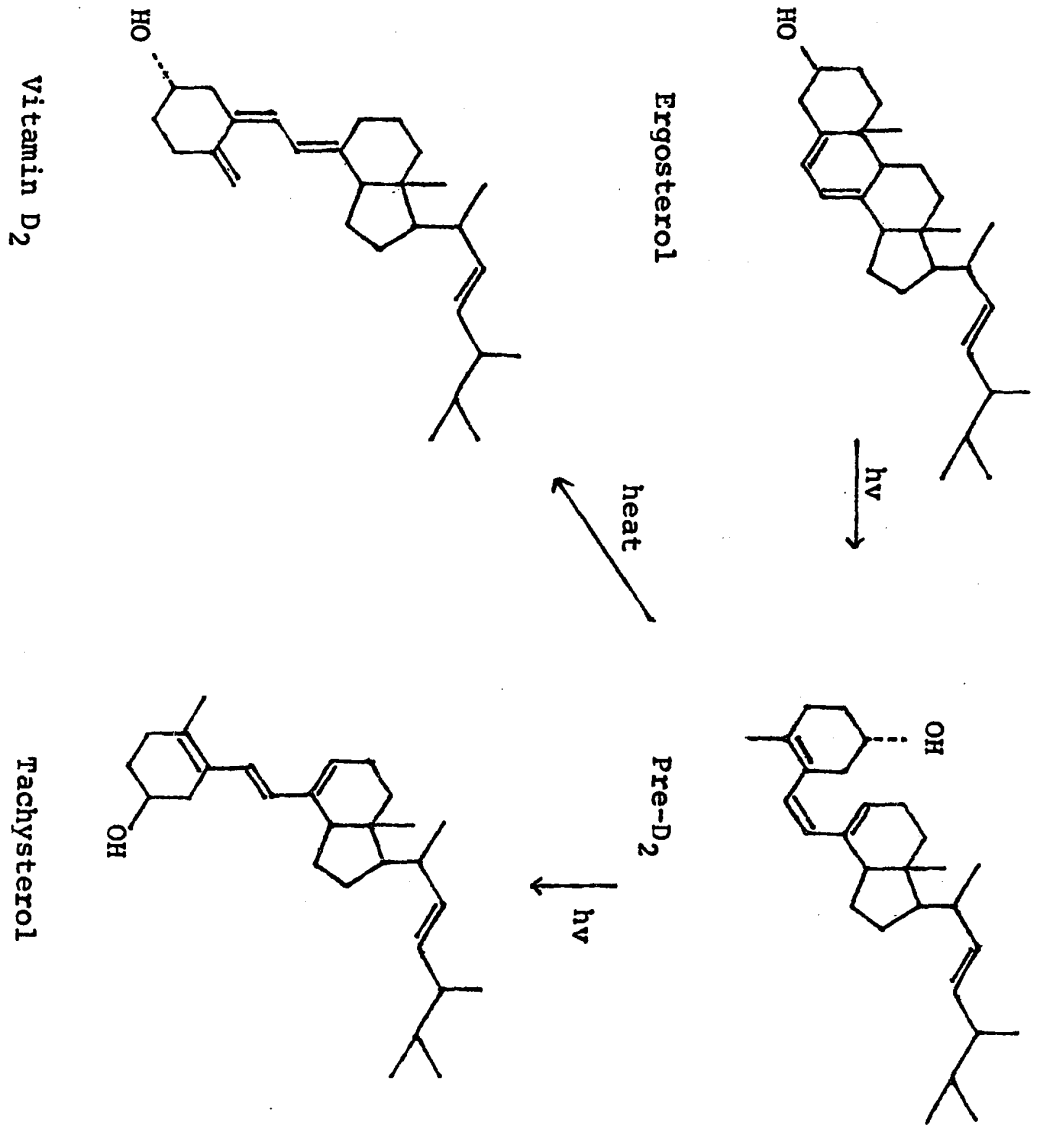
Photoi = photoisopyrocalciferol

Supra = suprasterols

All arrows indicate photochemical transformations except where marked by delta.

The number of literature reports on this topic is so extensive that no effort has been made to include a complete list of all the references. The reader is directed to several review papers[22-25].

The structures of the major products from the U.V. irradiation of ergosterol are shown below :



The final steps in the proposed sequence of reactions leading to 25-hydroxydihydrotachysterol involve an ultraviolet irradiation and succeeding reduction. The wide variation in experimental details quoted in the literature[22-29] for carrying out these reactions made it necessary to investigate the reaction conditions (via the use of the available photolysis apparatus) for maximum yield of tachysterol, and to set up chromatographic systems for isolating the products.

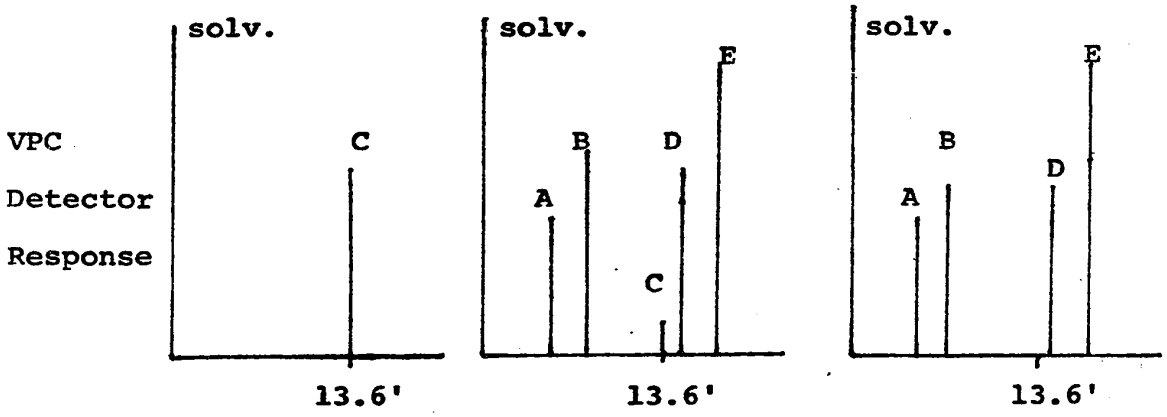
Ergosterol was utilized as a model compound until the appropriate precursor containing the 25-hydroxyl function would be available. Because of the readily occuring thermal transformation of pre-vitamin D_2 into vitamin D_2, all photolyses were carried out at reduced temperatures in a cold room (approximately 0-5 ^{o}C. throughout).

In order that vapor phase chromatography could be used to monitor the progression of the irradiation, ergosterol was acetylated before photolysis. In those cases where ergosterol was used, the trimethyl silyl ethers of the irradiation products were prepared before injection into the VPC [30].

Experimentally it was found that irradiation of ergosterol acetate in ether or ethanol for four and one-half

hours resulted in the virtual disappearance of starting material as analyzed by VPC, and the appearance of four major peaks labelled A,B,D, and E in Figure II.

Figure II :



Ergosterol acetate, standard

Irradiation products, 4 hours

Irradiation products, 4 and 1/2 hours

Retention time in minutes

Peaks A and B increase to a maximun after 30 minutes irradiation, and steadily decrease thereafter; peak E appears at 30 minutes and steadily increases to a maximun (approximately 50% of the total area) after 4 and 1/2 hours.

The thermal cyclization derivatives of pre-vitamin D_2 are pyro- and isopyro-calciferol, identical to those of vitamin D itself [31]. Hence a known sample of D_2 was compared on the VPC under the same conditions , and was found to exhibit the identical retention times of peaks A and B.

In addition, pre-D_2 can be transformed chemically by traces of iodine to tachysterol [32]. When an aliquot of the irradiated solution was stirred one hour with iodine and the reaction followed by VPC, peaks A and B gradually decreased to a neglible amount while peak E increased in area about 10%. Therefore, peaks A and B represent most likely pre-vitamin D_2 and peak E tachysterol. The nature of peak D was not investigated but is thought to be lumisterol.

Photolysis for longer periods than four and one-half hours caused an increase in over-irradiation products, ultraviolet absorption maxima at less than 250 millimicrons, and an accompanying decrease in the amount of tachysterol present.

The data listed in Figure III indicate the retention times of the various components produced by irradiation.

Figure III :

VPC Analysis of Crude Irradiation Products

Reagent	Solvent	Retention Times (in min.) - Major Components				
		Peak A	Peak B	Peak C*	Peak D	Peak E
ergo-sterol acetate	Ether	10.0 9%	14.4 11%	15.6 -	16.0 14%	17.0 36%
ergo-sterol acetate	Ethanol	11.4 10%	14.8 10%	16.2 -	16.6 2%	17.2 50%
ergo-sterol	Ether	8.6 9%	9.6 7%	13.9 -	12.2 6%	13.2 50%
ergo-sterol	Ethanol	8.8 7%	9.8 5%	14.2 -	12.2 6%	13.4 48%

C* - represents the starting material standard run immediately after injection of the photolysis products (not to be confused as a component of the crude product sample).

It appeared that varying the solvent from ether to ethanol caused no significant change in the nature of the photolysis products, at least by VPC and U.V. spectral evidence. However, later difficulties encountered in the reduction step lead us to suspect this evidence.

The ultra-violet absorption spectra of the samples (in absolute ethanol) being photolysed show a gradual change from the diene absorption with maxima at 262, 272, 282.5, and 293 mμ, corresponding to ergosterol, to a triene chromophore with maxima at 272, 282, and 292 mμ as well as broad absorptions from 305 to 330 mμ.

Isolation of the Irradiation Products :

Various chromatographic procedures were examined to effect the best separation of irradiation products. The fact that these compounds differ primarily in olefin position rather than in functional group or molecular weight makes their isolation difficult, and their extreme sensitivity to heat, light and to oxidation by air leads to extensive decomposition.

THIN LAYER CHROMATOGRAPHY :

Thin layer chromatography of the irradiated ergosterol solutions as reported by Norman and DeLuca[33] affords good separation of the components on an analytical scale with 10% acetone in hexane on silica gel.

Our results, summarized in Figure IV, indicate that hexane-benzene eluent (1:1 v/v) with 5% silver nitrate impregnated silica gel preparatory plates gives the best development for the irradiation products.

Figure IV :

Reaction Products from:	Adsorbant	Eluent	No. of Components-	Rf Values
ergosterol acetate	silica gel	hexane-benzene 1:1	3	0.72,0.61,0.53
ergosterol acetate	silica gel	hexane-ether 1:1	1	0.92
ergosterol acetate	silica gel 5% $AgNO_3$	hexane-benzene 1:1	5	0.67,0.51,0.46, 0.24, 0.00
ergosterol acetate	silica gel 5% $AgNO_3$	hexane-ether 9:1	5	0.47,0.34,0.19, 0.12, 0.00
ergosterol	silica gel 5% $AgNO_3$	hexane-ether 1:1	1	0.89
ergosterol	silica gel	hexane-benzene 1:1	-	streak
ergosterol	silica gel	hexane-ether 9:1	3	0.55,0.47,0.39
ergosterol	silica gel 5% $AgNO_3$	hexane-ether 9:1	5	0.51,0.41,0.31, 0.20, 0.00

Extraction of the tlc bands was performed by chloroform, and the ultraviolet and NMR spectra were determined on the components where possible.

The lowest band, which did not move with the solvent front, had no ultraviolet absorption above 250 millimicrons, indicating peroxide formation. Band 2 contained unreacted ergosterol. The third band showed U.V. absorption in the 240 millimicron range. Band 4 absorbed at 260-270 mμ and consisted probably of pre-vitamin D_2 or vitamin D_2. Band 5 exhibited absorption maxima at 272, 282, and 292 mμ, corresponding to a conjugated triene chromophore, possibly tachysterol.

In all extractions, not more than 50% of the material was recovered from the plates (presumably due to a binding agent in the adsorbant.) Phosphomolybdic acid (10% in ethanol) was found to be the most sensitive detector - limit of detection : 50 ng.

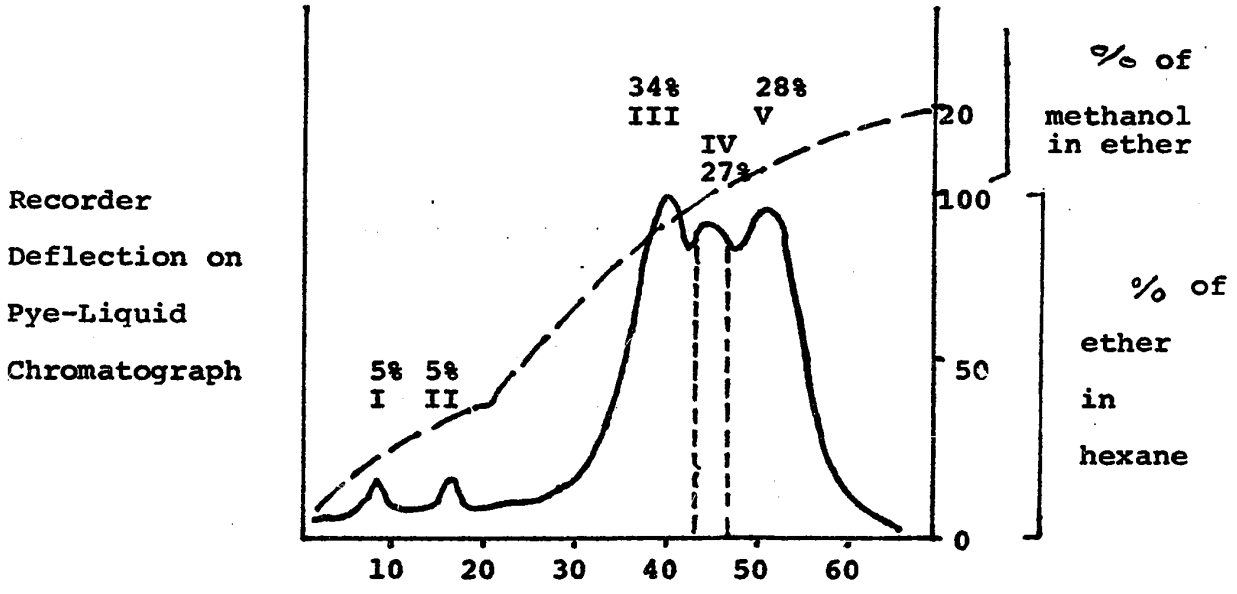
The NMR spectra of the components, compared with those reported in the literature [42], showed decomposition and hence were inconclusive. Because it would have been necessary to set up conditions for working totally in a nitrogen atmosphere under red light, it was decided to examine alternate systems.

COLUMN CHROMATOGRAPHY :

Elegant liquid chromatography procedures have been published for the isolation of Vitamin D metabolites using gradient elution devices, since traditional column methods gave relatively inferior separations [27].

Adapting these procedures for isolation of the products from the photolysis of ergosterol resulted in the typical separation indicated in Figure V.

Figure V : Products from the Irradiation of Ergosterol in Ether



Fraction number : 5 ml fractions collected

Although precautions were taken to minimize decomposition of the components from the column which was water-cooled throughout [35], complete recovery and characterization was found not possible without setting up an elaborate nitrogen atmosphere system.

Peak III (from Figure V) , absorption maximum at 264 mμ , was pre-vitamin D_2 ; peak IV consisted of tachysterol, absorption maxima at 271.5, 282, and 290 mμ ; peak V was unreacted ergosterol, U.V. absorption maxima at 262, 272, 282, and 290 mμ . Peaks I and II were shown to be impurities from the elution solvents. Extinction coefficients were low. Peroxide formation was assumed to be the major factor in decomposition since the U.V. absorption coefficients decreased steadily with time until virtual disappearance within one hour.

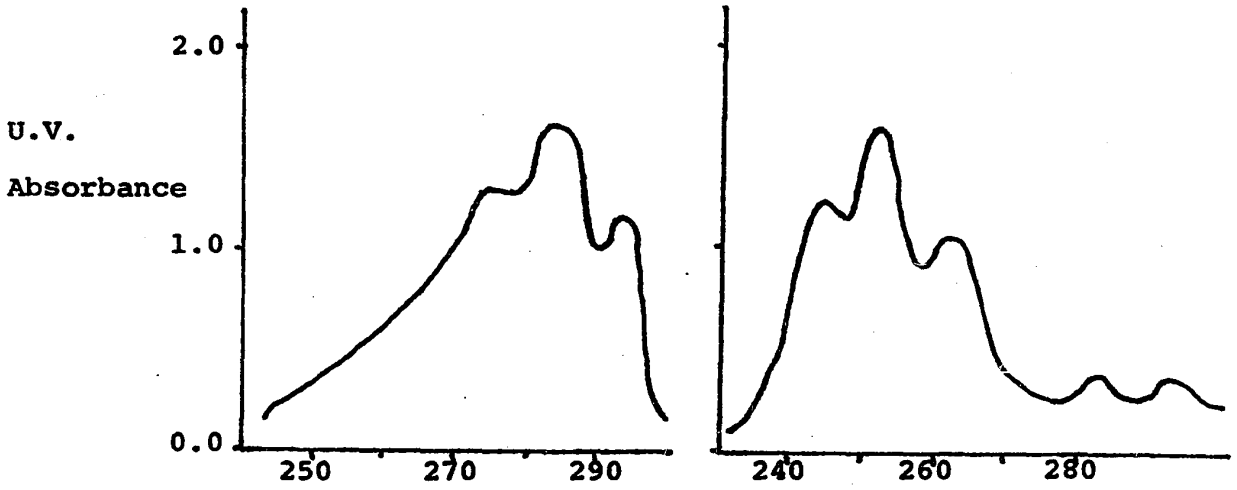
When acetylated ergosterol was photolyzed and chromatographed under identical conditions, no separation of components occured at all. A 5% silver nitrate - silica gel column with hexane-benzene gradient also was ineffective. In all cases the irradiation products were recovered quantitatively from the columns.

Since a practical and efficient isolation of tachysterol proved futile, the irradiated sample was taken to dryness in vacuo and reduced directly to the more stable dihydro-tachysterol.

REDUCTION :

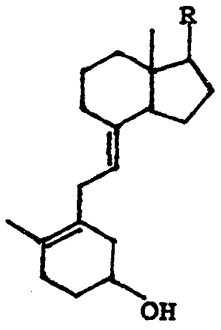
The method used to effect reduction was essentially that reported by Westerhof and Buisman [36]. The ultraviolet absorption spectrum of the crude material before and after reduction (Figure VI) demonstrates the change from a conjugated triene to a conjugated diene; however, it was found that this does not necessarily indicate the production of desired compound.

Figure VI : Photolyzed Sample Before and After Reduction

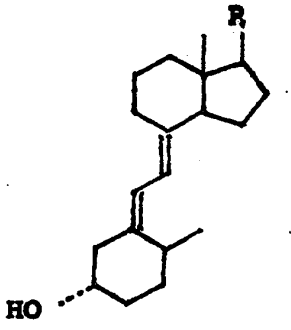


Vitamin D, tachysterol, and pre-vitamin D each undergo reduction to produce in varying yields all of the dihydrovitamins D and dihydrotachysterol.

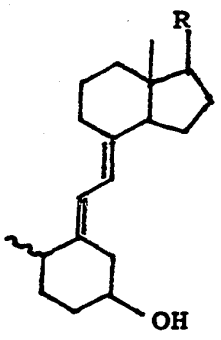
Some of the suggested stereochemical structures of the dihydrovitamin isomers (DHD_2) reported in the literature [37] are given below :



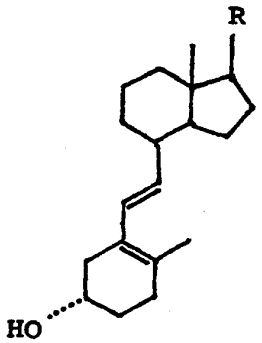
DHD_2 - I



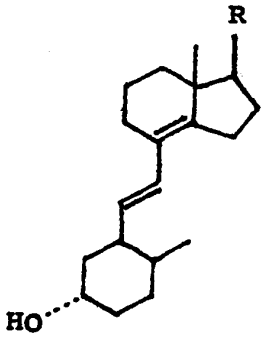
DHD_2 - II



DHD_2 - IV or DHT_2



DHD_2 - V



DHD_2 - VI

$R = C_9H_{17}-$

Isomer III is not included on the previous page with the other DHD_2 isomers because it is not yet sufficiently characterized. The configuration at C-10 in DHT and DHD_2-IV is not known*. It is still not clear which factors are most responsible for determining the proportions of individual products formed from each of the trienes.

It is suggested that much of the inconsistancy of results we experienced is due to the very close similarity in structure of possible reduction products, and the resulting sensitivity of reaction mechanism to reaction conditions.

Vapor phase chromatography was used to monitor the results after reduction of the crude photolyzed samples. The trimethylsilyl ethers were prepared before injection into the VPC since, in those cases where ergosterol acetate was irradiated, the conditions of reduction removed the acetate group.

The data listed in Figure III (page 26) appear to indicate no solvent influence on the nature of products yielded by photolysis of ergosterol in ether or ethanol. Yet reduction of the photolysis samples in the two

*Note: The latest evidence by ^{13}C NMR indicates that the C-10 methyl is trans with respect to the 3-hydroxyl.[43]

solvents gave different results.

Reduction of all the samples photolyzed in ethanol led consistantly to the production of dihydrotachysterol. However, reduction of the photolyzed ether solutions sometimes led to the production of dihydrotachysterol, but in at least 50% of the cases gave a different product; this was thought to be DHD_2-II or DHD_2-IV because the ultraviolet absorption maxima were identical to those of DHT_2.

The factor most responsible for determining consistancy of results was the purity of the solvent used for the irradiation. Because ether is extremely sensitive to the acquisition of peroxides by atmospheric oxidation and the reliable methods for complete purification laborious, the use of spectroscopically pure ethanol as solvent avoided the problem.

The results from the VPC analyses of the crude reduction products are listed in Figure VII.

Figure VII :

VPC Analyses of the Reduction Products
of Irradiated Ergosteryl Acetate

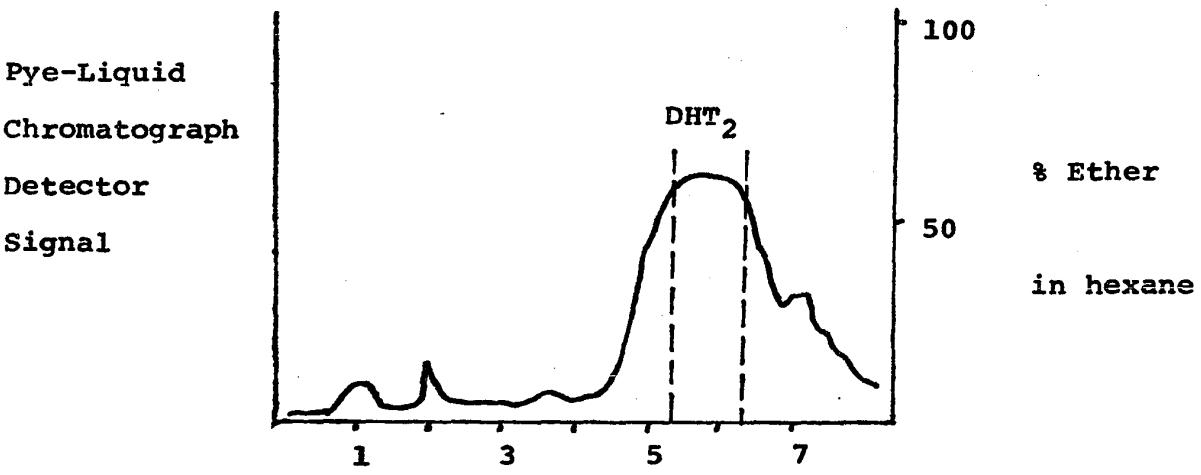
Major Reduction Products from	Retention time in minutes	Yields	DHT-standard (retention time)
Photol. in ether, acetate deriv.	11.0' 17.2'	55% 16%	11.8'
Photol. in ether, TMS derivatives	9.2' 10.2'	30% 31%	10.2'
Photol. in ether, TMS derivatives	8.8' 9.4'	41% 16%	9.4'
Phot. in ethanol, TMS derivatives	8.8' 9.6'	42% 25%	9.6'
Phot. in ethanol, pure alcohols	10.9' 12.0'	25% 42%	10.9'

The first reaction cited above is an example of the production of DHD_2-II or DHD_2-IV. The second and third reactions which were supposedly identical yielded varying amounts of DHT.

Isolation of dihydrotachysterol from the crude reduction products was straightforward, owing to the greater stability of the diene function to atmospheric oxidation. No attempt was made to characterize the products occuring in addition to DHT.

The column chromatography system found most effective for isolating DHT utilized Bio-Sil-Ha silica gel (325 mesh) and a linear solvent gradient of hexane-ether. The results are represented in Figure VIII.

Figure VIII:



The dihydrotachysterol obtained in this way was authenticated by spectral data, mixed melting point and acetate derivative as compared with a known sample of pure DHT_2.

THIN LAYER CHROMATOGRAPHY :

Preparative tlc was also investigated as a means of isolating DHT. The crude reduction products were acetylated with acetic anhydride and pyridine at room temperature and applied to silica gel preparative plates with development by hexane-ether (5% v/v) . Good separation is achieved on an analytical scale under these conditions.

Rf values of the reduction products: 0.98, 0.70, 0.61, 0.52 . Rf of DHT-acetate standard: 0.98 .

On a preparative scale, the band with the highest Rf was removed, yielding after extraction, a single compound shown to be DHT-acetate. Recrystallization from methanol gave white crystals of constant m.p. 104-105 ^{o}C.

EXPERIMENTAL

1) The melting points were taken on a GallenKamp apparatus and are not corrected.

2) The I. R. spectra were determined on an SP 1000 Unicam Instrument.

3) The U. V. absorption spectra were recorded on an SP 800 Unicam Spectrophotometer.

4) N.M.R. spectra were recorded on a Varian T-60 spectrometer with peak positions given in ppm from tetramethylsilane as internal standard.

5) Mass spectra were taken with an AEI MS 902 instrument, the samples being introduced directly by by probe; electron energy, 70 eV; electron current, 20 A; a secondary electron multiplier as the detector.

6) Microanalytical determinations were carried out by Daessle MicroAnalyses, 5757 Decelles Ave., Montreal.

3-Stigmasteryl tosylate : (1B)

To a minimum amount of pyridine required for solution were added a sample of recrystallized stigmasterol, m.p. 170-71 °C. (ethanol), and 1.1 equivalents of para-toluenesulphonyl chloride. The solution was left stirring at room temperature for fifteen hours and then pipetted slowly onto a large volumn of ice. The crystalline tosylate, m.p. 147-48 °C., from dry acetone, was filtered off, washed thoroughly and dried in a vacuum desiccator. Melting point and NMR spectral data agreed with literature values [39].

i-Stigmasterol : (2A)

Stigmasteryl tosylate, 1.0 g . and 1.1 equivalents of sodium carbonate were dissolved in 500 ml of 50% aqueous acetone and refluxed for five hours. The acetone was removed under reduced pressure. Ether extraction gave a sample, the NMR spectrum of which exhibited high field peaks (0.3 - 0.6 ppm) indicative of the cyclopropyl protons, and showed the olefin protons at C-5,6 essentially disappeared.

This sample was chromatographed on alumina, activity III - 6% water. Elution with hexane-benzene (4:1, 2:1, 1:1, 1:2, 1:4) yielded the pure i-stigmasterol as an oil in 70% overall yield.

NMR spectrum (chloroform-d) :

multiplet	0.2-0.6 ppm	cyclopropyl protons
singlet, broad	3.3 ppm	H alpha to the 6-OH
doublet, broad	5.2 ppm	olefin H's at C-22,23

i-Stigmasteryl acetate : (2B)

The i-steroid, 1.0 g , was dissolved in approximately 4 ml pyridine to which was added 10 ml acetic anhydride, and the solution was stirred overnight at room temperature. This was then poured onto ice, left standing 4 hours, which gave a precipitate. After filtering off, washing and drying, the sample failed to crystallize; it was used directly in the succeeding reaction.

NMR spectrum (choloform-d) :

singlet	0.5 ppm	cyclopropyl protons
singlet	2.1 ppm	methyl H's of acetate

NMR spectrum : continued

singlet, broad	4.5 ppm	H alpna to the 6-OAc
doublet, broad	5.2 ppm	olefin H's at C-22,23

6β-Acetoxy-3α,5-cyclo-23,24-bisnor-5α-cholan-22-al :

The procedure for the ozonolysis of i-stigmasterol or of its acetate derivative is the same. The material was dissolved in dry methylene chloride (up to 2.0 g in 100 ml solvent), cooled to -70 °C., and two equivalents of ozone were allowed to pass through the solution which was stirred vigorously. The cold solution was poured immediately into 100 ml of 0.5 N hydrochloric acid. The organic layer was washed several times with water and dried over sodium sulphate.

The solvent was distilled off under reduced pressure with external bath temperature not exceeding 70 °C. This removed the side-chain aldehyde, b.p. 67-69 °C., which is produced in conjunction with the steroid aldehyde. Attempted crystallizations of the steroid aldehyde, a colourless oil, proved futile. The 22-aldehyde was unstable to silica gel chromatography (acid catalyzed rearrangement of the i-steroid to the steroid) and it was also unstable

to alumina chromatography (epimerization of the proton alpha to the C-22 aldehyde).

NMR spectrum (chloroform-d) :

doublet, sharp	9.6 ppm (J=2.0)	aldehyde proton

There was no evidence of either the C-22 olefin protons or of the complex multiplet between 0.80 and 0.95 ppm due to the side-chain protons of the starting material. After thorough drying, the steroid aldehyde (3) was used in the succeeding Wittig reactions without further purification.

2,4-Diacetoxy-2-methylbutane : (10)

A dry 500 ml, three neck flask was set up with a pressure equalizing dropping funnel, condenser and drying tube outlet. Zinc dust, 13.0 g , was hexane washed and vacuum dried, and was placed in the flask under nitrogen. A solution of 23.4 g of ethyl bromoacetate and 23.2 g acetone in 80 ml of sodium dried benzene/20 ml anhydrous ether was added slowly by the dropping funnel. External heating was applied until gentle refluxing occured.

After one and a half hours, the flask was cooled. Half of the grey slurry was poured into a 10% sulphuric acid-ice

solution with vigorous stirring over a period of two hours. The organic layer was separated from the aqueous layer and washed with saturated sodium bicarbonate solution. It was then dried and distilled to yield a fraction boiling at 48-50 °C. (6 mm Hg) shown to be ethyl 3-hydroxy-3-methylbutanoate (9A) by its NMR spectrum. Attempted reduction of this material with 5.3 g of lithium aluminum hydride and quenching by dropwise addition of acetic anhydride in ether failed to give the desired product.

The remaining portion from the Reformatsky reaction was reduced by addition of solid lithium aluminum hydride, 5.3 g , and left stirring overnight. Dropwise addition of ethyl acetate quenched the excess hydride. The material was divided into two parts. The first was worked up directly by pouring onto 5% sulphuric acid-ice and extracting the organic layer with ether in the usual way. An NMR spectrum of the crude product indicated a very small amount of 2-methyl, 2,4-butandiol (10A) was possibly present but yields were very low. Attempted purification by distillation resulted in essentially complete decomposition.

The second portion was stirred with acetic anhydride for fifteen hours to replace -OLi by -OAc. After filtration of the precipitated material, the filtrate was washed with dilute acid, base, and saturated brine, and dried over

sodium sulphate. Distillation under reduced pressure yielded a wide variety of products resulting from decomposition, and no more than 1-2% of the desired 2-methyl-2,4-diacetoxybutane (10B). Because of the numerous side-products, the reaction was not investigated further.

4-Bromo-2-methyl-2-butanol : (4)

Two 3-neck flasks were joined consecutively such that nitrogen could be swept continuously throughout the apparatus. The diborane was generated in one flask by dropwise addition of 5.3 g of sodium borohydride dissolved in 100 ml of diglyme into 15.4 g of boron trifluoride-etherate in 100 ml diglyme. The second vessel contained 12.9 g of the 2-hydroxy-2-methyl-3-butene in 200 ml of freshly distilled tetrahydrofuran kept at 0 ^{0}C. After evolution of the diborane ceased the reaction flask was allowed to warm to room temperature over a period of one hour.

About 2 ml of methanol was added via a separatory funnel to destroy any excess hydride. The solution was then cooled to -5 ^{o}C. and 10 ml of bromine added dropwise, followed by 100 ml of 4.2 M solution of sodium methoxide.

This was left stirring an additional half hour while warming to approximately 10 °C. The bromine colour discharged completely and a white precipitate appeared. The solution was placed in a separatory funnel, diluted with 100 ml hexane and washed with saturated sodium bicarbonate solution and with brine. The organic fractions were dried over sodium sulphate and distilled under reduced pressure (about 6 mm Hg) to yield 500 mg of a colourless liquid, b.p. 32-34 °C.

NMR spectrum (carbon tetrachloride) :

singlet, sharp	1.3 ppm	methyl H's; integrates to 6 protons
multiplet	2.6-3.6 ppm	integrates to 5 H's; contains one deuterium exchangeable H.

The extremely poor yield of compound (4) by this method, only 2%, is thought to result from interference by the hydroxyl group with the boron hydride complex.

All attempts to protect the hydroxyl function as the tetrahydropyranyl ether or as the acetate derivative resulted in the polymerization of the starting olefin.

Methyl 3-bromopropanoate and methylmagnesium iodide :

Diazomethane in ether solution was prepared in the usual way from diazald and added to a solution of 3-bromo-propionic acid in ether until a faint yellow colour just persisted. The NMR spectrum of the sample after removal of the solvent shows disappearance of the acid peak at 12.1 ppm , and the appearance of the methyl ester protons at 3.8 ppm.

Into a dry 3-neck flask fitted with condenser, dropping funnel and nitrogen inlet, was placed 0.26 g of magnesium turnings (1.1 molar excess in terms of the ester) in 3 ml ether. Methyl iodide, 1.5 g , in ether solution was allowed to add slowly to the magnesium. Gentle warming began the reaction, and it was left at reflux for one and a half hours. Into a second flask set up as the first, was placed 1.6 g of the methyl 3-bromopropionic ester dissolved in 20 ml ether at 0 ^{o}C. The methylmagnesium iodide was pipetted quickly into the reaction flask through glass wool along with an additional 10 ml of anhydrous ether.

The reaction flask was allowed to come to room temperature, and left stirring for fifteen hours. The reaction was quenched with saturated ammonium chloride solution

which precipitated the magnesium salts. After filtration and extraction with ether, the solvent was removed in vacuo with the moisture azeotroped by a few ml of benzene.

The NMR spectrum in carbon tetrachloride indicated a wide range of products, due to magnesium complexation with the bromo function present in the starting material. When the next method was found a more favorable means of producing compound (4), no separation of the products was attempted.

This reaction, performed by a co-worker at a later date, proved very successful when carried out with 3-chloropropionyl chloride and methylmagnesium iodide [15].

2-Methyl-4-halo-2-butanol : (4A) and (4B)

Twelve ml of 3-chloropropionyl chloride was dissolved in 100 ml anhydrous ether under a nitrogen atmosphere, and added by syringe to a dry 3-neck flask set up with condenser, dropping funnel and nitrogen inlet. A 1.66 molar solution of methyl lithium, 250 ml, was placed in the dropping funnel inside a glove bag containing nitrogen, and transferred to the reaction vessel. The methyl lithium solution was added dropwise with stirring over a period of one and a half

hours while the reaction flask was kept at 0 °C.

After warming to room temperature over approximately four hours, the excess methyl lithium was destroyed by dropwise addition of saturated ammonium chloride solution. The lithium salts were filtered off and the solution extracted with ether. The organic fractions were dried over sodium sulphate and evaporated to strip the solvent. The NMR spectrum showed the presence of two major compounds: 2-methyl-4-chloro-2-butanol (4B) and 2-methyl-2-hydroxy-3-butene (4').

Distillation yielded the pure (4B), b.p. 60-63 °C. at 14 mm Hg, in 40% overall yield. Literature b.p. 62-63 °C, at 14 mm Hg [40].

NMR spectrum (chloroform-d) :

		Integral:	
singlet, sharp	1.25 ppm	6 H's	methyl H's
triplet, mid-point	2.0 ppm	2 H's	methylene adjacent to CMe_2OH
triplet, mid-point	3.7 ppm	2 H's	methylene adjacent to chloro group
singlet	3.0 ppm	1 H	-OH, deuterium exchangeable

The bromo analogue, that is, 4-bromo-2-methyl-2-butanol (4A) was prepared exactly as above from the 3-bromopropionyl chloride, except that yields were somewhat lower, about

30 %. The boiling point of (4A) was noted to be 38-40 °C. at 6 mm Hg. There was a sharp absorption in the infrared region at 3600 cm^{-1} corresponding to the tertiary hydroxyl function. The carbonyl absorption at 1780 cm^{-1} in the starting material had completely disappeared.

NMR spectrum (chloroform-d) :

singlet	1.10 ppm	6 H's	methyl protons
triplet, mid-point	2.00 ppm	2 H's	methylene adjacent to $-CMe_2OH$
triplet mid-poimt	3.40 ppm	2 H's	methylene adjacent to bromo group
singlet	2.80 ppm	1 H	-OH, deuterium exchangeable

Chemical Analysis:

Calc'd : C = 35.92; H = 6.57; Br = 47.90

Found : C = 36.47; H = 6.38; Br = 48.02

PREPARATION of the THP ETHERS : (4C) and (4D)

The alcohols were dissolved respectively in a three molar excess of freshly distilled dihydropyran at -15 °C. and

left stirring for one-half hour. One crystal of para-toluene -sulphonic acid was added to each solution with stirring continued for a further two hours while the temperature was maintained at -15 ^{o}C. The solutions were diluted with ether, washed with sodium bicarbonate and with brine. The organic fractions were dried and evaporated to yield the respective tetrahydropyranyl ether essentially quantitatively.

$Cl-CH_2-CH_2-CMe_2-OTHP$ (4D)

NMR spectrum (carbon tetrachloride) :

doublet	1.2 ppm	6 H's	methyl protons
singlet	1.6 ppm	4 H's	THP protons
triplet, mid-point	2.0 ppm	2 H's	$-CH_2-$ adjacent to the $-CMe_2OTHP$
triplet, mid-point	3.6 ppm	2 H's	$-CH_2-$ adjacent to the chloro group
multiplet	4.0-3.8 ppm	4 H's	THP protons
singlet, broad	4.8 ppm	1 H	H adjacent to the two oxygens

The peaks at 4.8, 4.0-3.8, and 1.7-1.4 ppm are characteristic of the tetrahydropyranyl ether function. The methyl peaks, formerly a singlet, are split slightly, due to the epimeric forms of the ether.

The tertiary hydroxyl function at 3600 cm^{-1} in the infrared spectrum of the starting material had disappeared completely for the ether derivative.

$Br-CH_2-CH_2-CMe_2-OTHP$: (4C)

NMR spectrum (carbon tetrachloride)

doublet	1.2 ppm	6 H's	methyl protons
singlet, broad	1.5 ppm	4 H's	THP protons
triplet, mid-point	2.0 ppm	2 H's	$-CH_2-$ adjacent to the $-CMe_2OTHP$ group
triplet, mid-point	3.4 ppm	2 H's	$-CH_2-$ adjacent to the bromo group
multiplet	4.0-3.7 ppm	4 H's	THP protons
singlet, broad	4.7 ppm	1 H	H adjacent to the two oxygens

The NMR spectra of (4C) and (4D) correspond exactly except for the shift upfield of the methylene protons adjacent to the less electronegative group. The infrared spectrum of (4C) also exhibits the loss of the tertiary hydroxyl group formerly at 3600 cm^{-1}.

4-Iodo-2-methyl-2-butanyltetrahydropyranyl ether :

The method used is essentially that reported by Bergel'son et al [16].

Sodium iodide, 750 mg, (a 1.5 molar excess) was dissolved in 20 ml of methyl ethyl ketone and refluxed at 80 ^{o}C. for thirty minutes. The chloro compound (4D), 400 mg, was added and the solution left refluxing for eighteen hours. A white precipitate, sodium chloride, was filtered off; the solvent was stripped and the residue taken up in benzene.

The resulting red solution was washed with 5% sodium thiosulphate solution until colourless, then washed with water and dried over sodium sulphate. Removal of the solvent resulted in an 80% yield of the iodo alkyl ether as a light brown oil.

The NMR spectrum of I-CH_2-CH_2-CMe_2-OTHP was identical to that of the chloro analogue except that the triplet for the methylene protons adjacent to the halo group shifted upfield from 3.6 to 3.2 ppm. Attempts to purify the the iodo compound on alumina resulted in the elimination of hydrogen iodide. Standing at room temperature also allowed decomposition.

Triphenylphosphonium salt of 4-iodo-2-methyl-2-butanyl tetrahydropyranyl ether : (14C)

A sample of 4-iodo-2-methyl-2-butanyl THP ether was dried thoroughly in a vacuum desiccator for 24 hours, and used without further purification. It was dissolved in dry benzene with an equimolar amount of triphenylphosphine and refluxed for approximately 32 hours. Evaporation of the solvent yielded a viscous light brown oil which appeared to contain the desired phosphonium salt (14C), as judged from its NMR spectral data.

NMR spectrum : (chloroform-d)

singlet	7.7 ppm	phenyl protons
doublet	7.38 and 7.33 ppm	

A complex multiplet replaced the original triplet corresponding to the methylene protons adjacent to the iodo group, due to phosphorus coupling. The remaining spectrum was identical to that of the starting compound.

The NMR spectrum of pure triphenylphosphine in chloroform-d exhibits a sharp doublet at 7.55 and 7.50 ppm.

All attempts to induce the oil to crystallize resulted only in crystalline triphenylphosphine. An NMR spectrum of the

mother liquors showed the presence of the starting alkyl compound with its THP protecting group lost.

Triphenylphosphonium salt of 1-bromo-3-methyl-2-butene : (15)

A sample of 4-bromo-2-methyl-2-butanol was dissolved in dry acetonitrile with an equivalent amount of triphenyl-phosphine and refluxed for 24 hours. The solvent was stripped by rotary evaporator to give a brown viscous oil which upon trituration with acetone yielded a white crystalline compound, deduced to be the triphenylphosphonium salt of 1-bromo-3-methyl-2-butene (15). Its m.p. was 232 ^{o}C.

Chemical Analysis:

Calc'd : C = 67.15; H = 5.84; P = 7.54; Br = 19.46

Found : C = 67.40; H = 6.00; P = 7.24; Br = 19.34

Infrared spectral analysis (nujol) indicated the loss of the hydroxyl group, and the NMR spectrum (chloroform-d) showed resonance absorptions at frequencies corresponding to methyl groups attached to an olefin (1.6 - 1.2 ppm). The remainder of the spectrum was complex, probably due to long range coupling with phosphorus.

Triphenylphosphonium salt of 3-halopropionic acid : (17A) and (17B)

Equimolar samples of 3-bromopropionic acid and triphenylphosphine were dissolved in acetonitrile and refluxed for eighteen hours. After the solvent was stripped trituration with acetone afforded a white crystalline product shown to be the triphenylphosphonium salt (17A), m.p. 203 °C.

NMR spectrum : (chloroform-d)

doublet	7.85 and 7.75 ppm	15 H's	phenyl protons
singlet	12.85 ppm	1 H	acid proton
quintet, mid-point	3.80 ppm	2 H's	$-CH_2-$ adjacent to phosphorus
quintet, mid-point	3.00 ppm	2 H's	$-CH_2-$ adjacent to the -COOH

Equimolar samples of 3-chloropropionic acid and triphenylphosphine were weighed into a flask equipped with condenser and stirring bar, and heated to 135 °C. for two hours. The reagents melted at approximately 90 °C. To the hot melt was added absolute ethanol and then anhydrous ether to precipitate the phosphonium salt. Several recrystallizations

from the ethanol and ether solution yielded a white crystalline compound with constant m.p. 192 ^{0}C. (literature value is 196 ^{o}C.).

NMR spectrum : (chloroform-d)

doublet	7.85 and 7.75 ppm	15 H's	phenyl protons
singlet	13.15 ppm	1 H	acid proton
quintet	3.75 ppm (mid-point)	2 H's	$-CH_2-$ adjacent to phosphorus
quintet	3.00 ppm (mid-point)	2 H's	$-CH_2-$ adjacent to -COOH

Wittig Condensation :

The procedure followed is adapted from a paper by E.J. Corey et al [19]. The sodium hydride, as a 50% oil dispersion, was weighed into a three neck flask and washed three times with sodium dried pentane under a constant stream of nitrogen. Freshly distilled dimethyl sulphoxide was added by syringe, such that the resulting solution would contain 1 mM of the methyl sulphinyl carbanion per ml. The mixture was heated to not more than 80 ^{o}C. until the evolution of hydrogen ceased, approximately 45 minutes. A dark green, almost black solution resulted.

Two millimoles of the base were then added to one mM of the phosphonium salt (17A) in 10 ml of dimethyl sulphoxide. The ylid, as a dark red solution, formed instantaneously, and was left standing sealed under nitrogen at room temperature about 30 minutes before use.

One millimole of the steroid aldehyde (3) was placed in a three neck flask and dissolved in 10 ml dimethyl sulphoxide under a nitrogen atmosphere. The ylid solution was added dropwise over 20 minutes at room temperature with stirring. It was immediately discoloured upon addition of the aldehyde. The resulting pale yellow solution was left stirring for fourteen hours at R.T., and then heated to 60 ^{o}C. for three hours.

After cooling, the solution was poured into a large volumn of water and extracted with pentane to yield a small amount of the hydrocarbon material from the sodium hydride oil dispersion. The aqueous layer was then brought carefully to a neutral pH with dilute hydrochloric acid and extracted with ether.

The steroidal product obtained in this way was presumed to be the cyclic phosphorane (23). Its NMR spectrum in chloroform-d showed evidence of phenyl peaks but no aldehyde

or olefinic protons. The material was dissolved in dimethyl sulphoxide-d_6 and heated at 70 °C. while following the reaction by NMR over an 18 hour period. No change occured. A similar attempt carried out in pyridine-d_5 also failed to produce any changes in the NMR spectrum.

Chromatography on alumina, activity III, with hexane-benzene elution resulted in three major fractions: epimerized aldehyde, triphenylphosphine, and a small amount of triphenylphosphine oxide.

PHOTOCHEMICAL IRRADIATION :

The standard procedure followed in all the irradiations is outlined below:

Samples of the recrystallized reagent were dissolved respectively in either anhydrous ether or in absolute ethanol in quartz irradiation vessels at a concentration of 1-2 mg per ml of solution. The solutions were allowed to equilibrate to 0 ^{o}C. in the cold room under continuous nitrogen flushing. The Rayonet Photochemical Reactor consisted of a circular array of low pressure lamps generating light of 2537 angstroms placed around the irradiation vessel. Back-ground reflectors and a foil cover were situated so as to minimize the loss of light to the surroundings.

Once the photolysis had begun, all external lighting was removed, and after completion of the irradiation the samples were kept as much as possible covered with foil at a low temperature. A thermometer placed inside the irradiation vessel and subjected to an equivalent amount of irradiation under identical conditions registered between 5 - 8 ^{o}C.

All vapor phase chromatographic analyses were run on a Hewlett-Packard Instrument (#5750) using an eight foot column containing 4% HCW 98 on Chromosorb W and heated to 260 ^{o}C.

The experimental results have been summarized previously.

Silica gel (Brinkmann F_{254}) preparatory plates were used in all thin layer chromatography separations. Phosphomolybdic acid, a 10% solution in ethanol, was found to be the best detector : limit of detection was 50 ng. As much as possible, the plates were handled in the cold room (0-3 ^{o}C.) under a nitrogen atmosphere. The results are listed in Chapter II.

Except where indicated, all column separations were effected with Mallinckrodt silicic acid (100 mesh A.R.) activated at 200 ^{o}C. for 24 hours. The columns were maintained at a low temperature by means of a water-cooling jacket. The gradient elution techniques were adapted from those reported in the literature [42].

Ten or five ml fractions were collected by use of an

automatic fraction collector, and simultaneously detected by a Pye-Liquid Chromatograph. The convex gradient was obtained by running successively into a mixing chamber containing 200 ml pure hexane, 200 ml each of 1:1 hexane-ether, pure ether, 1:1 ether-methanol, pure methanol.

The isolation of products obtained by column chromatography is discussed in Chapter II.

REDUCTION OF THE PHOTOLYSIS PRODUCTS :

The reductions of the products resulting from irradiation of both ergosterol and ergosterol acetate were carried out under identical conditions according to a procedure by Westerhof and Buisman [36].

Ammonia gas was dried and condensed to a volumn of 50 ml in a 3-neck flask under nitrogen. About 5 ml of anhydrous ether was introduced by syringe; then small pieces of lithium metal previously washed in benzene were added until a lasting blue colour was achieved. From a dropping funnel, 200 mg of the irradiation products in ether solution were added slowly to the lithium - liquid ammonia solution. The colour was discharged immediately. Additional lithium was

introduced until the blue colour was just maintained again.

The solution was left stirring for 20 minutes at -80 °C., and then quenched by the dropwise addition of saturated ammonium chloride solution. The reaction vessel was allowed to warm to room temperature until all the ammonia had evaporated. The remaining material was diluted with 100 ml water and extracted with ether. The organic fractions were washed again with brine, dried over sodium sulphate and evaporated to yield 185 mg product.

Isolation of Dihydrotachysterol :

The column chromatographic separation indicated in Figure VIII, page 38, was carried out with 10 g of Bio-Sil-Ha silica gel (325 mesh) used directly without thermal activation in a 20 cm column of bore 1 cm.

Thirty-three mg of the reduction product were applied to the column in hexane. The linear gradient of 20 ml ether into 20 ml hexane was obtained by means of metal mixing chambers which allowed the solvents to be eluted through the column under a nitrogen pressure of approximately 10-12 pounds. Five ml fractions were collected and simultaneously

detected on a Pye-Liquid chromatograph recorder.

Fraction V contained 8 mg of a white crystalline compound which co-chromatographed on the VPC with a known sample of pure dihydrotachysterol (obtained from Mann Research Laboratories). Its ultraviolet absorption maxima were measured at 244 mμ (ξ = 24,610) ; 252 mμ (ξ = 29,463) ; 262 mμ (ξ = 20,104) .

The preparatory tlc plates used in separating the re-acetylated reduction products were made from Brinkman F_{254} silica gel, 0.75 mm thickness, and activated at 200 °C. for one hour. Development was best effected by a hexane-ether solution (95:5 v/v) with detection of the components by U.V.

The bands were extracted by chloroform; the band with the highest Rf containing mainly dihydrotachysterol acetate. This was re-applied for further purification, resulting in a crystalline compound which recrystallized from methanol at constant m.p. 104-105 °C. Literature value for DHT-OAc is 106-108 °C.

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