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# THE SYNTHESIS OF POSSIBLE POLYENE INTERMEDIATES IN PHYTOSTEROL BIOSYNTHESIS

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**Abstract**—The previously unknown steroids 5, 22, 24(28)-ergostatriene-3β-yl-acetate, 5, 22, 24-ergostatriene-3β-yl acetate, 5, 7, 22, 24-ergostatetrene-3β-yl-acetate, and 4, 22, 24(28Z)-stigmastatriene-3β-yl-acetate have been synthesized. The naturally occurring 5, 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 sterol 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 stigmasteryl acetate. The effect that several new side chain groups exert on the chemical shift (NMR) the angular Me groups has been determined.

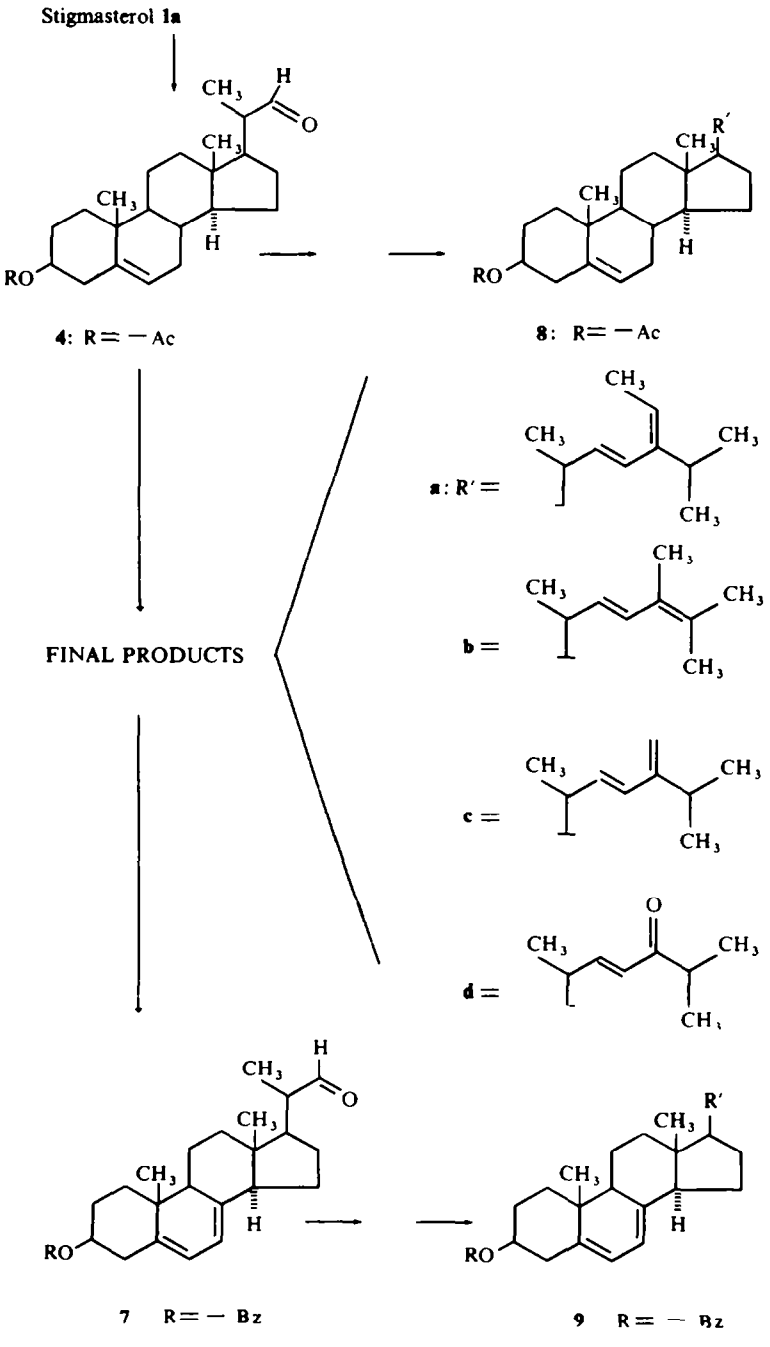
RECENT interest in the biosynthesis of phytosterols has been directed toward the elucidation of the steps involved in the modifications of the side chain in fungi and higher plants.[1–3] Several biosynthetic schemes have been proposed but the exact sequence of events has not yet been established. Available results seem to point to the possibility of two parallel pathways; one which could involve steroids with fully saturated side chains as key intermediates and another which could proceed via several steroid intermediates possessing varying degrees of side chain unsaturation.[4,5] Which of the two operates may well depend on the plant system as well as the condition under which it is investigated. Of interest in this connection is the role which steroidal compounds with side chains possessing conjugated double bonds play in the biosynthetic process. Only one such compound, Δ5, 7, 22, 24(28)-ergostatetraene-3β ol (**9c**, R = H), has, up to this time, been isolated from yeast.[6]

In order to facilitate the study of the possible intermediacy of this type of steroid in the biosynthesis of ergosterol in yeast and of the major phytosterols in plants,[7] we have synthesized several steroids with such doubly unsaturated side chains.

The synthesis of these compounds follows the route shown in Scheme 1. Basically it involves the preparation of two key intermediates, aldehydes **4** and **7** followed by their transformation to final products via Wittig reactions.

The synthesis of **4** from stigmasterol (**1a**) was accomplished by the route shown in Scheme 2. This aldehyde has been obtained previously by Centolella *et al.*[8] The present synthesis follows essentially the sequence used by these workers with the exception that bromination of stigmasteryl acetate was performed using iodobenzene dibromide. Bromination of stigmasteryl acetate (**1b**) 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 stigmasteryl 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 selective. This has been indicated in the

SCHEME 1




literature since 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 e.g., by hydrochlorination,[3] by formation of i-stigmasteryl-methyl ether,

by the formation of the 5,6 dichloro compound[10] or by transforming stigmasterol into the corresponding 4, 22-stigmastadiene-3-one.[11]

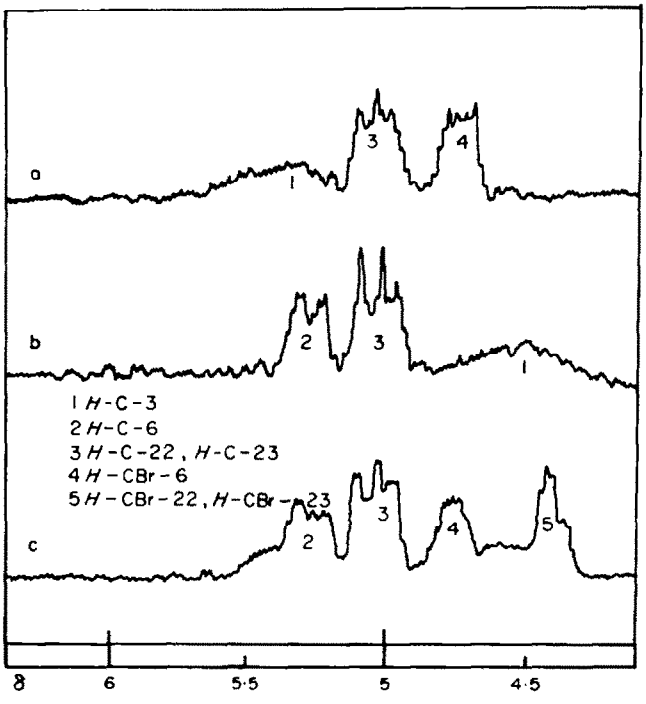
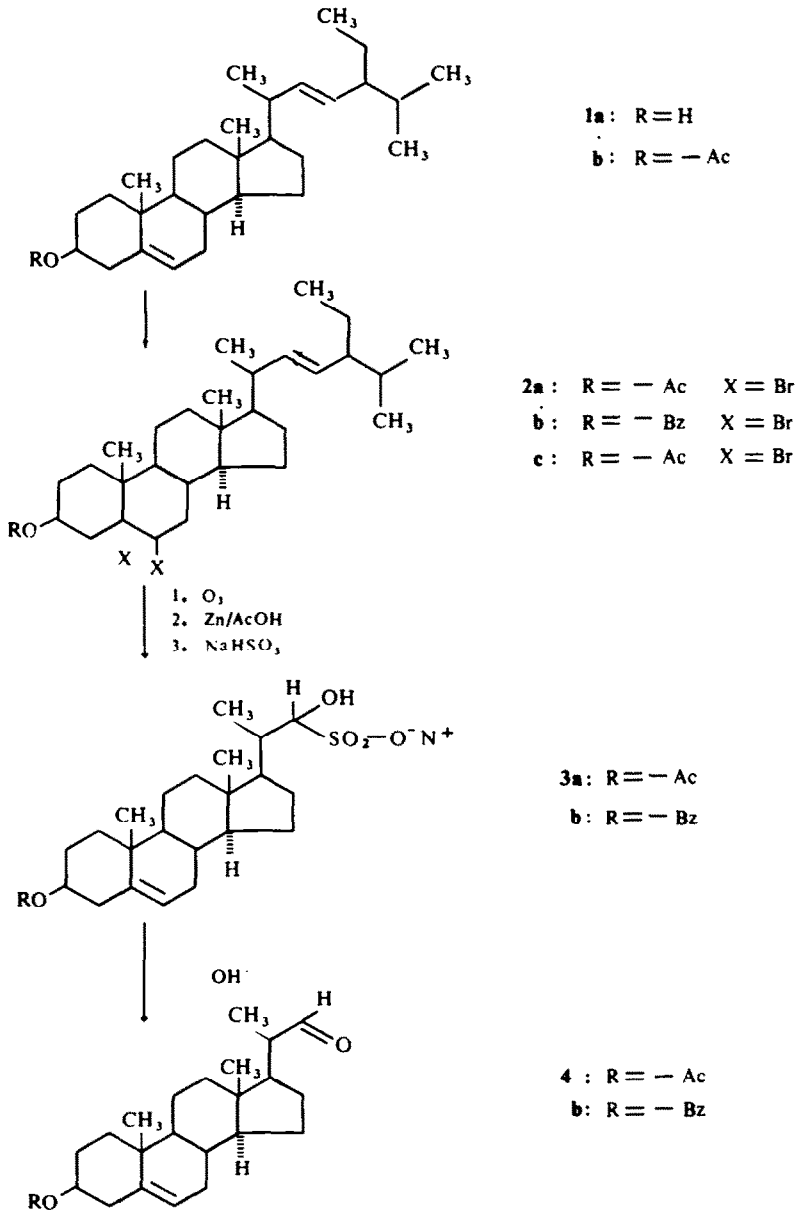



FIG 1. (a) NMR spectrum δ (4·5–6) of 5,6-dibromostigmasterol acetate obtained upon reaction of **1b** with iodobenzene dibromide; (b) Stigmasterol acetate (**1b**); (c) Reaction mixture obtained on bromination of stigmasterol acetate with molecular bromine

The specificity of the bromination of stigmasterol acetate with bromine was therefore investigated. Fig 1c shows the δ 3·5–6 region of the NMR spectrum of the mixture of bromosterols obtained after bromination according to the method of Fernholz.[12] Figs 1b and 1a show the same regions of the NMR spectra of stigmasteryl acetate (**1b**) and of the dibromostigmasteryl acetate obtained using iodobenzene dibromide. Comparison of the spectra allows the unambiguous assignment of the peak at δ 4·48 to the protons at C-22 and C-23 of the brominated side chain. Calculation of the extent of ring and side chain bromination revealed approximately 60% reaction of Δ5 and 40% reaction of Δ22. No absorption due to C-22 and C-23 of the brominated side chain could be detected in the crude bromination product using iodobenzene dibromide (Fig 1c). Halogenation of the 22, 23 double bond would also be expected to shift C-18 and C-19 Me resonance to lower field. No such absorptions as observed for the C-18 and C-19 Me resonance of 22, 23-dibromostigmasteryl acetate obtained by bromination with molecular bromine could be detected in crude product obtained with iodobenzene dibromide.

Iodobenzene dibromide has been described by Thiele *et al*[13] as an oil at room temperature, crystallizing at −45°. It gives off bromine rapidly at room temperature but is quite stable at 0°. It is best prepared in a solution of hexane and added to the

SCHEME 2

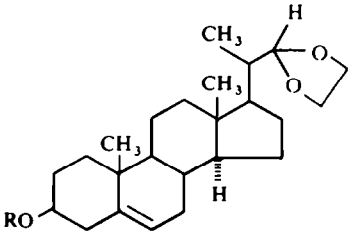



alkene in hexane solution. Bromination of stigmasteryl acetate (**3b**) using iodobenzene dibromide gives the 5,6-dibromide (**2a**) in a high state of purity which can be used without further purification for the ozonolysis.

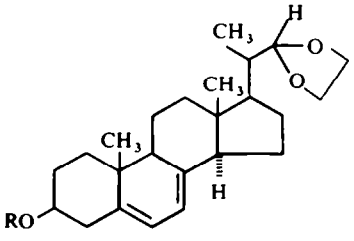
Stigmasterol esters have been chlorinated in the 5,6 position specifically, using iodobenzene dichloride as chlorinating agent.[10] Ozonolysis of the 5,6 dichlorostigmasterol acetate (**2c**), obtained by this method gave low yields of aldehyde **1a**,

since the ozonolysis reaction mixture must be heated with zinc and acetic acid in order to achieve dechlorination. Since the 5,6-dibromo stigmasteryl acetate (**2a**) can be dehalogenated at room temperature, selective 5,6-bromination using iodobenzene dibromide provides an efficient step in the synthesis of **4a**. Aldehyde **4a** was converted to the cholestatrienyl acetates **8a–c** by the appropriate Wittig reactions.

The conversion of aldehyde **4a** to aldehyde **7a** involved protection of the aldehyde function by acetal formation prior to introduction of the Δ7-double bond. Treatment of **4a** with ethylene glycol and $BF_3$-etherate in glacial acetic acid by the method of Fieser *et al*[14] gave high yields of acetal **5a**. 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.




**5a**: R = — Ac
**b**: R = — Bz




**6a**: R = — Ac
**b**: R = — Bz

The Δ7-double bond was introduced into **5** according to the slightly modified method described by Hunziker *et al*[15] e.g. bromination of **5** with NBS followed by dehydrohalogenation with trimethyl phosphite to give the 5, 7-diene, **6**. The reaction can be performed on either the C-3 acetate (**5a**) or C-3 benzoate (**5b**), however, when the acetate was used, it was found that up to 30% of the 4, 6-diene was formed. This was evident from the u.v. absorption maxima at 232, 238 and 248 mμ as well as from the C-18 and C-19 Me resonances in the NMR spectrum of the crude reaction product. Since no detectable amount of the Δ4, 6 isomer was found when the C-3 benzoate (**5b**) was subjected to the bromination/dehydrobromination sequence, the acetate was hydrolyzed and the C-3 OH benzoylated before introduction of the Δ7-double bond.

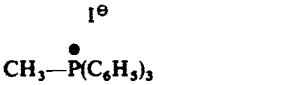
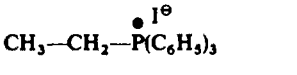
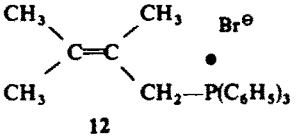
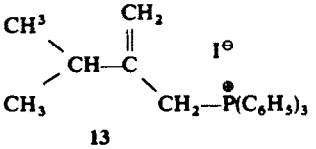
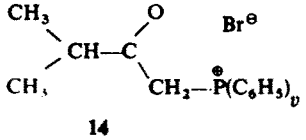
Hydrolysis of the acetal **6b** in the usual manner by refluxing in $H_2SO_4$–water–methanol gave a poor yield of the free aldehyde. A considerable amount of the isolated product seemed to consist of the 22-Me hemiacetal, (strong OH absorption in the IR and OMe peak at $\delta$ 3·5 in the NMR). Furthermore the **7** produced under these conditions was epimerized at $C_{20}$ to a 1 : 1 mixture of 20(*S*) and 20(*R*) aldehydes. Thus two doublets due to the aldehyde hydrogen resonance ($J$ = 3 Hz) at $\delta$ 9·59 and $\delta$ 9·40 were distinguishable in the NMR spectrum of **7** derived from the above reaction whereas only one doublet ($J$ = 3 Hz) at $\delta$ 9·50 was observable when the reaction was carried out in $H_2SO_4$–water–THF.

The position[16] of the aldehyde hydrogen resonance in the NMR of **7** produced from **6a** via reaction in $H_2SO_4$–water–THF as well as the occurrence of only one

doublet signal due to this hydrogen requires that in the entire reaction sequence from stigmasteryl acetate that the $C_{20}$ configuration remain intact (*S*). This requirement fixes the configuration in **4** as *S*. Since the conditions of the Wittig reactions used for the conversion of **4** to polyenes **8a–c** and **7** to polyenes **9b–c** are known to leave the $C_{20}$ configuration intact,[6d] the configuration at $C_{20}$ in these polyenes is *S*.

The ylides used in the Wittig reactions were prepared from the corresponding triphenylphosphonium salts. The triphenylphosphonium salts were prepared by treating the corresponding halide in a suitable solvent, most often benzene, with triphenylphosphine. The salt of methyl iodide (**10**) precipitated in 92% yield after reaction at room temperature for two hours whereas the salt of ethyl iodide (**11**)[17] was formed (52%) only after refluxing (Table 1). The allylic bromide 1-bromo-2,3-dimethyl-2-butene formed the corresponding phosphonium salt (**11**) spontaneously on mixing with triphenylphosphine. The isomeric bromide 1-bromo-2-methylene-3-methylbutane 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 **12** and **13** (halogen = Br). Heating of this mixture in $CHCl_3$ under pressure did not alter the ratio of the two isomers. When the corresponding iodide was reacted at room temperature with triphenylphosphine the expected salt **13** was formed within

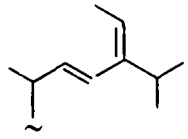
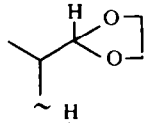
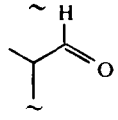
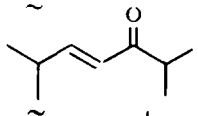
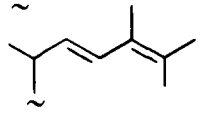
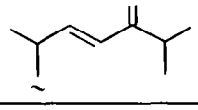
TABLE 1. TRIPHENYLPHOSPHONIUM SALTS PREPARED

| Salt | M.P. |
|---|---|
|  **10** | 184–184·5° |
|  **11** | 164–165° (Lit.[17] 163–164·5°) |
|  **12** | 164–166° |
|  **13** | 184–187° |
|  **14** | 235–237° |

one hour in good yield and no isomeric salt was formed. The structure of each salt was determined by analysis of its NMR spectrum.

Based on Schneider's[18] analysis of the stereochemistry of the Wittig reaction, the Δ22, 23-double bonds introduced in the formation of polyenes **8** and **9** were expected to be *trans*. This expectation was confirmed by analysis of the NMR spectra of the

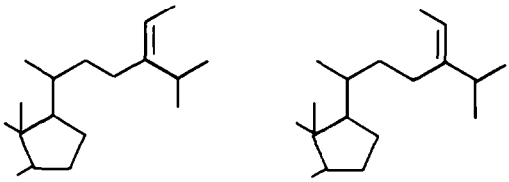
TABLE 2. EFFECT OF SIDE CHAIN SUBSTITUENTS ON THE CHEMICAL SHIFT OF C-18 AND C-19 METHYL RESONANCES

| Functional group (Δ5 or 5.7 steroid nucleus) | | C-19 H Hz | C—18 H Hz |
|---|---|---|---|
| 17β |  | −1·5 | −2·0 |
| 17β |  | −2·5 | −2·5 |
| 17β |  | 0 | 0 |
| 17β |  | 0 | 0 |
| 17β |  | −1·0 | −1·5 |
| 17β |  | −1·0 | −1·5 |

polyene steroids (**8a–c** and **9b–c**). Thus the NMR spectra of the steroid polyenes synthesized showed a doublet ($J = 15 \pm 1$ Hz) for the proton at C-23 and a quartet ($J = 16 \pm 1 \cdot 8$ Hz) for the proton at C-22.

All the polyenes (**8a–c**, **9b–c**) synthesized 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 *cis*-5, 22, 24-ergostatriene-3β-ol, these bands are missing entirely. Two new bands appear in this case at 745 and 690 $cm^{-1}$. Bands in this region have been assigned to *cis* double bonds.[19]

Compound **8a** can have two possible side chain isomeric forms if the 22, 23-double bond is *trans*. The nomenclature, put forward by Blackwood *et al*[20] designates the two isomers as E and Z. Differentiation between the two isomers based on IR absorption is not clear cut and it is necessary to rely on NMR spectra for a definite structure assignment.



E Z

The stereochemistry at C-28 in compound **8a** has been assigned as **Z** on the following grounds. Frost *et al*[21] have isolated 7, 24 (28Z)-stigmastadiene-3β-ol and found it to be identical with isofucosterol. In the former compound, H—C-25 resonates at δ 2·82. Goodwin *et al*[22] compared fucosterol and isofucosterol and found H—C-25 resonance to be δ 2·2 and δ 2·8 respectively. Rowe *et al*[23] correlated several model compounds and 24-ethylidene sterols and in accord with the foregoing they have determined that the septet signal, due to the isopropyl methine proton, absorbs at δ 2·2 for those ethylidene sterols possessing the **E**-configuration and at δ 2·8 for those with the **Z**-configuration. The NMR of compound **8a** shows a septet at δ 2·84 for H—C-25 and was therefore assigned the **Z**-configuration.

The chemical shifts of the angular Me groups of the new steroids encountered in this study were used in conjunction with published[24–26] values for the effects of nuclear substituents on the positions of these resonances to calculate the effect of the new side chain substituents introduced (Table 2).

## EXPERIMENTAL

*Instruments and materials.* M.pts were obtained on a Fisher–Johns m.p. apparatus and are uncorrected. Spectra were obtained on the following instruments: Perkin–Elmer 457 (IR), Unicam SP 800 (UV), Varian A 56/60 (NMR) and Hitachi–Perkin–Elmer RMU-7 double focusing mass spectrometer. NMR results are reported as δ using TMS as internal standard (δ = 0). Inlet voltage for MS was 80 eV if not specifically mentioned otherwise. Stigmasterol was generously supplied by the Upjohn Company, Kalamazoo, Michigan.

*Preparation of* 5α, 6β-*dibromostigmastan*-3β-*yl-acetate*, **2a**

(a) *Preparation of iodobenzene dibromide.* To iodobenzene (7 g, 34 mmole) in dry hexane (10 ml) $Br_2$ (4·8 g, 33 mmole) was added. The soln was mixed at r.t. and cooled to below − 5°.

(b) *Bromination.* A soln of stigmasteryl acetate[27] (15 g, 33 mmole) in dry hexane (900 ml) was cooled to below − 5°. The soln was stirred vigorously with a magnetic stirrer and the reagent prepared as above was added at such a rate (3–4 hr) as to maintain the soln pale yellow. The resulting slightly turbid soln 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 soln refrigerated for several hr. The 5,6-dibromostigmasteryl acetate which crystallized, was filtered off, washed thoroughly with ice cold MeOH and dried under vacuum to yield 13·5 g, m.p. 130–132°. The filtrate and the washings were concentrated to dryness. The solid was triturated with hot MeOH, cooled and filtered giving an additional 6·2 g of product, m.p. 124–128°. Recrystallization of the total bromosterol from 98% EtOH gave 18·5 g (91·5%) m.p. 131–135°. (lit.[12] m.p. 132–135°); $\nu_{max}$ (Nujol): 1732 (C=O), 975 and 965 $cm^{-1}$, (C-22, C-23 double bond); NMR ($CDCl_3$): δ 0·70 ($CH_3$—C-18, s), 1·44 ($CH_3$—C-19, s), 2·01 ($CH_3COO$—, 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 acetate with molecular bromine.* The bromination was carried out according to Fernholz.[12] The crude bromosterol obtained after evaporation of the $CHCl_3$ was used for the NMR spectra. NMR for the 5,6-dibromosterol ($CDCl_3$): δ 0·70 ($CH_3$—C-18, s), 1·45 ($CH_3$—C-19, s), 2·01 ($CH_3COO$—, s); for the 22, 23-dibromosterol: δ 0·77 ($CH_3$—C-18, s), 1·0 ($CH_3$—C-19, s). The region δ 4·0–6·0 is reproduced in Fig 1.

*Ozonolysis of* 5α, 6β-*dibromomostigmastan*-3β-*yl acetate*

A solution of **2a** (15 g, 0·0244 mole) in dichloromethane (600 ml) containing pyridine (8 ml) was cooled to − 70°. A mixture of $O_3$ and $O_2$ was introduced at a constant flow rate of 2 l/min (0·5 mmole ozone/min). The $O_3$ passing through the soln unreacted was monitored by titrating the $I_2$ liberated from a 5% KI soln against $Na_2S_2O_3$. The reaction was terminated after 1·7 molar equivts of $O_3$ were absorbed. Zn powder (40 g) and glacial AcOH (60 ml) were added. The soln was warmed to r.t. and stirred vigorously for 3–4 hr. The solution was filtered, washed twice with water then with $NaHCO_3$ aq and finally with 5% NaOH aq. The salt of 3β-acetoxy-5-cholestene-24-oic acid which concentrated at the interface was discarded. The dichloromethane soln 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 MeOH then 200 ml saturated $NaHSO_3$ aq was added and the mixture shaken for several hr. 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 **3a**. Ozonolysis of **2d**, was carried out in an analogous manner.

*Conversion of bisulfite adduct* **3a** to 3β-*acetoxy-5-cholestene-22-ethyleneacetal*, **5a**

To **3a** (7 g) suspended in 20 ml water, 500 ml ether and 300 ml 10% $Na_2CO_3$ aq were added. The mixture was shaken until two clear layers formed (5–10 min). The aqueous layer was drawn off and washed twice with ether. The combined ether solns were washed with water until neutral and dried over $Na_2SO_4$. The solvent was removed under vacuum at r.t. to give a white, crystalline residue of **4a** m.p. 116–118° (lit.[8] m.p. 116–117°); $\nu_{max}$ (Nujol): 1724 $cm^{-1}$, aldehyde C=O; NMR ($CDCl_3$): δ 0·74 ($CH_3$—C-18, s), 1·03 ($CH_3$—C-19, s), 2·01 ($CH_3COO$, s), 5·34 (H—C-6, broad m), 9·55 ( −CHO, d, $J$ = 3 Hz). The aldehyde, **4a**, was immediately dissolved in a boiling soln of 8 ml diethylene glycol and 25 ml glacial AcOH. The soln was cooled to 40° and 4 ml $BF_3$-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 MeOH and dried under vacuum for several hr to give 5 g (82%) of **5a**. Recrystallization from EtOAc gave m.p. 209–210°; NMR ($CDCl_3$): δ 0·68 ($CH_3$—C-18, s), 0·92 ($CH_3$—C-21, d, $J$ = 6·5 Hz), 1·01 ($CH_3$—C-19, s), 2·00 ($CH_3COO$—, s), 3·85 (4H, ethylenedioxy H, d, $J$ = 2 Hz), 4·81 (H—C-22, d, $J$ = 2 Hz), 5·35 (H—C-6, broad m). (Found: C, 74·63; H, 9·56. Calcd. for $C_{26}H_{40}O_4$: C, 74·96; H, 9·68%).

*Preparation of* 3β-*benzoxy-5-cholestene-22-ethylene acetal*, **5b**

The acetate, **5a** (5 g) was refluxed for 1 hr in 300 ml 5% alcoholic KOH, poured into ice/water and extracted with $CH_2Cl_2$. The extract was washed with water until neutral, dried over $MgSO_4$, and evaporated to dryness. The residue was taken up in 80 ml pyridine and 10 ml benzoyl chloride was added. After standing at r.t. for 12 hr the soln was poured into ice. The ppt was collected, washed free of pyridine with water and recrystallized from EtOAc to give 5·2 g (90·5%) of **5b**, m.p. 178–180°. Three recrystallizations gave an analytical sample which melted at 182·5–183°; $\nu_{max}$ (KBr): 1715, 1600, 1585, 1260, 1250, 1105, 1030, 1010, 800 and 710 $cm^{-1}$; NMR ($CDCl_3$): δ 0·71 ($CH_3$—C-18, s), 0·92 ($CH_3$—C-21, d, $J$ = 6·5 Hz), 1·08 ($CH_3$—C-19, s), 3·96 (—O—$CH_2$—$CH_2$—O—, d, $J$ = 2 Hz), 4·87 (H—C-22, d, $J$ = 1·5 Hz), 5·44 (H—C-6, m) 7·7 (arom. H, m). (Found: C, 78·04; H, 8·90. Calcd. for $C_{31}H_{42}O_4$: C, 77·79; H, 8·84%).

*Preparation of* 3β-*benzoxy-5,7-cholestadiene-22-ethylene acetal*, **6b**

A soln of **5b** (4 g, 0·084 mole) in 100 ml $CCl_4$ was heated to reflux and NBS (1·65 g, 0·093 mole) added. The mixture was refluxed for 8 min then cooled in ice and the solid (995 mg, m.p. 122–126°) filtered and washed with cold light petroleum. The filtrate was evaporated at r.t. under vacuum and the obtained crystalline residue taken up in 75 ml xylene. The soln containing the bromosterol was added dropwise to a vigorously boiling soln of trimethyl phosphite (5·2 g, 0·042 mole) in 25 ml xylene. After refluxing for 90 min the xylene was distilled off at 75° under vacuum and the crystalline residue was treated with little EtOAc in the cold, filtered and washed on the filter with little ice cold light petroleum. The crude product was then recrystallized from EtOAc to give 2·77 g (69%) of **6b** m.p. 155–158°. The analytical sample had m.p. 166–167° after three recrystallizations from the same solvent; $\nu_{max}$ (Nujol): 1715, 1600, 1585, 1270, 1110, 835, 807, and 710 $cm^{-1}$; $\lambda_{max}^{Hexane}$ 226 mμ (ε = 15,400), 260 mμ (ε = 13,300), 271 mμ (ε = 13,200), 282 mμ (ε = 13,300), and 293 mμ (ε = 7,200); NMR ($CDCl_3$): δ 0·63 ($CH_3$—C-18, s), 0·98 ($CH_3$—C-19, s), 0·96 ($CH_3$—C-21, d, $J$ = 7·0 Hz), 3·85 (ethylenedioxy, H, d, $J$ = 1·8 Hz), 4·81 (H—C-22, d, $J$ = 2·0 Hz), 5·54 (H—C-6, H—C-7 b broad m), 7·84 (arom. H, m). (Found: C, 78·24; H, 8·53. Calcd. for $C_3H_{40}O_4$: C, 79·12; H, 8·46%).

*Preparation of* 3β-*acetoxy*-5,7-*cholestadiene*-22-*ethylene acetal*, **6a**

The reaction was carried out in the manner described for the 3β-benzoxy analog. The crude product was a mixture of 4, 6-diene (*ca* 30%); $\lambda_{max}$ 232, 238, and 248 mμ; 5,7-diene (*ca.* 65%) $\lambda_{max}$ 261, 273, 282 and 295 mμ and *ca* 5% of a more highly conjugated compound $\lambda_{max}$307 and 320 mμ; NMR 5,7-diene ($CDCl_3$): δ 0·63 ($CH_3$—C-18, s), 0·94 ($CH_3$—C-19, s); 4, 6 diene: δ 0·69 ($CH_3$—C-18, s), 1·03 ($CH_3$—C-19, s).

*Preparation of* 3β-*benzoxy*-5,7-*cholestadiene*-22-*al*, **7**

To a soln of **6b** (2·0 g, 4·2 mole) in 50 ml THF 5% $HSO_4$ was added until a ppt appeared. The soln was clarified by addition of THF. The reaction vessel was flushed for 10 min with a stream of $N_2$, sealed and stirred vigorously at r.t. for 12 hr. The soln was neutralized with $Na_2CO_3$ and most of the THF distilled off. The product was extracted with $CH_2Cl_2$, dried over $MgSO_4$ and the solvent evaporated. The crystalline residue was taken up in little cold ether and filtered to give 1·57 g (86·7%) of **7**, m.p. 179·0 0–182·5°; $\nu_{max}$ (KBr) 2930, 2860, 2710, 1724, 1715, 1600, 1585, 1500, 1312, 1270, 1205, 1110, 1070, 835, 807 and 710 $cm^{-1}$; $\lambda_{max}^{Hexane}$ 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); NMR ($CDCl_3$): δ 0·68 ($CH_3$—C-18, s), 0·96 ($CH_3$—C-19, s), 1·10 ($CH_3$—C-21, d, $J$ = 7·0 Hz), 4·90 (H—C-3, broad m), 5·51 (H—C-6, H—C-7, broad m), 7·84 (arom. H, m), 9·60 (—CHO, d, $J$ = 3 Hz). Mass Spec.: Found mol. wt. 432. $C_{29}H_{36}O_3$ requires: mol. wt. 432. (Found: C, 80·81; H, 8·25. Calcd. for $C_{29}H_{36}O_3$: C, 80·52; H, 8·39%).

*Preparation of* 2,3-*dimethyl*-2-*butene*-1-*triphenylphosphonium bromide*, **12**

To tetramethylethylene (8 g, 0·09 mole) in 100 ml $CCl_4$ NBS (14·3 g, 0·08 mole) and benzoyl peroxide (0·1 g) were added and the mixture refluxed for 24 hr. The soln was cooled in ice and filtered (7·5 g, m.p. 122–125°). The solvent was evaporated to give 1-bromo-2,3-dimethyl-2-butene as a clear, colourless liquid which was kept under vacuum (5 mm) for 1 hr and used without further purification for the salt formation.

The bromo alkene prepared above was taken up in 100 ml dry benzene. To this soln was added (21 g, 0·08 mole) triphenylphosphine. The ppt formed immediately, as an oil which crystallized. The mixture was stirred at 30° for 3 hr and filtered. The product was purified by stirring several times with fresh benzene and finally dried under vacuum, m.p. 164–166°; NMR ($CDCl_3$): 1·14 ($CH_3$—C-2, broad d, $J$ = 3 Hz), 1·62 (6H, $CH_3$—C-3, broad d, $J$ = 5·5), 4·54 ($2H_1$—$CH_2$—P, d $J$ = 14 Hz), 7·7 (15H arom. H, m).

*Preparation of* 2-*methylene*-3-*methylbutan*-1-*triphenylphosphonium iodide*, **13**

The 2-methylene-3-methylbutan-1-iodide used for the salt formation was prepared in the following manner. A mixture of LAH 6·4 g and 200 ml dry ether was refluxed for 30 min and the ether soln of LAH decanted from undissolved material. To this ether soln 2-methylene-3-methylbutanoic acid ethyl ester[28] (10 g, 0·07 mole) was added in such a way as to maintain gentle refluxing. After addition was completed the mixture was refluxed vigorously for 30 min. The excess LAH was decomposed by addition of water and 300 ml 10% $H_2SO_4$ was added under cooling. The mixture was stirred until two clear layers were obtained. The layers were separated and the aqueous phase extracted 3 times with ether. The combined ether extracts were dried over $MgSO_4$. The ether was evaporated to give 8·5 g crude 2-methylene-3-methylbutan-1-ol which was shown by NMR and VPC to contain about 10% of the fully saturated alcohol. The crude product was distilled to give pure 2-methylene-3-methylbutan-1-ol (5·1 g, 73%), b.p. (4·6 mm) 39°; $\nu_{max}$ (film): 3300, 1040 (−OH) and 1645 (>C=CH) $cm^{-1}$; NMR ($CDCl_3$): δ 1·05 [$(CH_3)_2$—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).

To the alcohol prepared above (4 g, 0·04 mole) cooled to below 10°, $PBr_3$ (4·6 g, 0·017 mole) was added gradually so that the temp did not rise above 10°. After addition was completed the mixture was kept at r.t. for 30 min. $NaHCO_3$ aq 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 2-methylene-3-methyl-butan-1-bromide 6·4, 71%) which darkened rapidly on standing at r.t. Spectra of the crude product showed the presence of a terminal methylene group (IR 1645 and 895 $cm^{-1}$ and NMR two doublets, $J$ = 1 Hz at δ 4·9 and δ 5·09). The product was used without further purification for further reactions.

A soln of the crude bromide (6 g) in benzene (200 ml) was added to a soln of triphenylphosphine (10 g) in benzene (50 ml) and refluxed for 10 hr. No ppt formed. The soln was then heated at 80° under pressure for 24 hr and the ppt collected, washed thoroughly with benzene and dried under vacuum. The NMR showed the product to be a mixture of 46% of the phosphonium salt of **13** (halogen=Br) and 54% of **12**.

The salt mixture was redissolved in $CHCl_3$ and heated at 80° under pressure for 12 hr. The ratio of the two isomers did not change.

A soln of NaI (6 g) in acetone (25 ml) was added to a soln of 2-methylene-3-methylbutan-1-bromide (6 g). A ppt of NaBr formed immediately. The mixture was kept at r.t. for 40 min. Water was added and the compound extracted with ether. The ether extract was washed with water, aq $Na_2S_2O_3$ and again with water and dried over $MgSO_4$ for 24 hr. Dry benzene was added to the ether soln. The ether and most of the benzene was evaporated under vacuum at 18–20°. The residue 2-methylene-3-methylbutan-1-iodide was taken up in dry benzene and the soln added to a soln of triphenylphosphine (10 g) in benzene (50 ml). The salt, **13**, began to precipitate in fine needles at r.t. after 1 hr. The mixture was left standing at r.t. for 48 hr, filtered and washed with benzene to give 13·9 g (59%) of **13**. After drying under vacuum for several days it melted at 184–187°; $\nu_{max}$ (KBr): 3045, 1645, 1585, 1435, 1110, 995, 910 and 865 $cm^{-1}$; NMR ($CDCl_3$): $\delta$ 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).

*Preparation of 3-methylbutan-2-one-1-triphenylphosphonium bromide*, **14**

Triphenylphosphine, (25·5 g, 0·07 mole) was dissolved in dry benzene (80 ml). Isopropylbromomethyl ketone[29] (10 g, 0·06 mole) was added and the mixture was left at r.t. for 24 hr then filtered. The solid was taken up in fresh benzene, filtered and dried under vacuum to give phosphonium salt, **14** (29·1 g, 82%). The product was recrystallized from EtOAc-hexane, m.p. 235–237°; $\nu_{max}$ (Nujol): 1690 $cm^{-1}$ (C=O); NMR ($CDCl_3$): $\delta$ 1·1 [$(CH_3)_2$C, 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).

*General procedure for the Wittig reaction using the nonketonic phosphonium salts*

Carefully dried and pulverised phosphonium salt was suspended in dry diethyl ether in a 500 ml pressure bottle. The bottle was flushed for 10 min with dry $N_2$ and capped with a serological septum. The calculated amount of BuLi 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 soln. The addition was again made with a syringe through the septum. The mixture was shaken at r.t. for 1–2 hr. After cooling, moist ether was added until excess reagent was decomposed. The crystalline solid was filtered and the ether soln, containing the product, was dried over $Na_2SO_4$, 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.

*Preparation of 5,22,24(28)-ergostatriene-3β-yl-acetate*, **8c**

*Preparation from aldehyde* **4a** *via ylide of* **13**. To a suspension of **13** (6·24 g, 0·0132 mole) in ether (30 ml) BuLi (0·013 mole) in heptane was added. After 30 min at r.t. **4a** (1·0 g, 2·64 mmole) was added. The mixture was then treated as above to give **8c** (510 mg, 44%). After two recrystallizations from alcohol the products had m.p. 143·0–144·0°. The IR, UV and NMR spectra obtained were superimposable with those obtained in preparation described below.

*Preparation from aldehyde* **4** *via the ketoacetate*, **8d**. Bisulfite adduct, **3a**, (3·5 g, 7·4 mole) was decomposed as described previously to give **4a** (2·1 g, 77·5%). To a soln of the aldehyde in DMSO (150 ml) **15** (12 g, 35 mmole) 3-one, prepared from **14** as described below, was added and the mixture heated at 95° for 65 hr. Then water (500 ml) and 10% $H_2SO_4$ (100 ml) were added and the product extracted with ether. The ether extract was washed several times with water and dried over $MgSO_4$. The crude product was purified on a $SiO_2$ column, hexane:ether (95:5) as eluting solvent to give **8d** (1·7 g, 68·4%). Recrystallization from MeOH gave m.p. 141·5–142·5°; $\nu_{max}$ (KBr): 1732 (acetate C=O), 1695, 1670 (sh), 1625 and 990 $cm^{-1}$ (—C=CH—CO—); $\lambda_{max}^{EtOH}$ 222 mμ ($\varepsilon$ = 19,500); NMR ($CDCl_3$): $\delta$ 0·73 ($CH_3$—C-18, s) 1·14 ($CH_3$—C-19, s), 1·15 ($CH_3$—C-21, $2CH_3$—C-25, d, $J$ = 7·0 Hz), 2·00 ($CH_3COO$—, 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, $C_{29}H_{44}O_3$ requires: mol wt. 440. (Found: C, 79·00; H, 10·04. Calcd. for $C_{29}H_{44}O_3$. C, 79·04; H, 10·06%).

*2,4-Dinitrophenylhydrazone derivative of* **8d**. This derivative was made in the usual fashion by refluxing **8d**, and ethanolic $H_2SO_4$ solution of 2,4-dinitrophenylhydrazine. The product was recrystallized twice from EtOAc-MeOH and had m.p. 214·0–214·5°.

Compound **10**, (1·7 g, 4·2 mmole) in ether (25 ml) was treated with BuLi (4·1 mmole) in heptane. This

mixture was reacted with **8d** (270 mg, 0·6 mmole) in ether (20 ml). The mixture was shaken for 1 hr at r.t. and 3 hr 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 MeOH to give **8c** (86 mg, 32%) m.p. 143·5–144°; $[\alpha]_D^{20} = -58°$ (C = 0·99); $\nu_{max}$ (KBr): 3095, 1600, 975 (sh), 965, 965, 890 (—HC=CH—C=$CH_2$), 1732 $cm^{-1}$ (C=O); $\lambda_{max}^{EtOH}$ 231 ($\varepsilon$ = 21,400), 226 sh ($\varepsilon \sim$ 20,000) and 238 sh ($\varepsilon \sim$ 16,000); NMR ($CDCl_3$): $\delta$ 0·71 ($CH_3$—C—18, s), 1·02 ($CH_3$—C-19, s), 1·07 (12H, $CH_3$—C-21, 2$CH_3$—C-25, d, $J$ = 6·5 Hz), 2·01 ($CH_3COO$—, s), 4·3–4·7 (H—C-3), 4·79 =$CH_2$, broad, s), 5·30 (H—C-6, m), 5·62 (H—C-22, quart., $J$ = 15 and 8 Hz), 5·91 (H—C-23, d, $J$ = 15 Hz). Stripping the column with ether gave 65 mg of **8d**.

*Preparation of triphenylphosphine isobutanonemethylene*, **15**, *from* **14**

A soln of **14** (18 g, 0·042 mole) in water (650 ml) was treated with sat $Na_2CO_3$ aq at r.t. for 12 hr. The solid was collected, washed with water and dried for 12 hr under vacuum. The product was recrystallized from EtOAc–hexane to give **15** (13·4 g, 91·5%) m.p. 170–171°; $\nu_{max}$ (Nujol); 1510 $cm^{-1}$ (C=O); $\lambda_{max}^{EtOH}$ 266 m$\mu$ ($\varepsilon$ = 6,700), 273 m$\mu$ ($\varepsilon$ = 6,500) and 287 m$\mu$ ($\varepsilon$ = 5,930); NMR ($CDCl_3$): $\delta$ 1·18 (($CH_3$)$_2$C, 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).

*Preparation of* 5,22,24(28Z)-*stigmastatriene*-3$\beta$-*yl-acetate*, **8a**

Compound **11** (1·66 g, 3·97 mmole), suspended in 35 ml dry ether, was reacted with BuLi (3·85 mmole) in heptane at r.t. for 1 hr. Ketone, **8d** (350 mg, 0·795 mmole) in dry ether (20 ml) was added and the reaction allowed to proceed at r.t. for 1 hr. The mixture was then shaken at 60° for 10 hr. After cooling, excess reagent was decomposed with moist ether and the reaction worked up in the usual manner. The product **8a** (C-3—OH) was reacetylated with $Ac_2O$ in pyridine. The acetate was purified by preparative TLC ($SiO_2$, benzene). After recrystallization from EtOH, **8a** had m.p. 122–122·5°; $[\alpha]_D^{20}$ ($c$ = 1·1) − 54°; $\nu_{max}$(KBr): 1732 (C=O), 970, 962, 903, 886, 844, 810, 803 and 795 $cm^{-1}$; $\lambda_{max}^{EtOH}$ 234 ($\varepsilon$ = 24,000); NMR ($CDCl_3$): $\delta$ 0·70 ($CH_3$—C-18, s), 1·01 ($CH_3$—C-19, s), 1·00 ($CH_3$—C-26, C-27, d, $J$ = 7 Hz); 1·625 $CH_3$—C-29, d, $J$ = 7 Hz), 2·00 ($CH_3COO$—, 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. $C_{31}H_{48}O_2$ requires: mol wt. 452. (Found: C, 82·07; H, 10·41. Calcd. for $C_{31}H_{48}O_2$. C, 82·25; H, 10·69%).

*Preparation of* 5,22,24-*ergostatriene*-3$\beta$-*yl-acetate*, **8b**

To a suspension of **12** (1·6 g, 3·8 mmole) in dry ether (30 ml) of BuLi (3·8 mmole) in heptane was added. The mixture was left to react at r.t. for 1 hr then **4a** (1·0 g, 2·64 mmole) was added. After 1 hr, all the ylide had reacted. The mixture was then shaken at 55–60° for 10 hr, 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 had some triene-alcohol which remained near the origin.) Both recovered components had identical UV absorptions. The major product was **8b**. After recrystallization from EtOH, it analyzed as a single component on TLC ($SiO_2$/$AgNO_3$), 735 mg (63%), m.p. 110–110·5°; $[\alpha]_D^{4U}$ ($c$ = 1·05) − 51°; $\nu_{max}$ (KBr): 1732 (C=O), 1140, 1045, 965, 810, 840, 855 and 890 $cm^{-1}$; $\lambda_{max}^{EtOH}$ 241·5 m$\mu$ ($\varepsilon$ = 28,300); NMR ($CDCl_3$); $\delta$ 0·71 (CH—C-18, s), 1·01 ($CH_3$—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. $C_{30}H_{46}O_2$ requires: mol wt. 438. (Found: C, 82·01; H, 10·49. Calcd. for $C_{30}H_{46}O_2$. C, 82·01; H, 10·49%).

*Preparation of* 5,7,22,24(28)-*ergostatetraene*-3$\beta$-*yl-benzoate*, **9c**

(a) *Preparation from aldehyde* **7** *via ylide of* **13**. A soln of **1** (1·1 g, 2·55 mmole) in THF was added to a soln of 2-methylene-3-methylbutan-1-triphenylphosphorane (12·7 mmole), prepared from salt **13** and BuLi. The mixture was treated as in the preparation of **8c**. 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 **9c** (0·69 g, 54·5%) m.p. 148–149°. After two recrystallizations from EtOAc-MeOH the m.p. was 149·0–150·5°. (lit.[6] m.p. 149–151°); $\nu_{max}$ (KBr), 1715 (C=O), 1600, 1585, 970, 962, 886, 835, 803, and 710 $cm^{-1}$; $\lambda_{max}^{EtOH}$ 229 m$\mu$ ($\varepsilon$ = 36,900), 271 m$\mu$ ($\varepsilon$ = 12,200), 281·5 m$\mu$ ($\varepsilon$ = 12,900) and 293 m$\mu$ ($\varepsilon$ = 7,000); NMR ($CHCl_3$): $\delta$ 0·65 ($CH_3$—C-18, s), 0·95 ($CH_3$—C-19, s), 1·06 ($CH_3$—C-21, 2$CH_3$—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-6, m H—C-7, m), 5·91 (H—C-23), d, $J$ = 15 Hz), 7·7 (arom. H, m). Mass Spec Found: mol. wt. 498. $C_{35}H_{46}O_2$ requires: mol. wt. 498. (Found: C, 84·25; H, 9·22. Calcd. for $C_{35}H_{46}O_2$. C, 84·29; H, 9·30%).

(b) *Preparation from aldehyde* **7** *via ketoester* **9d**. A soln of **1** (1·4 g, 3·24 mmole) and **15** (7 g, 20 mole) in DMSO (200 ml) were treated in the same way as in the preparation of **8d** to give **9a** (0·68 g, 42%) m.p. 148–149°; $\nu_{max}$ (KBr): 1715 (C=O), 1695 (sh), 1670, 1625, 995, 985 (—C=CH—CO—), 1600, 1585, 835 and 710 $cm^{-1}$; $\lambda_{max}^{CH_3OH}$ mμ 293 ($\varepsilon$ = 7,800) 281 ($\varepsilon$ = 14,400), 270·5 ($\varepsilon$ = 14,600), 261 ($\varepsilon$ = 11,800) and 228 ($\varepsilon$ = 29,000); NMR ($CDCl_3$): $\delta$ 0·67 ($CH_3$—C-18, s), 0·99 ($CH_3$—C-19, s), 1·10 ($2CH_3$—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), 6·80 (C-22, quart., $J$ = 16, 8 Hz), 7·82 (arom. H, m). Mass spec Found: mol. wt. 500. $C_{34}H_{44}O_3$ requires: mol. wt. 500. (Found: C, 81·40; H, 8·75. Calcd. for $C_{34}H_{44}O_3$. C, 81·56; H, 8·86%).

A soln of **9d** (1·0 g, 1·97 mmole) in THF was added to methylenetriphenylphosphorane (10 mmole) prepared in ether from **10** (2·47 g, 10 mmole) and BuLi (9·8 mmole). The reaction product was rebenzoylated and purified on preparative TLC to give **9c** (0·72 g, 72%) m.p. 148·5–150·0°.

*Isolation of* 5,7,22,24(28)-*ergostatetraene*-3β-*ol from* Saccharmyces cerevisiae

Brewers yeast was digested in alcoholic KOH at reflux temp for 1 hr. 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 benzolated in the usual way in pyridine benzoyl chloride. The major part of the ergosterol benzoate was removed by crystallization. The mother liquor was chromatographed 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 EtOAc-MeOH to give **9c**, m.p. 149·5–150·5°. (lit.[6] m.p. 149–151°.) The m.p. was not depressed on mixing with the synthetic **9c**. The IR, UV and NMR spectra of the natural and the synthetic sterol were essentially identical. Hydrolysis with alcoholic KOH as described above gave the free alcohol **9c** (R = H), m.p. 116–116·5° (lit.[6] m.p. 116–117°).

*Preparation of* 5,7,22,24-*ergostatetraene*-3β-*yl-benzoate*, **9b**

A soln of **7** in THF (1·1 g, 2·55 mmole) was added to a soln of the ylide (12·1 mmole) prepared in the usual way from **12** and treated as in the preparation of **8b** to give **9b** (0·925 g, 73%) m.p. 147·5–148°; $\nu_{max}$ (KBr): 1716 (C = O), 1600, 1585, 1500, 965, 890, 835 and 710 $cm^{-1}$; $\lambda_{max}^{EtOH}$ 234 mμ ($\varepsilon$ = 43,500), 271 mμ ($\varepsilon$ = 12,100) and 293·5 mμ ($\varepsilon$ = 6,200); NMR ($CDCl_3$): $\delta$ 0·66 ($CH_3$—C-18, s), 1·00 ($CH_3$—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. $C_{35}H_{46}O_2$ requires: mol. wt. 498. (Found: C, 84·25; H, 9·32. Calcd. for $C_{35}H_{46}O_2$. C, 84·29; H, 9·30%).

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